MISSION STATEMENT

The mission of the journal is to serve the need of the internist to practice up-to-date medicine and to keep track with important issues in health care. With this purpose we publish editorials, original articles, reviews, controversies, consensus reports, papers on speciality training and medical education, book reviews and correspondence.

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Alphen aan den Rijn, the Netherlands
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Co-publication of articles from the
Netherlands Drug Bulletin

D. Bijl

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As a result of a fruitful cooperation between the editorial boards of the Netherlands Journal of Medicine and the Netherlands Drug Bulletin (NDB) (Geneesmiddelenbulletin) we are proud to present the article by Professor Verheugt on platelet aggregation inhibitors in this issue of the Journal. The article was previously published as ‘Preventie en behandeling van coronair trombose met plaatjesaggregatieremmers’ by F.W.A. Verheugt in NDB 2002;36:133-40.

For the future it is planned that articles from NDB that may be of interest to specialists in internal medicine will also be translated and published in the Netherlands Journal of Medicine.

Here we would like to take the opportunity to highlight the unique system of peer review the NDB uses and give some information and background on how the NDB works.

AIM AND PURPOSE

The NDB Foundation published the first issue of NDB in 1967 under the auspices of the former Ministry of Social Affairs and Public Health. The American Medical Letter on Drugs and Therapeutics (1959) and the British Drug and Therapeutic Bulletin (1962) were used as models for the new journal.

The NDB Foundation publishes a monthly bulletin targeting everyone involved in the prescribing and provision of pharmaceuticals. Its purpose is to promote a more rational approach to pharmacotherapy and it strives to put the principals of ‘evidence-based medicine’ into practice. Furthermore, it aims to provide impartial information to counterbalance the enormous amounts of money the commercial sector spends on information for pharmaceutical products. NDB makes every effort to protect its contents from any influence from the pharmaceutical industry or even the suspicion thereof. To guarantee its freedom, the bulletin is not financed by profit from advertising. This leaves the editors free to comment critically on issues such as new drugs, side effects and promotional activities.

DISTRIBUTION

Of each bulletin some 50,000 issues are printed and distributed to members of the Dutch medical association, dental association and that of pharmacists, as well as members of professional specialist associations.

Furthermore, the bulletin is distributed to all medical students who are in the clinical training part of their study, and is available free of charge on the world wide web.

CONTENTS OF THE JOURNAL

The main article forms the basis of the NDB. It usually deals with a specific clinical condition, such as heart failure or vaginal infection. The creation of a new group of drugs at the time that a second competitor comes onto the market or specific problems that arise in the prescribing of drugs, such as side effects and interactions, may also be an indication for a review article.

STRUCTURE OF THE FOUNDATION

The Foundation has an editorial board and an advisory council, each with its own part to play. The editorial board is
responsible for the content of the journal. This is composed of seven people: general practitioners, pharmacists (both hospital-based and community) and several medical specialists. They meet monthly to discuss the ongoing publications. The editorial board is supported by an advisory council, which meets twice a year and has 17 members consisting of prescribing specialists, pharmacists, and general practitioners. The advisory council gives advice on the contents of the journal, policy concerning articles, choice of articles and also offers critical comments on each article. The preparatory and actual editorial work takes place in the editorial office, staffed by two scientific editors and three editorial assistants.

SOURCES OF LITERATURE

The NDB uses standard and consensus reports as its literature sources, providing these are available on the works in question. The consensus of opinion is, however, not always completely independent from the pharmaceutical companies, and standard works are sometimes in need of revision. For this reason NDB always tries to draw independent conclusions. To this end we use only trials which have been well structured and competently carried out and whose results have been published in journals that practice a system of peer review. In principle, NDB does not publish results gained from abstracts, posters, papers read at conferences, data on file and expert’s reports, as this material has not been checked by independent reviewers. Only in cases where there is no source of reliable, published information whatsoever, will NDB use the less well-reviewed information. This is always pointed out explicitly in the article.

EDITORIAL PROCEEDINGS

The main articles are usually written by external authors at the request of the editorial board. In the interests of impartiality we endeavour to opt for authors who are not associated with any particular pharmaceutical company. The potential author (if necessary) is sent articles of the randomised, double-blind and controlled trials published on the subject in question. Reports of these trials are obtained by the editorial office by systematic search operations in (mostly) the Medline, Embase and Cochrane libraries. Information is also obtained from, for instance, sister publications, review articles and textbooks. The first draft received from the author(s) is checked on its content and adapted to the house-style by the editorial staff. The editors also look for uniform usage of medical terminology and literature listings, using the Vancouver style. The version of the article thus created is then sent to the editorial board, the advisory council and to a minimum of five experts in the field (external referees) for peer review. A number of permanent referees are also asked for their views. These include representatives of the Farmacotherapeutisch Kompas (national formulary), the Dutch scientific associations of general practitioners and pharmacists, as well as professional specialist associations. This system ensures that every article is reviewed by at least 20 experts. The companies who make the products also get a chance to comment on the article. The name of the author is not printed on the draft article at this stage. The editorial office inventorises and considers the reviews of the article and then performs a further literature search. Suggestions on processing of reviews are laid before the editorial committee. At its monthly meetings, the committee decides which suggestions to submit to the author. The summarised and undesignated comments and text suggestions are then discussed with the author personally. The resulting second revised version of the article is returned to the referees and drug companies with an accompanying explanation of the revisions. They again have the chance to review and comment, should they wish to do so. It is only then, usually about six months after the delivery of the first draft, that the article is considered ready for publication. Sometimes, even more time is necessary. This is an inherently laborious process as it involves the opinions and arguments of many experts who all have the right to hear and be heard. The main articles are meant to serve as guides for a number of years. The article by M.E.R. Gomes and Professor F.W.A. Verheugt is the first from NDB to be included in the Netherlands Journal of Medicine and underlines very well the quality of the peer-review process of NDB.

More information can be found on www.geneesmiddelenbulletin.nl.
Platelet aggregation inhibitors in prevention and treatment of coronary thrombosis

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ABSTRACT

Platelet aggregation plays a key role in the development of complications of atherosclerosis. By inhibiting platelet aggregation in a pharmacological way complications such as myocardial infarction and sudden death may be prevented. This goes for primary as well as secondary prevention. The most relevant substances for this goal are aspirin, clopidogrel and the new glycoprotein IIb/IIIa inhibitors.

INTRODUCTION

In the Western world atherosclerosis is the leading cause of severe morbidity and mortality. Though much of the pathophysiological mechanism of atherosclerosis is still unknown, a multifactor model seems appropriate. In many cases this chronic condition will be complicated by thrombosis, the so-called atherothrombosis. Antithrombotic medication is the cornerstone in the prevention and treatment of this severe complication of atherosclerosis. Within the arsenal of antithrombotic medication, platelet aggregation inhibitors have taken a prominent position, because they are highly effective and easy to use. In this review we summarise the latest developments in coronary thrombosis, discussing successively the pathophysiology, causes and consequences of atherothrombosis on one hand and the pharmacology, efficacy, most commonly occurring side effects, contraindications and interactions of the main antithrombotic agents on the other hand. Finally, the place of antiplatelet therapy within the total spectrum of antithrombotic treatment is discussed.

PATHOPHYSIOLOGY, CAUSES AND CONSEQUENCES OF ATEROTHROMBOSIS

Pathophysiology

Atherosclerosis causes arterial stenosis. Although the early stages of atherosclerosis are usually benign, sudden changes in flow can result in rapid progression of the stenosis and can even give total occlusion of the vessel by thrombus. This total occlusion will result in ischaemia and ultimately infarction. When the onset of the ischaemia is sudden and there is no collateral circulation, the infarcted area will be large and may result in pump failure, severe ventricular arrhythmias, (fatal) cerebral infarction or the loss of limbs, gut or retina. The reason for thrombus formation on an atherosclerotic plaque of a vessel with reduced diameter is not yet clarified. Atherosclerotic plaques are covered with an incomplete endothelial layer. The uncovered subendothelial structures such as collagen and tissue factor seem to stimulate platelet adhesion and aggregation and the activation of the coagulation system by the aggregated platelets, all of which cumulate in the formation of platelet-rich thrombi at the site of the atherosclerotic plaque. This thrombus can in turn result in total occlusion.

Causes of coronary thrombosis

There are two main theories on the cause of coronary thrombosis. The first theory assumes that local factors induce coronary thrombosis. This theory is supported by the findings of pathologists in patients with diseases due to coronary thrombosis and those who have received fibrinolytic therapy. In these patients total coronary artery occlusion has almost always occurred at a site with severe stenosis. The second theory assumes that there is a state
of elevated platelet aggregability. This is supported by the fact that some patients have a myocardial infarction without significant coronary artery disease. Also the circadian variation with a morning increment in the frequency of myocardial infarction, sudden death and ischaemic cerebrovascular events seems to support this theory. Elements of both theories probably play a role in coronary thrombosis.

**Consequences of coronary thrombosis**

Thrombus formation in coronary arteries can give severe complications. Among these complications acute coronary syndromes with ST elevation (acute transmural infarction) and without (unstable angina pectoris or non-Q-wave infarction) can be distinguished. Randomised controlled trials have shown that mortality rates of acute myocardial infarction were 30% within the first hour after onset in patients prior to hospital admission, 10% during hospital admission and 10% during the first year after discharge. About 10% of patients with unstable angina pectoris develop a myocardial infarction.

**PHARMACOLOGY**

Three groups of platelet aggregation inhibitors are available.

**Aspirin and dipyramidole**

The first group is represented by aspirin and dipyramidole. Aspirin reduces the synthesis of prostaglandins by acetyllating cyclo-oxygenase. As a result the formation of prostacyclin (PGI₂), which has a vasodilatory and platelet aggregation inhibiting function, and thromboxane A₂ (TxA₂), which has a vasoconstrictive and platelet aggregation stimulating function, is inhibited (figure 1). Due to the fact that cyclo-oxygenase production is regulated on a nuclear level, the effect of aspirin on nucleus-containing cells is only of short duration (a few hours). For cells without a nucleus, such as platelets, the effect is sustained for its lifetime, which is about seven days. The effect of aspirin is immediate and continuous until at least 50% of the platelets have been replaced. At a very low dose (from 9 mg/day) aspirin removes all thromboxane A₂ from plasma, without reducing prostacyclin levels. The clinical effectiveness of this dosage is, however, still under debate. A dose of 30 mg/day has shown to be clinically effective in neurology and 75 mg/day in cardiology.

**Clopidogrel and ticlopidine**

The second group consists of clopidogrel and ticlopidine. Due to its side effects ticlopidine is not registered in the Netherlands and is therefore not discussed in this article. Clopidogrel is a so-called prodrug which is metabolised in the liver. It is a powerful inhibitor of adenosine diphosphate (ADP) induced platelet aggregation, without reducing thromboxane A₂ induced platelet aggregation. It also has an indirect inhibitory effect on the glycoprotein IIb/IIIa receptor. It has no influence on platelet collagen adhesion (no reduction of intracellular cyclic adenosine monophosphate), humoral haemostasis or fibrinolysis. Its effect is best assessed by measuring bleeding time. It has a stronger effect on bleeding time than aspirin. Clopidogrel reaches a maximum effect three to five days after administration, which may reduce its usefulness in the acute setting. Clinical effectiveness can be enhanced using a loading dose.

**Glycoprotein IIb/IIIa inhibitors**

The third group contains the glycoprotein IIb/IIIa inhibitors. By preventing fibrinogen mediated cross-linkage of platelets by means of the platelet glycoprotein IIb/IIIa receptor, these agents inhibit the final common pathway of platelet aggregation, independent of the kind of stimuli (thromboxane, collagen and cathecholamines). Glycoprotein IIb/IIIa inhibitors do not influence platelet adhesion. Theoretically, glycoprotein IIb/IIIa inhibitors can prolong bleeding time indefinitely. By recombinant
EFFECTIVENESS AND MOST COMMONLY OCCURRING SIDE EFFECTS

We will now describe the effectiveness of antiplatelet agents in primary prevention of coronary heart disease, the direct treatment of acute coronary syndromes and in secondary prevention. Additionally, their most important side effects will be discussed. For the explanation of the abbreviations used, see table 1.

Effects on primary prevention

In 2001 a meta-analysis was performed from four randomised controlled clinical trials concerning the effectiveness of aspirin in the primary prevention of coronary heart disease.4 This meta-analysis showed that aspirin gives a significant reduction of cardiovascular events and myocardial infarction, without affecting mortality. Treatment was useful in patients with an absolute risk for cardiovascular events of 1.5% or more per year (NNT=44 for five years). For patients with an absolute risk of 1% per year or less, treatment with aspirin was of limited value. A more recent meta-analysis, assessing the efficacy of aspirin in the primary prevention of cardiovascular disease events, showed that aspirin reduces myocardial infarction by an average of 28% (95% CI 11 to 42%) in healthy individuals with a wide range of cardiovascular risk.5 Neither mortality nor stroke were significantly decreased in either of these trials. They calculated that for preventing 6-20 myocardial infarctions, one has to treat 1000 patients with an absolute risk for coronary heart disease of 5% for a period of five years with aspirin (number needed to treat=NNT=50-167). On the other hand in this group 0-2 cerebral haemorrhages will be induced (number needed to harm=NNH= 2500) as well as 2-4 severe gastrointestinal bleedings (NNH=250-500). In patients with an absolute risk of 1% for a period of five years, 1-4 myocardial infarctions will be prevented (NNT=250-1000), at the cost of 0-2 cerebral haemorrhages (NNH=2500) and 2-4 gastrointestinal bleedings (NNH=250-500), resulting in a benefit to harm ratio <1.0. With regards to the major side effects, excess gastrointestinal bleeding in the above trials ranges from 0.4 (pNS) to 1.7 (p<0.05) per 1000 patients treated per year. In the HOT trial excess risk was similar in males and females. In the same trial elderly (>65 years) had slightly more excess bleeding (1.7%, p=0.03) than younger individuals (1.4%, p=0.002) per 1000 person-years. Excess cerebral bleeding in the above trials varied from -0.12 (pNS) to 0.2 (pNS) per 1000 patients treated per year. Regarding the benefits and haemorrhagic risks, aspirin should not be given to each healthy individual in the primary prevention of cardiovascular disease events. Only in those with a certain amount of cardiovascular risk will the benefit of aspirin outweigh the haemorrhagic risk. In the Physicians’ Health Study, of all the parameters assessed (age, smoking, diabetes, family history, plasma lipid level, blood pressure, alcohol used, exercise and body mass), only blood lipid levels and age showed a significant interaction with aspirin benefit. Whereas lipid levels showed a negative interaction (the higher the plasma cholesterol, the less benefit from aspirin), age was a clear indicator of increased benefit. Aspirin showed to be effective from the age of 50, with the protective effect of aspirin for the elderly (over the age of 65 to 70) seeming likely. Protective benefit in women below the age of 50 seems unlikely, but above this age their protective benefits seem to be similar to those in men.6 Additionally individuals at risk can be identified using some form of risk calculation.

In conclusion low-dose aspirin is indicated in the prevention

<table>
<thead>
<tr>
<th>TERM</th>
<th>DEFINITION</th>
<th>FORMULA</th>
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<tbody>
<tr>
<td>Relative risk (RR)</td>
<td>The risk for a certain event in the experimental group (Y) divided by the risk in the control group (X)</td>
<td>( RR = \frac{R_y}{R_x} )</td>
</tr>
<tr>
<td>Absolute risk (AR)</td>
<td>The frequency of occurrence of an event in a specific period of time</td>
<td></td>
</tr>
<tr>
<td>Absolute risk reduction (ARR)</td>
<td>The risk for an event in the control group (X) minus the risk in the experimental group (Y)</td>
<td>( ARR = R_x - R_y )</td>
</tr>
<tr>
<td>Number needed to treat (NNT)</td>
<td>The number of patients needed to be treated for a period of time to prevent one event</td>
<td>( NNT = \frac{1}{R_y - R_x} )</td>
</tr>
<tr>
<td>Number needed to harm (NNH)</td>
<td>The number of patients in which during active treatment (H_y) in one extra person a complication occurs compared with the controls (H_x)</td>
<td>( NNH = \frac{1}{H_y - H_x} )</td>
</tr>
</tbody>
</table>
of myocardial infarction in persons at higher cardiovascular risk, i.e. males and females over the age of 50 years with a cardiovascular risk of more than 1%/year. Additional risk analysis can be performed using the risk calculation tables.

Direct treatment of acute coronary syndromes
Aspirin should be administered to every patient under suspicion of acute coronary syndrome, because it is the only substance that gives an instant inhibition of platelet aggregation. In most countries 75 to 325 mg/day is administered after a loading dose of 160-200 mg. After admission clopidogrel or a glycoprotein IIb/IIIa inhibitor can be added.

Effects on secondary prevention
General
In 1994 a meta-analysis of 145 randomised trials (over 100,000 patients) comparing platelet aggregation inhibitors against each other and against placebo in patients with an increased risk for vascular events, a protective effect in secondary prevention of cardiovascular or cerebrovascular events was found when treated with aspirin (75-325 mg/day) or another platelet aggregation inhibitor for a few years. For the endpoints of myocardial infarction, CVA and mortality, the following NNTs were calculated: nonfatal myocardial infarction NNT=25 (one month of treatment), prior myocardial infarction NNT=25 (two years of treatment), unstable angina pectoris NNT=20 (six months treatment), miscellaneous with an increased risk, e.g. operation, stable angina pectoris, NNT=50 (one year of treatment). There was no relation with age, sex, hypertension or diabetes. In 2002 a consecutive meta-analysis was performed by the same group of investigators. They now analysed 287 randomised trials (over 200,000 patients) comparing different doses of platelet aggregation inhibitors with placebo in patients with an increased risk for vascular events. The authors came to a similar conclusion as in their meta-analysis of 1994, with NNTs now being respectively: 26 for myocardial infarction (one month of treatment), 28 for patients with a prior myocardial infarction (27 months of treatment) and miscellaneous 45 (22 months of treatment). The authors could not show a dose-effect relationship for aspirin, when comparing a dose of less than 75 mg with a dose of 75 mg or more in the secondary prevention of cardiovascular events. They stated that in the literature there was insufficient evidence of the efficacy of an aspirin dose of less than 75 mg, to conclude that the effectiveness was similar to that of a dose of 75 mg or more.

Aspirin and clopidogrel
Only one study compared the efficacy of clopidogrel with aspirin. In this study (with a mean follow-up time of 1.9 years) the effects of clopidogrel and aspirin were compared in 19,000 patients with a recent CVA, myocardial infarction or peripheral vascular disease. The authors concluded that in 9.8% of the patients treated with clopidogrel the primary combined endpoint had occurred (nonfatal cerebral or myocardial infarction or death by a cardiovascular event), compared with 10.7% of the patients treated with only aspirin (NNT=110). For total mortality these numbers were 5.8% and 6.0% respectively (NNT=500). Severe haemorrhages occurred in 112 patients in the clopidogrel group and 149 patients in the aspirin group. In another study assessing 12,000 patients with acute coronary syndrome without ST elevation, patients were administered clopidogrel with aspirin or aspirin alone within 24 hours after the initial symptoms (table 2). After 12 months a nonfatal CVA, nonfatal myocardial infarction or death by cardiovascular event had occurred in 9.3% of the patients using clopidogrel, compared with 11.4% of the patients without clopidogrel (NNT=48). There was a significantly larger number of severe haemorrhages (patients in need of transfusion) in the group using clopidogrel (3.7% versus 2.7%, NNH=100), but there was no difference in the number of life-threatening haemorrhages.

Dipyramidole
This agent is only used in the secondary prevention of CVA and TIA and will not be further discussed in this article.

Glycoprotein IIb/IIIa inhibitors and aspirin
The combination of aspirin with an intravenously administered glycoprotein IIb/IIIa inhibitor has been compared with aspirin alone in patients with acute coronary syndromes with or without ST elevation and in patients who underwent a PTCA (table 2). In almost all of these studies both groups (experimental and control) were given a combination of aspirin and heparin as the standard treatment. The mean follow-up was 30 days. In a meta-analysis the effect of an intravenously administered glycoprotein IIb/IIIa inhibitor was assessed in patients with acute coronary syndromes without ST elevation. Six randomised placebo-controlled trials were studied with a total of 31,402 patients. Five of these trials were double-blinded. The authors concluded that within 30 days after randomisation the primary combined endpoint of death or myocardial infarction was reached in 10.8% of the patients treated with the glycoprotein IIb/IIIa inhibitor, compared with 11.8% in the controls (NNT=106). There was no statistically significant difference with regards to the endpoint death (2.06% in the treated group versus 3.66% for the controls, RR 0.90, CI 0.80-1.02). After 30 days the treated group showed more severe haemorrhages than the control group (2.4% versus 1.4%, NNH=121). Of all patients 38% underwent a percutaneous coronary intervention (PCI) or bypass operation. In these patients, compared with the patients without intervention, the treatment seemed effective. The efficacy of oral glycoprotein IIb/IIIa inhibitors has also been assessed in...
Table 2

Large randomised trials on platelet aggregation inhibitors in acute coronary syndromes with or without ST elevation or PCI

<table>
<thead>
<tr>
<th>STUDY (YEAR) AGENT</th>
<th>DEATH AND (RE)INFARCTION (n/N)</th>
<th>RR</th>
<th>AR</th>
<th>ARR</th>
<th>NNT</th>
<th>NNH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ACUTE CORONARY SYNDROMES WITHOUT ST ELEVATION</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Clopidogrel CURE (2001)</td>
<td>450/5202</td>
<td>209/2598</td>
<td>0.80 [0.72-0.90]</td>
<td>0.02</td>
<td>0.02</td>
<td>48</td>
</tr>
<tr>
<td><strong>Intravenous glycoprotein IIb/IIIa receptor antagonists</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CURE (2001)</td>
<td>450/5202</td>
<td>209/2598</td>
<td>0.80 [0.72-0.90]</td>
<td>0.02</td>
<td>0.02</td>
<td>48</td>
</tr>
<tr>
<td>GUSTO-IV ACS (2001)</td>
<td>abciximab</td>
<td>450/5202</td>
<td>209/2598</td>
<td>0.80 [0.72-0.90]</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>PARAGON (1998)</td>
<td>lamifiban</td>
<td>172/1524</td>
<td>89/758</td>
<td>0.81 [0.70-0.94]</td>
<td>0.03</td>
<td>0.03</td>
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<tr>
<td>PARAGON-B (2002)</td>
<td>lamifiban</td>
<td>278/2628</td>
<td>296/2597</td>
<td>0.80 [0.72-0.90]</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>PRISM (1998)</td>
<td>tirofiban</td>
<td>94/1616</td>
<td>115/1616</td>
<td>0.67 [0.55-0.81]</td>
<td>0.01</td>
<td>0.01</td>
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<td>PRISM-PLUS (1998)</td>
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<td>96/797</td>
<td>0.77 [0.65-0.91]</td>
<td>0.01</td>
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<td>PURSUIT (1998)</td>
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<td>873/6209</td>
<td>745/4739</td>
<td>0.80 [0.72-0.90]</td>
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<td>Meta-analysis (2002)</td>
<td>1598/18297</td>
<td>1550/13105</td>
<td>0.93 [0.86-0.99]</td>
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<td><strong>Oral glycoprotein IIb/IIIa receptor antagonists</strong></td>
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<td>FROST (2000)</td>
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<td>17/401</td>
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<td>214/3074</td>
<td>0.72 [0.65-0.79]</td>
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<td>339/4406</td>
<td>136/2231</td>
<td>0.72 [0.65-0.79]</td>
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<td>1125/17769</td>
<td>487/8836</td>
<td>1.16 [1.04-1.28]</td>
<td>0.01</td>
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<tr>
<td><strong>ACUTE CORONARY SYNDROMES WITH ST ELEVATION</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>Intravenous glycoprotein IIb/IIIa receptor antagonists</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASSENT-3 (2001)</td>
<td>abciximab</td>
<td>177/2017</td>
<td>208/2038</td>
<td>0.80 [0.72-0.90]</td>
<td>0.02</td>
<td>0.02</td>
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<tr>
<td>ENTIRE (2002)</td>
<td>abciximab</td>
<td>14/241</td>
<td>20/242</td>
<td>0.72 [0.49-1.09]</td>
<td>0.02</td>
<td>0.02</td>
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<tr>
<td>GUSTO-V (2001)</td>
<td>abciximab</td>
<td>616/8328</td>
<td>726/8260</td>
<td>0.80 [0.72-0.90]</td>
<td>0.02</td>
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<tr>
<td>INTRO-AMI (2002)</td>
<td>eptifibatide</td>
<td>16/204</td>
<td>7/101</td>
<td>0.72 [0.49-1.09]</td>
<td>0.02</td>
<td>0.02</td>
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<tr>
<td>SPEED (2001)</td>
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<td>5/115</td>
<td>9/109</td>
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<td>TIMI-14 (2000)</td>
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<td>0.02</td>
<td>0.02</td>
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<tr>
<td>Meta-analysis</td>
<td>840/11055</td>
<td>979/10900</td>
<td>0.85 [0.78-0.93]</td>
<td>0.014</td>
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<tr>
<td><strong>PERCUTANEOUS CORONARY INTERVENTION</strong></td>
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<tr>
<td><strong>Intravenous glycoprotein IIb/IIIa receptor antagonists</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meta-analysis (2001)</td>
<td>0.82 [0.71-0.96]</td>
<td>0.02</td>
<td>0.02</td>
<td>50</td>
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</table>

The results mentioned in this table correspond in part with the Cochrane Collaboration. The primary endpoint (death and reinfection) is displayed for the experimental as well as the control group, as the number of patients with this primary endpoint compared with the total number of patients in each individual group (n/N). The result of the relative risk (RR) and the absolute risk (AR) are presented by way of graphics. The size of the squares represents the number of contestants in each study. The horizontal lines through each square correspond with the 95% CI. With regards to the AR, there is no statistically significant effect when the squares enclose the vertical line. The squares at the left side of the vertical line indicate a tendency to treatment preference, whereas the squares at the right side of the vertical line indicate control preference. The results of the intravenously administered glycoprotein IIb/IIIa inhibitors in acute coronary syndromes without ST elevation have been slightly adjusted. The same goes for the results of the oral glycoprotein IIb/IIIa inhibitors in acute coronary syndromes without ST elevation and those of the intravenous glycoprotein IIb/IIIa inhibitors. For all results statistical tests for heterogeneity were performed. These were all negative, which indicates that the results of all studies point in the same direction.

a. The number needed to harm regards to major bleedings. b. The values of the ARR and NNT are negative because the treatment is not effective. c. Only the data of the control group which were given heparin are displayed. d. Of these studies, in which ‘dose-finding’ was also assessed, only the results of the period hereafter (‘dose-confirmation’) were analysed. e. Only the results of the two largest trials are shown, due to the fact that of the remaining studies not all data could be calculated. f. Of this study only the number of patients who died were included. g. This meta-analysis gives insufficient data for some outcomes.

The authors concluded that the use of oral glycoprotein IIb/IIIa inhibitors gives an increase in mortality and myocardial infarction. The use of intravenously administered glycoprotein IIb/IIIa inhibitors has also been analysed in patients with an acute coronary syndrome without ST elevation. The use of oral glycoprotein IIb/IIIa inhibitors gives an increase in mortality and myocardial infarction.
During PCI, compared with cessation prior to the PCI showed an additional benefit when their use was continued (0.80–1.03) respectively. Glycoprotein IIb/IIIa inhibitors for bleeding for all doses of aspirin.34 When used for more than one year, there is a remarkable increase in the number of gastrointestinal bleedings compared with placebo (2.47% versus 1.42%, NNH=106).35 There was no evidence that a lower dose or tablets with slow release decreased the number of bleedings. For this reason it seems likely that the risk for bleeding is independent of the dose used. In this meta-analysis, studies were excluded that selected specific categories of bleedings, e.g. only large bleedings or bleedings that necessitated hospital admission. Another meta-analysis of five primary prevention studies showed that aspirin does not increase the risk for cerebral haemorrhage (OR 1.4, CI 0.9–2.0), but does increase the risk for a large gastrointestinal bleeding (OR 1.7 CI 1.4–2.1). Total mortality was not influenced. Yet another meta-analysis assessed the risk of cerebral haemorrhage in the use of aspirin.16

Aspirin and ticlopidine
Four randomised controlled open trials assessing the efficacy of ticlopidin and aspirin on the endpoints death and (re) infarction in patients who underwent a PCI with stent implantation showed a positive effect against stent thrombosis.39 40 There were significantly more haemorrhagic complications in the ticlopidin group.

Contraindications
The most important contraindications for aspirin use are gastric ulcers, erosive gastritis, haemorrhagic diathesis, recent cerebral haemorrhage, severe renal insufficiency (renal clearance of <30 ml/min), severe liver insufficiency and the use of anticoagulants. Further contraindications are gastric discomfort or pain, asthmatic attacks, collapse or allergic reactions in former use.

Interactions
The effect of oral anticoagulants can be enhanced by the use of aspirin, increasing the risk for bleeding. In a recently published study on patients with arthritis, authors found that ibuprofen antagonises the aspirin induced irreversible platelet aggregation inhibition. This effect was not found for diclofenac, rofecoxib or paracematol.37 The relevance of this finding for day-to-day practice is not yet clear. The simultaneous use of NSAIDs or corticosteroids with aspirin also increases the risk of gastrointestinal ulceration.

Clopidogrel
Side effects
Gastrointestinal side effects such as abdominal pain, dyspepsia, diarrhoea and nausea are the most common side effects of clopidogrel. Gastrointestinal bleedings, purpura or nose bleeds can also occur. Rash and itching have also been described, as well as central and peripheral nervous system disorders.
Contraindications
Severe liver insufficiency and haemorrhages such as peptic ulcer or cerebral haemorrhage are contraindications.

Interactions
The simultaneous use of clopidogrel with aspirin, heparin, thrombolytic agents or NSAIDs increases the chance of haemorrhage.

Ticlopidine
Side effects
The ticlopidine-induced bone marrow depression that is seen in almost all patients has prohibited large-scale use of this agent.38

Glycoprotein IIb/IIIa inhibitors
Side effects
The most frequent side effect of abciximab is haemorrhages within 36 hours after administration. Mild thrombocytopenia is seen in over 4% of the patients, where a severe thrombocytopenia occurs in 1% of the patients. The other common side effects are hypotension, nausea, vomiting, chest pain, bradycardia and fever. Some people have suggested the administration of an H2-receptor antagonist or a proton pump inhibitor for the prevention of gastrointestinal bleeding. There is no scientific proof to support this theory.

Contraindications
The most important contraindications are recent bleeding or an increased risk for bleeding: internal bleeding, recent CVA or operation, haemorrhagic diathesis, thrombocytopenia, retinopathy and hypertension. Additionally liver and renal insufficiency are absolute contraindications (except for tirofiban).

Interactions
There is little known about their interactions. Preferably, these agents should not be used simultaneously with thrombolytic therapy.

CONCLUSION
Platelet aggregation plays a key role in the thrombotic complications of atherosclerosis, such as unstable angina pectoris, myocardial infarction, stroke and sudden death. Platelet aggregation can follow many different pathways, of which thromboxane A2, thrombin, collagen and ADP are the most important. Eventually, by binding to the glycoprotein IIb/IIIa receptor, fibrinogen is used for the final common pathway to create the binding between the platelets. Pharmacological inhibition of platelet aggregation can be achieved by influencing thromboxane A2 (aspirin), ADP (clopidogrel) or by blocking the glycoprotein IIb/IIIa receptor (abciximab, eptifibatide and tirofiban). Aspirin reduces the risk of myocardial infarction and death in patients with arterial vascular disease. All patients with acute coronary syndromes should therefore be given aspirin as soon as possible and they should continue to use aspirin for the rest of their lives. The same goes for patients with chronic coronary syndromes not yet using aspirin. Aspirin gives an instant effect. If there are contraindications for aspirin, clopidogrel can be given. Ticlopidine is no longer used in the Netherlands because of its side effects. In one study assessing patients with a prior CVA or myocardial infarction, clopidogrel showed less reinfarction and death than aspirin. With aspirin a larger number of bleedings occurred. Additional research is necessary. In acute coronary syndromes without ST elevations the efficacy of clopidogrel with aspirin has been demonstrated compared with aspirin alone in one study, but additional research is also necessary. In clinical practice clopidogrel is not routinely used outside coronary intervention. The most important application of clopidogrel is in secondary prevention, when there is a contraindication for aspirin. A statistically significant decrease in death and reinfarction has been demonstrated with a combination of glycoprotein IIb/IIIa inhibitors plus aspirin, compared with aspirin alone in patients undergoing PTCA. In practice only specialists in cardiovascular medicine (such as interventional cardiologists or heads of coronary care units) use these substances. The value of glycoprotein IIb/IIIa inhibitors in the treatment of acute coronary syndromes is only small, while there is an increased risk for haemorrhages. It is obvious that there will be no further research on oral glycoprotein IIb/IIIa inhibitors, because of the increase in deaths and re-infarctions.

NOTE
This paper is slightly adapted from a previously published paper entitled ‘Preventie en behandeling van coronaire trombose met bloedplaatjesaggregatieremmers’ from prof. dr. F.W.A. Verheugt, under responsibility of the editorial office of the Geneesmiddelenbulletin, 2002;36:133-40.

REFERENCES


ABSTRACT

A 60-year-old woman presented with typical features of alkaptonuria.

CASE REPORT

A 60-year-old woman was referred by her general practitioner because of a bluish discoloration of both auricles, which had been present for some years (figure 1). A year earlier arthroscopy, performed because of pain in the right knee, showed dark-grey sediment on the cartilage but no further abnormalities. Except for the pain in the right knee, which was already decreasing, she had no symptoms of the skeletal system. The patient said that she had noticed that her urine showed a dark discoloration when the toilet had not been flushed for a number of hours. The family history revealed that two of her three brothers had also developed dark-coloured auricles. Besides the bluish discoloration of both auricles, physical examination revealed pigmentation of both sclerae (figure 2). No further discolorations of skin or cartilage were found.

WHAT IS YOUR DIAGNOSIS?

See page 212 for the answers to this photo quiz.

Figure 1
Ochronotic pigmentation of the conchae, anthelices and helices of the right and left ear, respectively

Figure 2
Ochronotic pigmentation of the sclerae of the right and left eye, respectively
Adipose tissue as an endocrine organ: impact on insulin resistance

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A B S T R A C T

It is well known that obesity is associated with insulin resistance and an increased risk for type 2 diabetes mellitus. Formerly it was postulated that increased lipolysis and consequently free fatty acid (FFA) production, from with triglycerides overloaded fat cells, would disrupt glucose homeostasis via Randle's hypothesis. Lipodystrophy, however, also leads to insulin resistance. Recently it has become clear that adipose tissue functions as an endocrine organ and secretes numerous proteins in response to a variety of stimuli. These secreted proteins exert a pleiotropic effect. The proteins that are involved in glucose and fat metabolism and hence can influence insulin resistance are discussed in this paper. They include leptin, resistin, adiponectin, acylation-stimulating protein, tumour necrosis factor-α and interleukin-6. The stimuli for production and the site and mechanism of action in relation to insulin resistance will be discussed. None of these proteins are, however, without controversy with regard to their mechanism of action. Furthermore, some of these proteins may influence each other via common signalling pathways. A theory is presented to link the interrelationship between these adipocyte secretory products and their effect on insulin resistance.

L I S T O F A B B R I E V I A T I O N S

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>Acrp 30</td>
<td>Complement-related protein 30</td>
</tr>
<tr>
<td>AgRP</td>
<td>Agouti-related protein</td>
</tr>
<tr>
<td>AMPK</td>
<td>Adenosine monophosphate kinase</td>
</tr>
<tr>
<td>ADD1/SREBP</td>
<td>Adipocyte determination and differentiation factor/sterol regulatory element-binding protein</td>
</tr>
<tr>
<td>aP2</td>
<td>Fatty acid-binding protein</td>
</tr>
<tr>
<td>apM1</td>
<td>Adipose most abundant gene transcript-1</td>
</tr>
<tr>
<td>ASP</td>
<td>Acylation-stimulating protein</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BAT</td>
<td>Brown adipose tissue</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CART</td>
<td>Cocaine-amphetamine-related transcript</td>
</tr>
<tr>
<td>C/EBP</td>
<td>CCAAT (is piece of DNA)/enhancer-binding proteins</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>COS cells</td>
<td>Monkey cells immortalised with simian V40 virus</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin-releasing hormone</td>
</tr>
<tr>
<td>Cys</td>
<td>Cysteine</td>
</tr>
<tr>
<td>DAG</td>
<td>Diacylglycerol</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>FAS</td>
<td>Fatty acid synthase</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acid</td>
</tr>
<tr>
<td>FIZZ</td>
<td>Found in inflammatory zone</td>
</tr>
<tr>
<td>Gdp 28</td>
<td>Gelatin-binding protein</td>
</tr>
<tr>
<td>GLUT-4</td>
<td>Glucose transporter-4</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>IRS-1</td>
<td>Insulin receptor substrate-1</td>
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<tr>
<td>JAK</td>
<td>Janus kinase</td>
</tr>
<tr>
<td>α-MSH</td>
<td>Alpha-melanocyte-stimulating hormone</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>NEFA</td>
<td>Non-esterified fatty acids</td>
</tr>
<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td>PEPCK</td>
<td>Phospho-enolpyruvate carboxykinase</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphatidylinositol-3 phosphate</td>
</tr>
<tr>
<td>POMC</td>
<td>Pro-opiomelanocortin</td>
</tr>
<tr>
<td>Ob-Rb</td>
<td>Long isoform of the leptin receptor</td>
</tr>
<tr>
<td>RELM</td>
<td>Resistin-like molecule</td>
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<tr>
<td>PPAR-γ</td>
<td>Peroxisome proliferator-activated receptor γ</td>
</tr>
<tr>
<td>RXR</td>
<td>Retinoid X receptor</td>
</tr>
<tr>
<td>STAT</td>
<td>Signal transducers and activators of transcription</td>
</tr>
<tr>
<td>TG</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor alpha</td>
</tr>
<tr>
<td>TZDs</td>
<td>Thiazolidinediones</td>
</tr>
<tr>
<td>WAT</td>
<td>White adipose tissue</td>
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INTRODUCTION

Type 2 diabetes mellitus is a chronic disease characterised by insulin resistance of the muscle, liver and adipose tissue and an impaired function of the β-cell of the pancreas. The incidence of type 2 diabetes mellitus (type 2 DM) has increased dramatically over the last decades. Nowadays it is the most frequently occurring metabolic disease, affecting over 140 million people worldwide with an expected rise to about 300 million patients in 2025.

Epidemiological studies assessing the explanation for this explosion point to an excess caloric intake over metabolic demand and decreased physiological activity as plausible causes. A chronic imbalance between energy intake and energy expenditure eventually leads to obesity, a condition predisposing to insulin resistance and type 2 DM. Of type 2 diabetic patients, 80% are obese as defined by a body mass index >27 kg/m².

In the past, adipose tissue was merely viewed as a passive organ for storing excess energy in the form of triglycerides. Recently, however, it has become clear that the adipocyte actively regulates the pathways responsible for energy balance and that this function is controlled by a complex network of hormonal and neuronal signals.

To discuss all the adipocyte secretory products (table 1) and all their effects is beyond the scope of this paper. In this review we will focus on the function of the adipocyte in relation to insulin resistance and obesity. First the differentiation process of the adipocyte will be discussed.

Then some of the adipocyte secretory products that are involved in energy balance regulation and their function will be considered. Finally, some interactions between adipocyte-derived factors that could be involved in inducing insulin resistance will be described.

ADIPOCYTE DIFFERENTIATION

There are two forms of adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT). BAT serves primarily to dissipate energy, which is done via uncoupling protein 1 (UCP-1) in the mitochondria of BAT. Adult humans only have a small amount of BAT. WAT stores energy in the form of triglycerides. It has recently become evident that WAT also secretes a vast amount of so-called adipokines, which are involved in maintaining energy homeostasis. This will be discussed in this article.

In humans, the formation of white adipose tissue (WAT) begins during late embryonic development, with a rapid expansion shortly after birth as a result of increased fat cell size as well as fat cell numbers. Even in adults the potential to generate new fat cells persists. The origin of the adipose cell and adipose tissue are still poorly understood. Our current understanding indicates that a

<table>
<thead>
<tr>
<th>MOLECULE</th>
<th>EFFECT</th>
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<tbody>
<tr>
<td>Leptin*</td>
<td>Feedback effect on hypothalamic energy regulation; maturation of reproductive function</td>
</tr>
<tr>
<td>Resistin*</td>
<td>Appears to impair insulin sensitivity</td>
</tr>
<tr>
<td>Adiponectin*</td>
<td>Improves insulin sensitivity if administered to rodent models of insulin resistance; improves fatty acid transport and utilisation</td>
</tr>
<tr>
<td>Adipsin*</td>
<td>Required for the synthesis of ASP, possible link between activation of the complement pathway and adipose tissue metabolism</td>
</tr>
<tr>
<td>ASP*</td>
<td>Activates diacylglycerol acyltransferase, inhibits hormone sensitive lipase, stimulates GLUT-4 translocation to the cell surface</td>
</tr>
<tr>
<td>TNF-α*</td>
<td>Mediator of the acute phase response. Inhibits lipogenesis, stimulates lipolysis and impairs insulin-induced glucose uptake, thus leading to insulin resistance and weight loss</td>
</tr>
<tr>
<td>IL-6*</td>
<td>Increases hepatic glucose production and triglyceride synthesis, role in insulin resistance unclear</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Potent inhibitor of the fibrinolytic system</td>
</tr>
<tr>
<td>Tissue factor</td>
<td>Initiator of the coagulation cascade</td>
</tr>
<tr>
<td>Angiotensinogen</td>
<td>Regulator of blood pressure and electrolyte homeostasis</td>
</tr>
<tr>
<td>PG₁_{α} and PGF₂_{α}</td>
<td>Implicated in inflammation and blood clotting, ovulation and menstruation, acid secretion</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Regulates growth and differentiation of numerous cell types</td>
</tr>
<tr>
<td>IGF-1</td>
<td>Stimulates cell proliferation and mediates many of the effects of growth hormone</td>
</tr>
<tr>
<td>MIF</td>
<td>Involved in proinflammatory processes and immunoregulation</td>
</tr>
<tr>
<td>aP2</td>
<td>Involved in intracellular trafficking and targeting of fatty acids</td>
</tr>
<tr>
<td>Agouti</td>
<td>Might be involved in inducing insulin resistance through increasing intracellular free calcium concentrations</td>
</tr>
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</table>

* Proteins discussed in this article.
pluripotent stem cell precursor gives rise to a mesenchymal precursor cell, which has the potential to differentiate along mesodermal lineages of myoblast, chondroblast, osteoblast and adipocyte (figure 1). Given appropriate stimuli the preadipocyte undergoes clonal expansion and subsequent terminal differentiation into a mature adipocyte. In vitro, adipogenesis follows an orderly and well-characterised temporal sequence. Initially there is growth arrest of proliferating preadipocytes induced by the addition of a prodifferentiative hormonal mixture (including insulin, a glucocorticoid, an agent that elevates cAMP levels and foetal bovine serum). Growth arrest is followed by one or two rounds of cell division, known as clonal expansion. At about the second day after differentiation induction there is a second, permanent period of growth arrest. Growth-arrested cells are committed to becoming adipocytes and begin to express late markers of adipocyte differentiation at day 3. Cells eventually become spherical, accumulate fat droplets and become terminally differentiated adipocytes by day 5 to 7.

Most of the changes that occur during adipocyte differentiation take place at the gene expression level. Several reports have attempted to schematise the stages of adipocyte differentiation as we have here in figure 1.

Three major classes of transcription factors that directly influence fat cell development have been identified: the peroxisome proliferator-activated receptor-γ (PPAR-γ), CCAAT/enhancer binding proteins (C/EBPs) and the basic helix-loop-helix family (ADD1/SREBP-1c). The C/EBPs belong to the basic-leucine zipper class of transcription factors which function through homodimeric and heterodimeric complexes with C/EBP family members. Six isoforms have been identified with varying tissue distribution. C/EBP α, β and δ are expressed in both white and brown adipose tissue and are involved in the regulation of adipogenesis.

The peroxisome proliferator-activated receptor (PPAR) belongs to the nuclear hormone receptor family. Three isotypes have been identified thus far, PPAR α, β and γ, each with a different tissue distribution, ligand and metabolic action. All PPARs form a heterodimer with the retinoid X receptor (RXR) and bind to a PPAR-RXR response element on the DNA. Their actions upon ligand binding, however, are completely different. PPAR-γ exists as three isoforms, γ1, γ2 and γ3. PPAR-γ2 is highly expressed in adipose tissue. The thiazolidinediones (a new class of oral blood glucose lowering drugs), which are high-affinity synthetic ligands for PPAR-γ, strongly induce adipogenesis and activate the expression of multiple genes encoding...
for proteins involved in lipid and glucose metabolism.\(^5\) Adipocyte determination and differentiation factor 1 (ADD1) and sterol regulatory element binding protein 1c (SREBP-1c), which are rodent and human homologues respectively, belong to the basic helix-loop-helix (bHLH) family of transcription factors. ADD1/SREBP-1c is expressed in brown adipose tissue, the liver, WAT and the kidney.\(^1\) The expression of ADD1/SREBP-1c is increased early during adipocyte differentiation.\(^4\) The protein seems to exert its adipogenic effect through upregulation of PPAR-\(\gamma\). Furthermore the protein might be involved in the production of an endogenous ligand for PPAR-\(\gamma\).\(^8\) In addition to its effect on adipogenesis, ADD1/SREBP-1c clearly stimulates many genes involved in fatty acid and cholesterol metabolism.\(^9\) A summary of the molecular events of adipocyte differentiation, based on our current knowledge, is depicted in figure 1 and 2.

**ADIPOCYTE SECRETORY PRODUCTS**

**Leptin**

*Discovery, structure, genetic locus and sites of expression of leptin*

The discovery of leptin (from the Greek *leptos* which means thin) in 1994\(^{10}\) has led to a renewed and intensified interest in the adipocyte and its role in energy homeostasis. Leptin acts on hypothalamic neuropeptide-containing regions and increased leptin signalling leads to decreased food intake, increased energy expenditure and increased thermogenesis, all promoting weight loss. Apart from these effects, leptin is also involved in glucose metabolism, normal sexual maturation and reproduction, and has interactions with the hypothalamic-pituitary-adrenal, thyroid and growth hormone axes.

Leptin is a protein consisting of 167 amino acids and has a helical structure similar to cytokines. Leptin is the product of the *ob* gene, which is located on chromosome 7q31. Leptin is expressed mainly in white adipose tissue. The protein circulates as both free and bound hormone and is cleared among others by the kidneys.\(^{11-13}\)

**Modulators of leptin production**\(^{14,15}\)

Leptin levels are positively correlated with the amount of energy stored as fat, so leptin levels are higher in obese people.\(^{14,15}\) Leptin levels rapidly decrease during fasting\(^{16}\) and remain low until four to six hours after eating when they begin to rise again.\(^{17}\) Plasma leptin levels show a diurnal pattern with a nocturnal peak shortly after midnight and a midmorning trough between 10 am and 12 noon.\(^{18}\) Insulin also plays a role in the regulation of leptin secretion: prolonged insulin infusions markedly increase serum leptin levels.\(^{19,20}\) Finally, even after adjustment for body fat mass, women have higher serum leptin levels than men.\(^{21}\) At the gene promotor level, it is known that stimulation of PPAR-\(\gamma\) downregulates leptin production\(^{21,22}\) whereas C/EBP-\(\alpha\) stimulates leptin production.\(^{23}\)

**Site of action of leptin and its role as part of an adipostat**

Leptin acts through binding at and activation of specific leptin receptor isoforms, which belong to the class I cytokine receptor family.\(^{41}\) Only the long isoform (ob-rb) is able to activate the JAK-(Janus kinase)-STAT (signal transducers and activators of transcription) signal transduction pathway upon leptin binding (figure 3). The long form of the leptin receptor is found in several peripheral tissues and in many areas of the brain, including the arcuate, ventromedial and dorsomedial hypothalamic nuclei.\(^{44}\) These hypothalamic regions are known to be involved in the regulation of appetite, food intake, temperature regulation and body weight. Intracerebral administration of leptin alters the expression of many hypothalamic neuropeptidies.\(^{45}\) By modulating these neurotransmitter systems, leptin has

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...a major role in maintaining energy balance and thus serves as part of an adipostat. During fasting, serum insulin levels fall and the uptake of glucose and lipids by the adipocyte diminishes. This leads to a decreased expression of the ob-gene, which is responsible for leptin formation and hence the plasma leptin concentration falls. Reduced leptin signalling leads to an increased expression of neuropeptide Y (NPY) and agouti-related protein (AgRP) in the arcuate nucleus of the hypothalamus. NPY and AgRP promote body weight gain by stimulating food intake and decreasing energy expenditure. Another neuronal cell type coproduces cocaine-amphetamine related transcript (CART) and pro-opiomelanocortin (POMC), from which α-melanocyte stimulating hormone (α-MSH) is cleaved. CART and α-MSH are both anorexigenic and reduced leptin signalling inhibits the synthesis of CART and POMC (figure 3).16,27

Finally, corticotropin-releasing hormone (CRH), which is also produced in the hypothalamus, might be important in mediating the effects of leptin, presumably via activation of sympathetic outflow to BAT, WAT, liver and muscle. Intracerebral injection of CRH stimulates thermogenesis and oxygen consumption and reduces food intake and body weight. CRH mRNA levels are increased by the intraventricular administration of leptin.28

Role of leptin in obesity

The initial conception of leptin as an anti-obesity hormone, whose primary role was to increase the metabolic rate and decrease food intake and appetite through action in the brain, was based on the following observations: 1) leptin deficient ob/ob mice and leptin receptor deficient db/db mice exert marked hyperphagia, decreased energy expenditure, morbid obesity and insulin resistance;29,30 2) administration of intravenous or intracerebroventricular leptin decreases body weight and fat mass through inhibition of food intake and increased energy expenditure in ob/ob but not in db/db mice;31 3) there is a threshold level of serum leptin (25-30 ng/ml) above which increases in serum levels are not translated into proportional increases in cerebrospinal or brain leptin levels, i.e. the transport system must be saturable;32 4) the discovery of leptin receptors in the hypothalamus, the region...
involved in regulation of food intake and energy balance.\textsuperscript{57} However, in most obese humans the gene encoding leptin is normal: up till now only two families with a mutation in the leptin gene have been identified.\textsuperscript{59,64} In contrast, most obese humans have increased serum leptin levels,\textsuperscript{54,55} indicating that obesity is a leptin-resistant state. Such a resistance could theoretically occur at several levels of the leptin signal transduction pathway, but this has not been resolved yet.

**Leptin and insulin resistance.**

Since obesity is associated with insulin resistance, it is interesting to look at the role of leptin in the development of insulin resistance and diabetes. A strong correlation between serum leptin and insulin levels, independent of body fatness, has been demonstrated in human studies.\textsuperscript{35,36} Hyperinsulinaemia induced by clamp techniques increases serum leptin levels, though not acutely.\textsuperscript{9} Serum leptin levels are increased by insulin therapy as well, both in type 1 and type 2 diabetic patients.\textsuperscript{36,37} Vice versa, a fair amount of evidence points to the fact that leptin has insulin- and glucose-lowering properties, although some studies find just the opposite. An extensive review on the association between leptin and insulin resistance has recently been published.\textsuperscript{38}

In both normal rodents\textsuperscript{39} and rodents with obesity and insulin resistance\textsuperscript{30-34} leptin therapy improves hyperinsulinaemia and hyperglycaemia. These effects are already apparent before weight loss occurs and are not due to energy restriction as was shown in pair-fed control studies.\textsuperscript{44,45} Most obese humans have increased serum leptin levels\textsuperscript{44,45} and thus far the overall effect of leptin therapy on weight loss and metabolic parameters has been modest.\textsuperscript{44} It is likely that very high plasma levels of the hormone are needed to overcome the leptin-resistant state. A final point pointing to an antidiabetogenic effect of leptin is that both in lipodystrophic rodents\textsuperscript{46} and humans (who have an extreme deficit of subcutaneous adipose tissue),\textsuperscript{46} a condition associated with severe insulin resistance with hyperglycaemia, hyperinsulinaemia and hypertriglyceridaemia, leptin therapy corrects all these metabolic abnormalities, independent of the accompanying reduction in food intake.

**Hypotheses with regard to the glucose and insulin-lowering effect of leptin**

As mentioned before, leptin seems to have an insulin-sensitising effect on the whole body level but conflicting results were reported when individual tissues were examined. Most in vivo experiments suggest a diabetogenic effect of leptin.\textsuperscript{59} Beside the differences between animals and humans, sources of leptin and time of exposure to this hormone might also play a causative role in the differences found. Furthermore, the fact that leptin exerts a glucose- and insulin-lowering effect and improves insulin sensitivity in vivo, suggests involvement of centrally acting mechanisms. This concept is further supported by the observation that leptin fails to reverse insulin resistance and lipid accumulation in mice with ventromedial hypothalamic lesions.\textsuperscript{67} The peripheral mechanism by which leptin exerts its glucose- and insulin-lowering effect might be via promoting fatty acid oxidation and triglyceride synthesis. Indeed, leptin administration activates 5’-AMP-activated protein kinase (AMPK) in skeletal muscle, leading to the inhibition of acetyl coenzyme A carboxylase and subsequently stimulation of fatty acid oxidation. The resulting intramyocellular lipid depletion will enhance insulin sensitivity.\textsuperscript{58} Apart from insulin-sensitising effects, leptin diminishes hyperinsulinaemia, probably via inhibition of insulin secretion. Functional leptin receptors have been demonstrated on insulin-secreting β-cells of the pancreas.\textsuperscript{49} Leptin inhibits glucose-stimulated insulin secretion both in vitro\textsuperscript{50} and in vivo.\textsuperscript{51} The mechanism involved is activation of the ATP-sensitive potassium channels in the β-cell. Finally, leptin shares intracellular pathways with insulin, both in peripheral tissues and in the central nervous system.\textsuperscript{54} Many effects of both insulin and leptin are mediated via activation of PI-3 (phosphatidylinositol-3-phosphate) kinase, so a degree of crosstalk between insulin and leptin may exist at the level of PI-3 kinase. Effects of leptin on insulin signalling have been studied and support an inhibitory effect of leptin on insulin signalling at the level of tyrosine phosphorylation of IRS-1 (insulin receptor substrate 1) and PI3-kinase binding to IRS-1.\textsuperscript{53} The effect of hyperinsulinaemia on intracellular leptin signalling has rarely been addressed but in one study supraphysiological concentrations of insulin completely cancelled out the leptin-induced insulin response.\textsuperscript{53}

**Conclusion**

Thus, leptin is an adipocyte secretory product that is not only involved in food intake and energy metabolism but clearly also has a role in glucose metabolism. Since plasma leptin levels are positively correlated with BMI, obesity seems to reflect a leptin-resistant state. Resistance for the action of leptin could promote obesity via decreased energy expenditure and a failure to diminish food intake. Furthermore, since leptin has a glucose- and insulin-lowering effect on the whole body level in vivo, resistance for this effect could induce insulin resistance. One explanation for the insulin resistance seen in obesity might be that the high leptin levels interfere with insulin signalling. Another possibility is that there is a diminished activation of AMPK in myocytes due to impaired leptin signalling. The resultant decrease in fatty acid oxidation will lead to an increase in intramyocellular lipids and thus to insulin resistance. Finally, both peripheral and central leptin resistance must be involved in insulin-resistant states since leptin treatment fails to correct insulin resistance in mice with ventromedial hypothalamic lesions.
Resistin

**Discovery, structure, genetic locus, sites and modulators of expression of resistin**

Resistin is a unique protein with cysteine-rich residues, which belongs to a class of tissue-specific secreted proteins termed the RELM (resistin-like molecule)/FIZZ (found in inflammatory zone) family. Resistin/FIZZ 3 is specifically expressed and secreted by adipocytes. The gene encoding resistin in mice has been named *Retn*. The regulation of resistin gene expression is controversial, see table 2.

**Resistin in obesity and insulin resistance**

The initial report by Steppan *et al.* suggested that resistin might constitute the link between obesity and insulin resistance. Resistin serum levels were increased in obese mice and resistin gene expression was induced during adipocyte differentiation. In addition, administration of resistin impaired glucose tolerance and insulin action in wild-type mice and in vitro in 3T3-L1 adipocytes whereas antiresistin antibody improved insulin sensitivity. The fact that thiazolidinediones suppressed resistin secretion led to the hypothesis that these insulin sensitisers exert their effect via downregulation of resistin gene expression. An increase in adipocyte gene expression during 3T3-L1 adipocyte differentiation and after the induction of high-fat-diet induced obesity was found in two other studies. Several other investigators, however, found a decreased resistin gene expression in WAT in different models of rodent obesity and insulin resistance, and resistin did not seem to be involved in the aetiology of insulin resistance in Fischer 344 rats, a good model for the metabolic syndrome in humans. Studies in humans are even more controversial. One study could not detect any resistin mRNA in human fat cells at all in subjects with varying degrees of insulin resistance and obesity. Another investigator found increased resistin mRNA in adipose tissue of obese humans, compared with lean controls, but decreased mRNA in freshly isolated human adipocytes. In addition resistin mRNA was undetectable in a severely insulin resistant subject. Janke *et al.* found an increased resistin gene expression in cultured human preadipocytes compared with mature adipocytes but again no relationship between resistin gene expression and either insulin resistance or body weight could be detected. Although the higher resistin mRNA levels found in abdominal fat tissue compared with thigh could explain the increased metabolic abnormalities in abdominal obesity, the fact that resistin mRNA expression is very similar in subcutaneous and omental adipose tissue suggests that it is unlikely that resistin is the link between (visceral) adiposity and insulin resistance.

**Conclusion**

The conclusion must be that many questions still have to be resolved. Conflicting results have been reported with regard to the factors regulating resistin gene expression (table 2). This is probably due to the difference between 3T3-L1 cell lines and in vivo models. Furthermore, the observed relation between resistin mRNA, serum resistin levels and insulin resistance in rodents cannot readily be extrapolated to humans. Murine resistin is only about 56% identical to human resistin at the amino acid level. Even in mouse models it is still unclear whether resistin plays a causal role in insulin resistance. Experiments in resistin knockout mice and in transgenic mice (which overexpress resistin) will be needed to solve this problem, but even then the relevance of resistin to human diabetes remains unclear, especially because some groups have found only minimal expression of the hormone in human fat. Furthermore it would be interesting to know how resistin exerts its presumed insulin-antagonising effects and what its target organs are. For that purpose the resistin receptor would have to be found and downstream signalling pathways have to be unravelled.

**Adiponectin**

**Discovery, sites of expression and stimuli leading to adiponectin production**

Adiponectin is a recently identified adipocyte-specific secretory protein of about 30 kD that appears to be involved

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**Table 2**

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>DECREASING RESISTIN</th>
<th>INCREASING RESISTIN</th>
<th>NO EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiazolidinediones</td>
<td>[54,56,58]</td>
<td>[59]</td>
<td>[60]</td>
</tr>
<tr>
<td>Insulin</td>
<td>[56,58]</td>
<td>[59,61]</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td>[58]</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>[56,58]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-adrenergic agonists</td>
<td>[62]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>[58,63]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epinephrine</td>
<td>[58]</td>
<td></td>
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</tbody>
</table>

Factors that have been reported to increase or decrease resistin expression with their references.
in the regulation of energy balance and insulin action and also seems to have anti-inflammatory and anti-atherogenic properties. Adiponectin is the product of the adipose tissue most abundant gene transcript-1 (apM1), which is exclusively expressed in WAT and is located on chromosome 3q27. Adiponectin is specifically expressed during adipocyte differentiation and is not detectable in fibroblasts. The expression of adiponectin is stimulated by insulin,70 IGF-171 and the TZDs. Corticosteroids,72 TNF-α73 and β-adrenergic stimulation73 inhibit adiponectin gene expression in 3T3-L1 adipocytes.

Serum and mRNA levels of adiponectin in obesity and insulin resistance
Serum adiponectin levels are decreased in humans with obesity76-77 and type 2 diabetes76-78 as well as in obese and insulin-resistant rodents.79 In addition, adiponectin gene transcription is decreased in adipocytes from obese75 and diabetic86 humans and rodents.77-79 Plasma adiponectin concentrations increase after weight reduction in obese diabetic and nondiabetic patients.24 The degree of plasma hypoapoadiponectinaemia was more closely related to the degree of hyperinsulinaemia and insulin resistance than to the degree of adiposity.76 Low plasma adiponectin concentrations predicted a decrease in insulin sensitivity81 to the degree of adiposity.76,77,79 In nondiabetics plasma adiponectin concentrations of free fatty acids which will both improve insulin signalling; 2) direct improvement of insulin signalling; 3) inhibition of gluconeogenesis, partly via reduced substrate delivery and partly via reduction of molecules involved in gluconeogenesis by activation of AMPK.

Disappointingly, no positive correlation between plasma adiponectin levels and 24-hour respiratory quotient (RQ) measurement (pointing to an increase in carbohydrate metabolism) could be demonstrated in healthy nondiabetic Pima Indians.29 This does not rule out, however, that administration of adiponectin to subjects with low levels of this hormone will increase RQ and energy expenditure.

The acylation-stimulating protein (ASP) pathway
ASP production and site of action
Acylation-stimulating protein (ASP) is a 76 amino acid protein identical to C3adesArg, a cleavage product of complement factor 3 (C3) formed via interaction of C3 with factor B and adipsin. C3, factor B and adipsin are all components of the alternative complement pathway and are produced by the adipocyte in a differentiation dependent manner.91 The major site of action of ASP appears to be on the adipocytes themselves, which have a specific saturable receptor for ASP.92 In human adipocytes there are differentiation and site-specific differences in ASP binding which are proportional to the ASP response: differentiated adipocytes bind more ASP and have a greater response to ASP than undifferentiated adipocytes.93 Furthermore, subcutaneous adipose tissue has greater affinity and greater specific binding to ASP than undifferentiated adipocytes.94

ASP promotes triglyceride storage
ASP promotes triglyceride storage in adipocytes via three mechanisms. First, ASP increases fatty acid esterification in adipocytes by increasing the activity of diacylglycerol acyltransferase, which is the final enzyme involved in triglyceride synthesis.95 Second, ASP stimulates glucose transport in human and murine adipocytes and preadipocytes.95 This effect on glucose transport is accomplished via translocation of cell-specific glucose transporters to the cell membrane. Third, ASP decreases lipolysis via inhibition of hormone-sensitive lipase.95 The effects of ASP are independent of and additional to the action of insulin.95

Stimuli leading to ASP production
In vitro studies in cultured adipocytes indicate that insulin96 and even more so chylomicrons97-98 increase ASP production. In vivo, plasma ASP concentrations seem to show little change after an oral fat load.98 There is, however, post-

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prandially an increased venoarterial gradient of ASP across a subcutaneous abdominal tissue bed with a maximum after 3 to 5 hours, indicating increased adipose tissue ASP production. This increase in ASP postprandially is substantially later than the increase in insulin but shows a close temporal relationship with maximal plasma triacylglycerol clearance.108

Plasma ASP levels in obesity
An excellent review on the physiology of ASP in humans and rodents has recently been published.99 Plasma levels of ASP are 225-fold lower (weighted average 28.3 nM) than its precursor C3. Studies measuring plasma ASP levels should therefore be interpreted with caution while it might very well be that ASP acts as a paracrine hormone.99 Plasma ASP levels are increased in obese humans100-103 and are reduced after fasting or weight loss.104,105 ASP has also been shown to be significantly increased in type 2 diabetes106-108 but since type 2 diabetes is often associated with obesity this might be a confounding factor. On the other hand, plasma ASP levels were inversely correlated to glucose disposal during a euglycaemic clamp in humans.109 Adipocytes from obese humans are as responsive to ASP as adipocytes from lean people.105 Thus the increased levels of ASP in human obesity in the face of a similar responsiveness to ASP compared with lean subjects, may promote energy storage, leading to adiposity.

Relation between ASP enhanced triglyceride clearance and insulin resistance
ASP production is increased in obese mice. Intraperitoneal (i.p.) administration of ASP to normal mice resulted in accelerated postprandial triglyceride (TG) and nonesterified fatty acid (NEFA) clearance after an oral fat load.106 In addition, plasma glucose levels returned faster to basal levels. C3 knockout mice (KO), which are unable to produce ASP, showed delayed plasma triglyceride clearance after an oral fat load in the absence of any change in fasting plasma TG levels. Administration of exogenous ASP enhanced plasma TG clearance.107 Remarkably these C3 KO mice were more insulin sensitive, had a reduced fat mass and yet an increased food intake. It was later shown that the hyperphagia/leanness was balanced by an increase in energy expenditure.108

Conclusion
In summary, ASP promotes storage of energy as fat. Decreased ASP production decreases lipid storage and induces an obesity-resistant state and improved insulin sensitivity. Plasma ASP levels are increased in obese humans; whether this is the effect or cause of the increased adipose tissue mass remains to be elucidated. Post or propter, increased ASP levels together with a continuing responsiveness of the ASP receptor will lead to further triglyceride storage. Although enhanced fatty acid trapping will decrease free fatty acid levels and hence diminish hepatic gluconeogenesis, increased ASP functioning in skeletal muscle will lead to an increase in skeletal muscle triglyceride storage leading to insulin resistance.

Tumour necrosis factor-α (TNF-α)
Structure of TNF-α, sites of production and receptor interaction
TNF-α is a cytokine produced mainly by activated macrophages in response to invasive stimuli, but also by nonimmune cells such as muscle and adipose tissue. Furthermore, TNF-α has a variety of biological effects in various tissues and cell types, and can thus be considered a multifunctional cytokine.109 TNF-α is produced as a 26-kD membrane-bound precursor that is proteolytically cleaved to a 17-kD soluble form.109 The cytokine interacts with two membrane-bound receptors, a 60-kD and an 80-kD subtype also called type I and type II receptor (TNFR-1 and TNFR-2). These receptors have different cellular and tissue distribution patterns and can bind other cytokines as well. TNF-α has a higher affinity for TNFR-1 than for TNFR-2.109 Due to the high affinity for its receptor TNF-α can act either as an autocrine or paracrine cytokine at low concentrations or as an endocrine cytokine at high concentrations. In addition to the membrane-bound receptors, soluble forms of the two receptors exist for which TNF-α has an even higher affinity. When TNF-α is bound to these soluble receptors no interaction can take place with the membrane-bound forms and thus TNF-α action is inhibited. Therefore, the physiological role of the soluble receptors may be to regulate TNF-α action.

Modulators of TNF-α production
In macrophages and monocytes, the expression and production of TNF-α is stimulated by endotoxins such as lipopolysaccharide (LPS). LPS resulted in a fivefold stimulation of TNF-α in human adipose tissue and isolated adipocytes in vitro, the latter indicating that it is unlikely that the response is entirely due to macrophages and monocytes in the stromal vascular fraction of adipose tissue. Insulin and glucocorticoids did not have a significant effect on TNF-α release from human adipose tissue or isolated adipocytes in vitro.110 Thiazolidinediones reduced adipocyte TNF-α release in obese rodents111 but no effect was seen in human adipose tissue in vitro.112 Since high-fat diets resulted in a significant increase in TNF-α mRNA and protein in epidydimal and retroperitoneal fat pads in rats, free fatty acids and/or triglycerides may play an important role as inducers of TNF-α expression.112

Effect of TNF-α on glucose and lipid metabolism
Firstly, TNF-α inhibits preadipocyte differentiation by downregulating the expression of two important adipocyte
transcription factors: PPAR-γ and CEBP/α.113 Secondly, TNF-α reduces the expression of GLUT-4, glycogen synthase and fatty acid synthase, which are essential for insulin-mediated glucose uptake and the subsequent conversion of glucose to glycogen or fatty acids. Furthermore, genes involved in the uptake of free fatty acids and the subsequent conversion to triglycerides, such as lipoprotein lipase, long-chain fatty acyl-CoA synthetase and diacylglycerol acyltransferase, were also downregulated by TNF-α.114 The above-mentioned changes in gene expression lead to a diminished insulin-stimulated glucose uptake and an altered lipid metabolism which can, via accumulation of triglycerides in various organs, eventually lead to insulin resistance of the muscle and liver. In addition, insulin resistance can be induced via a direct toxic effect of TNF-α on intracellular insulin signalling.114 TNF-α reduces the insulin-stimulated autophosphorylation of the insulin receptor in a variety of cell types. It does so by phosphorylation of serine residues at the insulin receptor substrate-1 (IRS-1); this modified IRS-1 subsequently interferes with the insulin signalling capacity of the insulin receptor.114

Relation between TNF-α, obesity and insulin resistance
A positive relationship between obesity, insulin resistance and adipose tissue mRNA levels of TNF-α has clearly been established in rodent models.111 Furthermore, mice with no functional copy of the TNF-α gene (TNF-α-/-) although developing marked obesity on a high-fat, high-energy diet, remained highly insulin sensitive compared with their control litter mates (TNF-α+/+).115 In contrast to rodents, the role of TNF-α in the induction of insulin resistance in humans is less clear. Although there seems to be a positive relationship between obesity and TNF-α mRNA and protein levels in adipose tissue in humans in vitro,117-119 TNF-α is expressed at much lower levels in humans compared with rodents. In addition, no difference in TNF-α concentration was found in a vein draining subcutaneous adipose tissue compared with a peripheral vein, suggesting no or very low TNF-α production in vivo.120 Furthermore, circulating TNF-α concentrations in obese diabetic and nondiabetic patients are not substantially elevated.121,122 With regard to a direct relationship between TNF-α and insulin sensitivity in vivo, two studies found a strong and positive correlation between adipose tissue TNF-α mRNA levels and hyperinsulinaemia.123,124 When the relation between adipose tissue TNF-α secretion and insulin-stimulated glucose transport was examined, a strong inverse relationship was found that was independent of fat cell volume, age and BMI.122 However, other studies125,126 showed no significant relationship between adipose tissue mRNA for TNF-α and insulin sensitivity. Furthermore, treatment of insulin-resistant subjects with anti-TNF-α antibodies did not improve insulin sensitivity.124 All these results implicate that TNF-α might have an effect on insulin resistance but that it must be a local factor. Interestingly, TNF-α is also produced by muscle, and muscle TNF-α production is increased in obesity.125 Since adipose tissue dispersed within muscle is correlated with insulin resistance, the effect of fat cell secretory products on insulin signalling in skeletal muscle cells was recently studied in a model in which muscle cells were co-cultured with adipocytes. A disturbance of insulin signalling was found, but TNF-α did not seem to be involved.126

Conclusion
In conclusion, TNF-α is a multifunctional cytokine produced by adipocytes in proportion to the percentage body fat. TNF-α has a variety of metabolic effects, including increased lipolysis, decreased lipogenesis and decreased insulin-stimulated glucose transport, contributing to insulin resistance. These effects are induced by modulation of genes involved in glucose and lipid metabolism. Furthermore, TNF-α directly interferes with the early steps of insulin signalling. However, the role of TNF-α in obesity-induced insulin resistance in humans is not quite clear yet, as might be obvious from the contradicting results mentioned in the previous paragraph. The low plasma levels of TNF-α in humans indicate that the hormone most likely acts in a paracrine and or autocrine manner. This might be the reason why treatment with anti-TNF-α did not improve insulin sensitivity in humans in vivo.

Interleukin-6 (IL-6)
Structure, genetic locus and site of production of IL-6
IL-6 is a circulating, multifunctional cytokine that is produced by a variety of cell types including fibroblasts, endothelial cells, monocytes/macrophages, T-cell lines, various tumour cell lines and adipocytes. The protein has a molecular mass of 21 to 28 kD, depending on the cellular source and preparation. The gene encoding IL-6 is localised on chromosome 7p21 in humans.127 Although human adipocytes produce IL-6, adipocytes accounted for only 10% of total adipose tissue IL-6 production when IL-6 production by isolated adipocytes prepared from omental and subcutaneous fat depots was examined.128 This means that cells in the stromal vascular fraction of adipose tissue have a major contribution in adipose tissue IL-6 release. The concentrations of IL-6 in adipose tissue are up to 75 ng/ml, which is well within the range to elicit biological effects.129 Furthermore, plasma levels of IL-6 are markedly elevated in obesity and up to 30% of plasma levels could be derived from adipocytes.130

Modulators of IL-6 production
The stimuli leading to IL-6 production differ with the cell type; here only IL-6 production by adipocytes will be
discussed. Both in rodent and human adipocytes, IL-6 production is stimulated by catecholamines and inhibited by glucocorticoids, whereas insulin has no effect whatsoever.\textsuperscript{128,131,132} Finally, another stimulator of IL-6 release is TNF-\(\alpha\), which has been reported to produce a 30-fold\textsuperscript{113} increase in IL-6 production in 3T3-L1 adipocytes. Interestingly, IL-6 in turn inhibits the release of TNF-\(\alpha\).

**IL-6 acts via receptor interaction**

IL-6 acts through binding at and activation of a specific receptor, belonging to the class I cytokine receptors, which act through JAK-STAT signalling (see figure 4 where leptin signalling is explained).\textsuperscript{133} The IL-6 receptor consists of two membrane glycoproteins, a 80-kD ligand binding component and a 130-kD signal-transducing component (gp130). The 80-kD component binds IL-6 with low affinity; this complex subsequently binds with high affinity to gp130 after which signal transduction can take place.\textsuperscript{127} Soluble forms of the IL-6 receptor have been found but neither their functional significance nor the regulation of their production is understood.

**Effects of IL-6 on glucose and lipid metabolism**

IL-6 has pleiotropic effects on various cell types. Here we will only focus on its role in glucose and lipid metabolism. Infusion of rhIL-6 to humans increased whole body glucose disposal and glucose oxidation but increased hepatic glucose production\textsuperscript{134} and fasting blood glucose concentration in a dose-dependent manner.\textsuperscript{135} With regard to lipid metabolism, IL-6 decreases adipose tissue lipoprotein lipase (LPL) activity\textsuperscript{129} and has been implicated in the fat depletion taking place during wasting disorders, such as cancer, perhaps via an increase in plasma norepinephrine, cortisol, resting energy expenditure and fatty acid oxidation as was assessed in eight renal cancer patients.\textsuperscript{134} In rats, IL-6 increased hepatic triglyceride secretion partly because the increase of adipose tissue lipolysis resulted in an increased delivery of free fatty acids to the liver.\textsuperscript{136} This increased release of FFAs following rhIL-6 infusion was observed in humans as well.\textsuperscript{134}

**IL-6 in obesity and insulin resistance**

In both mice\textsuperscript{137} and humans, IL-6 mRNA in adipose tissue\textsuperscript{137,138} but even more so plasma levels of IL-6 are positively correlated with BMI.\textsuperscript{132,137,138} Weight loss is associated with a reduction in serum and IL-6 mRNA levels. After one year of a multidisciplinary programme of weight reduction, obese women lost at least 10% of their original weight and this was associated with a reduction of basal serum IL-6 levels from 3.18 to 1.7 pg/ml (p<0.01).\textsuperscript{139} In another study, both IL-6 mRNA in adipose tissue and IL-6 serum levels were reduced with weight loss after three weeks of a very low calorie diet in obese women.\textsuperscript{138} In this study, insulin sensitivity as assessed by the fasting insulin resistance index (FIRI= fasting glucose x fasting insulin/25) improved as well. The reduction in IL-6 levels could play a role in this improvement, since several studies found a significant correlation between circulating IL-6 levels and insulin sensitivity measured by either an intravenous glucose tolerance test\textsuperscript{137} or the fasting insulin resistance index.\textsuperscript{138} Recently this correlation between circulating IL-6 and insulin sensitivity was confirmed using the gold standard for insulin sensitivity: the hyperinsulinaemic euglycaemic clamp.\textsuperscript{140} In addition, a high correlation between adipose tissue IL-6 content and insulin sensitivity was found, both in vivo and in vitro. Furthermore, for the first time IL-6 receptors were demonstrated in 60% of the subcutaneous adipocytes suggesting that IL-6 can alter adipocyte metabolism via autocrine or paracrine mechanisms and have a local

![Figure 4](image-url)

The leptin receptor is a transmembrane receptor belonging to the class I cytokine receptors. The receptor consists of two parts. The intracellular domain is associated with the Janus kinase, a tyrosine kinase. Binding of leptin to the receptor results in the fusion of the two receptor parts, which results in trans-phosphorylation of the JAK molecules, which subsequently phosphorylate the terminus of the leptin receptor. The phosphorylated receptor then forms a docking site for a variety of Src homology 2 (SH-2) domain containing proteins, including a novel family of cytoplasmatic transcription factors termed STATs (signal transducers and activators of transcription). STATs are then phosphorylated on a single tyrosine residue by JAKs, after which the STATs dimerise, migrate into the nucleus and regulate gene transcription.
influence on insulin sensitivity.\textsuperscript{44} Further support for a relationship between IL-6 and insulin sensitivity comes from a genetic study. It appeared that subjects with an IL-6 gene polymorphism had lower IL-6 levels, a lower area under the glucose curve after an oral glucose tolerance test, lower glycosylated haemoglobin (HbA\textsubscript{c}), lower fasting insulin levels and an increased insulin sensitivity index compared with carriers of the normal IL-6 allele, despite similar age and BMI.\textsuperscript{141} Finally, basal serum IL-6 levels are higher in type 2 diabetic patients.\textsuperscript{44} In contradiction with the above-mentioned positive correlation of IL-6 with BMI and inverse relation with insulin sensitivity is the observation that a lack of IL-6 also leads to obesity and a disturbed glucose tolerance, at least in mice.

**Conclusion**

Various studies show a clear relationship between increased IL-6 levels and obesity,\textsuperscript{132-137,138} and between IL-6 levels and insulin resistance\textsuperscript{137,138,140} even when corrected for BMI.\textsuperscript{137} Furthermore, basal plasma IL-6 levels are higher in patients with type 2 diabetes\textsuperscript{44} and subjects with an IL-6 gene polymorphism clearly have lower serum IL-6 levels and this is correlated with improved insulin sensitivity and postload glucose levels.\textsuperscript{141} IL-6 does have different effects on the various end-organ tissues, however, with on the one hand improved glucose uptake in adipocytes and whole body glucose disposal, and on the other hand an increased hepatic glucose output, decreased LPL activity (leading to decreased triglyceride clearance) and increased hepatic triglyceride synthesis. How then does IL-6 fit in the insulin resistance syndrome? Is there a causal effect or are the increased IL-6 levels found in obesity and insulin resistance merely a reflection of the pathogenetic state or the increased adipose tissue mass? Is IL-6 detrimental to health or does it have a positive role in health. If we start from the principle that IL-6 production is increased in obesity and that it is involved in inducing insulin resistance, what would the mechanisms be by which IL-6 causes insulin resistance? Firstly, it has to be noted that omental fat produces threefold more IL-6 than subcutaneous adipose tissue.\textsuperscript{128} Because venous drainage of omental tissue flows directly to the liver and IL-6 is known to increase hepatic triglyceride secretion,\textsuperscript{194-196} this might explain the hypertriglyceridaemia associated with visceral obesity. As mentioned before, increased triglyceride content of muscle and liver leads to insulin resistance. Secondly, IL-6 signal transduction is mediated via JAK-STAT signaling; it is possible that feedback mechanisms interfering with insulin signalling exist. Thirdly, IL-6 has opposing effects to those of insulin on hepatic glycogen metabolism\textsuperscript{18} and increases hepatic glucose production.\textsuperscript{135} On the contrary, despite an increase of IL-6 in obesity, insulin resistance and type 2 diabetes, there is evidence that IL-6 improves insulin sensitivity: 1) IL-6 increases glucose uptake in \textit{Yt3-L1} adipocytes;\textsuperscript{144} 2) infusion of rhIL-6 to humans increased whole body glucose disposal and glucose oxidation;\textsuperscript{131} 3) IL-6 inhibits TNF-\alpha production, a cytokine with deleterious effects on insulin sensitivity; and 4) physical exercise, which is related to an improvement in insulin sensitivity, is coupled to an increased IL-6 secretion.\textsuperscript{145} It might be that muscle derived IL-6 down-regulates TNF-\alpha.\textsuperscript{44} So, in conclusion, it is still not clear whether IL-6 has a positive or a negative metabolic role in health. One of the reasons for the contradicting results might be that there is a difference in the acute and chronic exposure to IL-6 with regard to health implications. Furthermore, there might be differences in local and CNS-acting effects of IL-6. More transgenic mice studies can help shed light on the role of IL-6 in insulin resistance. Up until now, it is quite possible that the increased IL-6 levels observed in adiposity and type 2 diabetes are the cause of an increased production by the enlarged adipose tissue mass and/or an attempt to overcome either insulin resistance or another metabolic defect, for example IL-6 resistance.

**DISCUSSION**

Obesity, defined as a BMI >27, is the consequence of a chronic imbalance between energy intake and energy expenditure. This is partly due to the modern society with excess ('fast') food intake and a sedentary lifestyle. The role that should be ascribed to primary defects in energy storage caused by adipocyte secretory products or impaired hypothalamic functioning remains to be elucidated. At the moment a combination of the two seems the most likely. It is well known that obesity is associated with insulin resistance and type 2 diabetes mellitus. An overwhelming amount of evidence indicates that visceral fat is associated with glucose intolerance and insulin resistance,\textsuperscript{145-151} along with other facets of the metabolic syndrome such as dyslipidaemia. Therefore, in the past, the predominant theory used to explain the link between obesity and insulin resistance was the portal/visceral hypothesis,\textsuperscript{152} which states that increased visceral adiposity leads to an increased free fatty acid flux into the portal system and inhibition of insulin action via Randle’s effect.\textsuperscript{153} However, several investigators have challenged the singular importance of visceral adiposity in inducing insulin resistance. They found an independent association between total fat mass and subcutaneous truncal fat mass and insulin resistance.\textsuperscript{154-156} Furthermore, the observations that 1) triglyceride content within skeletal muscle cells is increased in obesity\textsuperscript{95} and type 2 diabetes mellitus\textsuperscript{152-156} and is a strong predictor of insulin resistance,\textsuperscript{95} and 2) lipodystrophy is associated with insulin resistance as
well\textsuperscript{62,63} obviated the need to develop new theories to explain the link between adipose tissue and insulin resistance.\textsuperscript{62} A well-accepted theory is that of ectopic fat storage.\textsuperscript{62,63} A limitation in the capacity of adipose tissue to store triglycerides would divert triglycerides to be deposited in liver cells and skeletal muscle cells.\textsuperscript{62,63} The cause of the ectopic fat storage is unclear. It might be due to impaired fat oxidation,\textsuperscript{62} since inhibition of fat oxidation in rodents increased intracellular lipid content and decreased insulin action.\textsuperscript{64} Furthermore, a mutation in the AGPAT2 gene encoding 1-acylglycerol-3-phosphate O-acyltransferase inhibits triacylglycerol synthesis and storage in adipocytes but not in hepatocytes, thus leading to hepatosteatosis, because the latter can accumulate triacylglycerol via AGPAT-1.\textsuperscript{65} Another possibility is the central and/or peripheral action of leptin, since leptin therapy has been associated with the reversal of insulin resistance and hepatic steatosis in patients with lipo-dystrophy\textsuperscript{66} and also with improvement of intramyocellular lipid content.\textsuperscript{66} Finally, a defect in the proliferation and/or differentiation of adipocytes, whether or not due to alterations in the expression of transcription factors,\textsuperscript{66} can lead either to impaired adipocyte triglyceride storage and/or adipocyte hypertrophy. This is where the third hypothesis emerges: the adipocyte as an endocrine organ.\textsuperscript{62} Adipocytes secrete a large number of cytokines and hormones that act in a paracrine, autocrine and endocrine manner on adipocyte and whole body metabolism. It is plausible that these enlarged adipocytes are deregulated in their transcriptional setting and secrete a different pattern of hormones or different amounts of them compared with small adipocytes. On the other hand, enlarged adipocytes might merely be a manifestation of other, yet to be defined, pathogenetic factors.\textsuperscript{62}

In obese humans and rodents there is, besides numerous other proteins and cytokines that have not been discussed here, overproduction of leptin,\textsuperscript{14,15} IL-6,\textsuperscript{127} TNF-\alpha,\textsuperscript{103-105} ASP\textsuperscript{100,101} and resistin,\textsuperscript{54,60} and a decreased production of adiponectin (see figure 5).\textsuperscript{71,77,78,80} Of leptin,\textsuperscript{15} TNF-\alpha\textsuperscript{74} and IL-6\textsuperscript{115} it is known that they act via receptors on the cell surface and subsequent intracellular signalling cascades. As can be seen in figure 5, all three cytokines decrease food intake and increase energy expenditure and lipolysis together with a decrease in lipogenesis. These are well-adaptive mechanisms to prevent further weight gain. Since all these cytokines are increased in adiposity it is unlikely that they are the cause of adiposity unless there is an impairment in cytokine signalling. Interestingly, leptin and TNF-\alpha have opposing effects with regard to insulin sensitivity. TNF-\alpha interferes with insulin signalling and downregulates many genes encoding for proteins involved in glucose and free fatty acid uptake.\textsuperscript{113} Leptin can act through some components of the insulin-signalling cascade as well.\textsuperscript{52} The relation between TNF-\alpha and leptin in humans is not clear. Infusion of TNF-\alpha to patients has been reported to acutely raise serum leptin levels,\textsuperscript{167} whereas chronic exposure of cultured human adipocytes to TNF-\alpha resulted in a decrease in leptin production.\textsuperscript{168} If TNF-\alpha increases leptin production this might be an adaptive mechanism to com-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Hyperplasia and hypertrophy of adipocytes as seen in adiposity leads to an increased (light grey arrow) production of leptin, TNF-\alpha, IL-6, resistin, ASP and many other proteins, and a decreased production (dark grey arrow) of adiponectin. The results of these increases, respectively decrease, are mentioned below each protein.}
\end{figure}
penetrate for the TNF-α induced impaired insulin signalling. When we take a further look at the mutual coherence of the adipocyte secretory factors it is striking that both insulin and TNF-α are, somehow, involved in the regulation of all of the adipocyte secretory products. Insulin increases the production of leptin, adiponectin and ASP, whereas no effect has been recorded with regard to TNF-α and a potentially positive effect on resistin levels. TNF-α downregulates resistin and stimulates the production of leptin, adiponectin and IL-6. The problem is that some of these factors lead to an improvement of insulin sensitivity whereas others have just the opposite effect. This makes it extremely difficult to elucidate which factors are most important in regulating insulin sensitivity. Furthermore, the time of exposure to a stimulus seems to be important. Thus it seems that leptin and insulin are long-term regulators with regard to food intake and energy expenditure whereas insulin has a direct effect on glucose uptake and lipolysis. How do these adipocyte-derived factors mediate their effects? What they all seem to have in common is a change in the expression of genes encoding for proteins involved in glucose and protein metabolism. Transcription of genes can only take place if they are activated, which always occurs via some kind of ligand-receptor interaction followed by an intracellular signal transduction. Cytokine signalling proceeds in part via the JAK-STAT pathway. The actions of leptin, TNF-α and IL-6 may influence each other via common signalling steps. Furthermore, it is known that leptin can signal through some components of the insulin signalling cascade such as IRS-1 and -2, PI3K and MAPK and can modify insulin-induced changes in gene expression in vitro and in vivo. TNF-α can interfere with the early steps of insulin signalling as well. So, more and more evidence exists that the adipocyte secretory cytokines leptin, IL-6 and TNF-α not only interact with each other but also with insulin on the level of intracellular signal transduction. In the case of obesity and hyperinsulinaemia, there is an increase in hormones and cytokines produced by the adipose tissue. These hormones subsequently mediate a change in the expression of genes encoding for proteins involved in glucose and lipid metabolism. In case of ASP these changes promote triglyceride uptake. However, in case of IL-6, TNF-α and adiponectin there is a deleterious effect on glucose uptake and fatty acid oxidation leading to insulin resistance. The effect of increased serum resistin levels remains to be elucidated. Everything seems to come down to interference with intracellular signal transduction, not only of insulin but also of the various adipocyte secretory products, with a subsequent change in the expression of genes involved in glucose and lipid metabolism leading to a diminished glucose uptake and fatty acid oxidation. The latter will, via accumulation of triglycerides in liver cells and muscle cells, enhance insulin resistance, thus further impairing glucose uptake.

Concluding remarks
It is now well established that adipose tissue not only has an important function in the storage and release of triglycerides but also has an important effect on whole body metabolism and energy homeostasis via the production of various hormones and cytokines. Adipose tissue not only responds to insulin, glucagon, cortisol and catecholamines but also to cytokines and products that it produces itself, thereby regulating its own metabolism and cell size. Some of the products produced by the adipocytes, such as TNF-α and leptin, are clearly involved in the induction of insulin resistance. The role of others (resistin, IL-6) has yet to be defined. Their increase in obesity is at least a manifestation of the increased adipose tissue mass itself. Further research is needed to come to a better understanding of the molecular pathways regulating the production of these hormones, their individual actions and target organs, and finally their mutual interaction and role in insulin resistance. These new insights provide the basis for the development of improved therapies for obesity and insulin resistance related diseases as type 2 diabetes and cardiovascular complications.

ACKNOWLEDGEMENT
The authors would like to acknowledge Professor J.A. Maassen of the Department of Molecular Cell Biology of Leiden University Medical Centre, The Netherlands, for critically reading our manuscript.

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Alkaptonuria is a rare autosomal recessively inherited disease, in which a deficiency of the enzyme homogentisate 1,2-dioxygenase leads to the secretion of homogentisic acid in the urine and to accumulation of oxidised homogentisic acid pigment in connective tissues (ochronosis). This ochronosis causes the typical grey-brown discolorations of the sclerae and the concha, anthelix, and finally, the helix of the ear. Ultimately, ochronosis also causes degenerative arthritis in middle age (achronotic arthropathy).

At the moment treatment is purely symptomatic: analgesia, NSAIDs, physiotherapy, orthopaedic treatment and intra-articular corticosteroid injections.1 Treatment with NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexadiene) combined with some restriction in the intake of phenylalanine and tyrosine is still in an experimental stage, although the results seem to be promising.2

**DIAGNOSIS**

Alkaptonuria

**REFERENCES**


Substitution therapy in immunodeficient patients with anti-IgA antibodies or severe adverse reactions to previous immunoglobulin therapy

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ABSTRACT

Background: Patients with common variable immunodeficiency often suffer from recurrent bacterial infections. Administration of immunoglobulins is a well-established treatment to reduce the frequency and severity of these infections. However, in patients with anti-IgA antibodies or side effects to previous immunoglobulin substitution therapy, administration of immunoglobulins may lead to anaphylactoid reactions.

Objective: To describe the feasibility of immunoglobulin substitution therapy in patients with anti-IgA antibodies or side effects to previous immunoglobulins.

Methods: A retrospective study was conducted in two university hospital outpatient clinics. Fourteen patients with common variable immunodeficiency were found to have circulating anti-IgA antibodies or have experienced severe reactions to previously administered blood products.

Results: In eight out of 15 patients side effects to immunoglobulins and/or blood transfusions had occurred previously. In four patients these reactions were due to anti-IgA antibodies. No side effects were observed when human immunoglobulin 16% was given by subcutaneous infusion. In all patients with anti-IgA antibodies, as well as in those without, subcutaneous immunoglobulins were well tolerated. In some patients antibodies disappeared and therapy could be changed into intravenous immunoglobulin administration.

Conclusions: Patients with serious side effects to previous immunoglobulin therapy and/or blood transfusions can be safely treated with subcutaneous immunoglobulins and, if necessary, with intravenous immunoglobulins at a later point in time.

INTRODUCTION

In patients with primary humoral immunodeficiencies, such as X-linked agammaglobulinaemia and common variable immunodeficiency disease (syn. CVID, late onset hypogammaglobulinaemia), administration of immunoglobulins is a well-established treatment to reduce the frequency and severity of infections. Originally such immunoglobulin substitution was given by the intramuscular route, but in the 1980s the subcutaneous route gained more acceptance because it met with fewer side effects and higher dosages could be given. Nowadays, most patients are treated with intravenous infusion of immunoglobulin preparations. Although this treatment is generally well tolerated, mild side effects such as malaise, nausea and headache occur in approximately 27% of patients and in about 5% of infusions. The more severe side effects, anaphylactoid reactions, are rare with the modern intravenous immunoglobulin preparations; these have been observed especially in patients with antibodies against IgA. Such antibodies are especially common in patients with selective IgA deficiency or IgA deficiency in the context of CVID. The antibodies may have arisen after

* J.W.M. van der Meer was not involved in the handling and review of this manuscript.

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exposure to blood products including immunoglobulins or because of mechanisms such as adsorption of animal IgA (cow’s milk) via the intestinal wall. Occasionally, severe anaphylactoid reactions occur in patients without circulating antibodies against IgA or other immunoglobulins. Immunoglobulin substitution in patients with anti-IgA antibodies or a history of severe reactions to intravenous immunoglobulin preparations is considered a difficult problem in the management of hypogammaglobulinaemic patient. Over nearly two decades, we have successfully substituted immunoglobulins in patients with primary humoral immunodeficiencies and circulating anti-IgA antibodies and/or severe adverse reaction to previous immunoglobulin therapy. The present retrospective analysis describes our experiences.

PATIENTS AND METHODS

The clinical and laboratory data of patients with primary humoral deficiencies with circulating anti-IgA antibodies and/or severe reactions to previous blood products were reviewed. Severe reactions consisted of:

- anaphylactoid reactions (angio-oedema, hypotension and tachycardia, with or without wheezing and/or urticarial rash);
- hypotension and tachycardia without angio-oedema;
- syncope.

These reactions had to occur within six hours following the administration of a blood product. All patients attended outpatient clinics at two university hospitals with extensive experience in the management of adults and adolescents with primary immunodeficiency. In these outpatient clinics patients were, as a rule, tested for antibodies against IgA before immunoglobulin substitution therapy was started if they were either completely IgA deficient or had experienced serious side effects of immunoglobulin preparations or other blood products in the past.

Two preparations were used for immunoglobulin substitution of these patients, normal immunoglobulin 16% for intramuscular administration (Immunoglobulin I.M.) and 6% intravenous immunoglobulin preparation (Immunoglobulin I.V.), both prepared by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service in Amsterdam, the Netherlands, using cold ethanol (modified Cohn method) fraction II, which is lyophilised to powder. The powder for the intravenous immunoglobulin preparation was dissolved and treated at pH 4 in the presence of mild pepsin proteolysis and then freeze dried. Powder for the intramuscular immunoglobulin preparation was dissolved and formulated in 0.3 mol/l glycin solution. IgA content in Immunoglobulin I.M. and Immunoglobulin I.V. was ±2.3% of total protein (160 g/l) and ±1.9% of total protein (60 g/l), respectively. Immunoglobulin I.M. was licensed for both intramuscular and subcutaneous administration.

As patients were studied in two hospitals and over an extended period of time, serum IgA was measured by several methods: Elisa, RIA, latex-enhanced nephelometry and Ouchterlony (electrophoresis). The sensitivity of Elisa, latex-enhanced nephelometry and RIA are identical. Serum IgG was measured by nephelometry. Anti-IgA antibodies were measured by passive haemagglutination.

RESULTS

The records of 15 patients (7 males, 8 females) were studied. All patients were classified as having CVID with IgA deficiency (serum IgA <0.001 g/l) (table 1). The median age was 48 years (range 26-75 years). Eight patients (#1-8) had experienced severe side effects, such as anaphylactoid reactions, after immunoglobulin therapy and/or blood transfusion. In four of them (#5-8), anaphylactic reactions were most likely due to circulating anti-IgA antibodies. At the time of the reactions, circulating anti-IgA antibodies were measured in the serum of patients 5, 6 and 8. Patient 7 tested positive for anti-IgA antibodies ten years after the anaphylactic reaction.

In patients 5 and 6, intramuscular immunoglobulin 16% therapy had been discontinued because of the side effects. Despite antibiotic therapy recurrent infections kept occurring, which meant that other ways of substitution had to be considered, i.e., a switch to subcutaneous immunoglobulin administration. Although circulating anti-IgA antibodies in these patients remained present, further adverse reactions did not occur. Subcutaneous immunoglobulin was administered to patient 7 more than 20 years after a severe anaphylactic reaction caused by blood transfusion. During therapy circulating anti-IgA antibodies disappeared and four years later treatment was changed into intravenous immunoglobulin infusion without any adverse reaction. The adverse reactions in patients 1 to 4 could not be attributed to measurable anti-IgA antibodies.

Patient 2 was treated with intramuscular immunoglobulin injections for a period of three years before severe anaphylactoid reactions occurred. Because no anti-IgG, -IgM, -IgA or -IgE were detected in his serum, it was suggested that these reactions were caused by aggregates in the normal immunoglobulin 16% preparation, which accidentally entered the bloodstream during the intramuscular injections. Transfusion of plasma also caused anaphylactoid reactions in this patient, even when blood
group compatible plasma and premedication with antihistaminic drugs were administered. When treatment was switched to subcutaneous immunoglobulin, no reactions occurred. After seven years of subcutaneous immunoglobulin, he was switched to intravenous immunoglobulin without side effects.

In patient 1, the second intramuscular immunoglobulin injection had caused an anaphylactic reaction (hypotension, oedema and dizziness). This reaction disappeared after treatment with adrenaline and corticosteroids. As no anti-IgG, -IgE and -IgA antibodies were detected in this patient, it was assumed that this reaction was complement-mediated through aggregates in the product. During therapy with slow subcutaneous immunoglobulin no side effects occurred for several years. Over the years compliance with subcutaneous home treatment waned, resulting in low serum IgG concentrations, and it was felt necessary to try the switch to intravenous immunoglobulin infusions; these were well tolerated.

Because of severe adverse reactions to previous intravenous immunoglobulin therapy, patients 3, 4 and 8 were hospitalised to receive the first immunoglobulin substitution. Treatment consisted initially of subcutaneous immunoglobulin. When after several days no side effects were observed, it was attempted to transfuse low-dose intravenous immunoglobulin. In patients 3 and 4 adverse reactions did not occur, whereas patient 9 developed fever (39°C) after the first intravenous immunoglobulin infusion, but not after subsequent infusions. Besides fever no other reactions occurred.

At the time of diagnosis, circulating anti-IgA antibodies were detected in patients 10 and 11. These antibodies disappeared during subcutaneous immunoglobulin therapy. Recurrent infections and local pain from the subcutaneous infusions necessitated a change to intravenous immunoglobulin in these two patients. Anti-IgA antibodies did not reappear during intravenous immunoglobulin therapy. In patients 12 and 13 circulating anti-IgA antibodies also disappeared during subcutaneous immunoglobulin, whereas these antibodies remained present in patients 14 and 15 during subcutaneous substitution.

**DISCUSSION**

The results of this retrospective analysis show that patients with serious side effects to previous immunoglobulin therapy and/or blood transfusions can be safely treated with subcutaneous immunoglobulin even when anti-IgA antibodies are present. If necessary, subcutaneous treatment can be replaced by intravenous immunoglobulins. When anti-IgA antibodies are present or when serious reactions to immunoglobulins have occurred in the past, our practice to start with subcutaneous immunoglobulin

**Table 1**

*Characteristics of patients challenged with s.c. immunoglobulins*

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>GENDER</th>
<th>ANAPHYLACTOID REACTION (CAUSATIVE PRODUCT)</th>
<th>ANTI-IgA</th>
<th>CURRENT TREATMENT</th>
<th>FOLLOW-UP (YEARS)</th>
<th>DISAPPEARANCE OF ANTI-IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>+ (imIg)</td>
<td>No</td>
<td>IvIg</td>
<td>7</td>
<td>n.a.</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>+ (imIg; plasma)</td>
<td>No</td>
<td>IvIg</td>
<td>11</td>
<td>n.a.</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>+ (ivIg)</td>
<td>No</td>
<td>IvIg</td>
<td>4</td>
<td>n.a.</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>+ (plasma; ivIg)</td>
<td>No</td>
<td>IvIg</td>
<td>4</td>
<td>n.a.</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>+ (imIg)</td>
<td>Yes</td>
<td>ScIg</td>
<td>9</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>+ (imIg)</td>
<td>Yes</td>
<td>ScIg</td>
<td>7</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>+ (blood transfusion)</td>
<td>Yes</td>
<td>IvIg</td>
<td>5</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>+ (ivIg)</td>
<td>Yes</td>
<td>ScIg</td>
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<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>-</td>
<td>Yes</td>
<td>IvIg</td>
<td>8</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>-</td>
<td>Yes</td>
<td>IvIg</td>
<td>12</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>-</td>
<td>Yes</td>
<td>IvIg</td>
<td>6</td>
<td>Yes</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>-</td>
<td>Yes</td>
<td>IvIg</td>
<td>12</td>
<td>Yes</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>-</td>
<td>Yes</td>
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<td>15</td>
<td>M</td>
<td>-</td>
<td>Yes</td>
<td>ScIg</td>
<td>11</td>
<td>No</td>
</tr>
</tbody>
</table>

imIg = intramuscular immunoglobulin treatment, scIg = subcutaneous immunoglobulin treatment, ivIg = intravenous immunoglobulin treatment, n.a. = not applicable.
The feasibility of subcutaneous immunoglobulin substitution, which has also been noted by others, may be explained by the gradual exposure to IgA due to slow resorption of the subcutaneous deposit of immunoglobulin into the circulation. We do in fact know that also IgG appears at a very slow rate after subcutaneous immunoglobulin substitution. The disappearance of anti-IgA antibodies from the circulation could be due to the formation of complexes between IgA in the product and circulating antibodies. These complexes might be removed rather rapidly from the circulation.

Cunningham-Rundles et al.
reported five patients with immunodeficiency and circulating anti-IgA antibodies who were treated (total of 170 infusions) with IgA-depleted intravenous immunoglobulin (IgA content of 3.4-14.4 mg/g IgG). In these patients only mild reactions occurred. Our results suggest that such an elaborate approach is not necessary.

In the absence of anti-IgA antibodies, adverse reactions to immunoglobulin are probably induced by complement activation. Aggregates in intramuscular immunoglobulin may induce complement activation. Welch and Stiehm suggested such a mechanism in a patient who had had several reactions to intramuscular immunoglobulin. The skin testing for immediate type hypersensitivity to intramuscular immunoglobulin was negative and no antibodies to IgG, IgA or IgE could be measured. In our study complement-mediated reactions probably occurred in patients 1 and 2. In these patients no reactions were observed after the introduction of subcutaneous immunoglobulin. Slow subcutaneous immunoglobulin in the patients presented here and in more than 40 additional patients without a history of previous reactions treated in our hospitals did not result in any systemic reactions.

Slow infusion of subcutaneous immunoglobulin probably does not give rise to sizable release of bioactive mediators, such as kinins, complement factors and cytokines. This retrospective analysis suggests induction of tolerance to subcutaneous immunoglobulin, and subsequently, to intravenous immunoglobulins. We are confident that in patients with (persistent) anti-IgA antibodies subcutaneous immunoglobulin can be administered on a weekly basis. In patients with a history of anti-IgA antibodies or who have experienced severe side effects to previous immunoglobulin and in whom subcutaneous immunoglobulin therapy is not satisfactory (e.g. recurrent infections, low serum IgG, painful injections), treatment can be changed into intravenous immunoglobulins. For clinical practice, we propose the following scheme. On days 1 and 2, 5 ml subcutaneous immunoglobulin at an infusion rate of 0.3 - 0.5 ml/h, followed by 10 ml subcutaneous immunoglobulin on the third and fourth day. On days 5, 6 and 7, 3 grams intravenous immunoglobulin can be transfused. During all the infusions, medication such as analgin, corticosteroids and antihistaminic drugs should be on stand-by.

REFERENCES


ABOUT THE COVER

‘Untitled’

Desire Haverkamp

Desire Haverkamp (1963) studied ‘autonomic composition/graphic art’ at the Academy of Art in Utrecht. At first she exposed her work in several solo exhibitions. Since 1987, she has been exhibiting mainly in group exhibitions, such as the ‘7 Sculptors of Utrecht’ presented by the ‘Love of Art Society’ in 2001 and the Graphic shop ‘INKT’ exhibition in The Hague in 2002. This year her work can be seen at Rhoon Castle in Rhoon and in the ‘Oude Haven’ museum in Amsterdam. In 1989 she became interested in using medical equipment in her work. This brought her to Adriaan van der Kuip’s shop in Utrecht, which specialises in medical instruments. That is where she developed her fascination for all kinds of syringes, pipettes, tubes and box injectors, which were exposed there. Nowadays Desire mostly composes spatial work and graphic art but there will always be a place for a few engravings inspired by medical instruments.

Original prints of this month’s printing are available at a price of € 200. You can order the print at Galerie Unita, Rijkstraatweg 109, 6573 CK Beek-Ubbergen, the Netherlands or by e-mail: galerie-unita@planet.nl.

Eijhout, et al. Immunoglobulin substitution in patients with anti-IgA antibodies or severe adverse reactions.
Comparison of four-day and seven-day pantoprazole-based quadruple therapy as a routine treatment for Helicobacter pylori infection

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ABSTRACT

Background: H. pylori eradication is usually performed with three or four drugs for at least seven days. Recently four reports have shown a cure rate of approximately 90% using a four-day quadruple therapy. The objectives of this prospective study were: 1) to evaluate the efficacy of pantoprazole-based quadruple therapy, and 2) to compare the efficacy and tolerability of four-day with seven-day quadruple therapy.

Methods: The study was performed in a single centre. The first 56 consecutive patients with nonulcer dyspepsia or peptic ulcer disease and proven H. pylori infection received seven days of quadruple therapy (pantoprazole, bismuth, tetracycline and metronidazole). At least six weeks after treatment, endoscopy was repeated with six biopsies of the antrum and corpus for histology, urease test and culture. The next 59 consecutive patients followed the same protocol but received four-day quadruple therapy.

Results: Using an intention-to-treat analysis, the cure rate in the seven-day treatment group was 54/56 (96.4%, 95% confidence interval (CI) 87.7-99.6%). In the per protocol analysis the cure rate was 53/55 (96.3%, 95% CI 87.5-99.6%). Primary metronidazole resistance was observed in seven patients. All were cured. Using an intention-to-treat analysis, the cure rate in the four-day treatment group was 51/59 (86.4%, 95% CI 75.0-94.0%). In the per protocol analysis the cure rate was 50/58 (86.2%, 95% CI 74.6-93.8%). Primary metronidazole resistance was observed in seven patients, four of whom were cured. In three out of eight patients in whom four-day treatment failed, secondary metronidazole resistance was induced. Both treatment regimens were well tolerated. The difference between cure rates of both regimens did not reach statistical significance (p=0.0585).

Conclusion: Routine use of both four-day and seven-day pantoprazole-based quadruple anti-H. pylori treatment is effective and well tolerated. The results of both regimens reach the required eradication standard, but results with the seven-day regimen were slightly but not significantly better. Seven-day treatment may be superior, especially in case of metronidazole resistance, and should be preferred.

INTRODUCTION

Curing Helicobacter pylori infection has become the standard treatment for patients with Helicobacter-associated peptic ulcer disease. Antibiotic treatment, however, is still not standardised and a variety of treatment schedules are used. Most regimens consist of three or four drugs, usually administered for between seven to 14 days. The success rate of a given treatment depends on the potency of its components and their dosing schedule, but also on other factors such as antibiotic resistance of H. pylori, the infective strain and patient compliance, which is partly determined by the occurrence of adverse effects. To achieve a simpler treatment with less adverse effects and better patient compliance, shortening the duration of treatment has been proposed. Adding a proton pump inhibitor (PPI) to bismuth triple therapy (quadruple therapy) improves the cure rate. Although this is probably a class effect of the PPIs, pantoprazole has not yet been used in
a quadruple regimen. Recently, four papers reported the results of a four-day quadruple treatment with omeprazole and lansoprazole.\(^5\)\(^\text{a}\) The results of these studies showed a cure rate of approximately 90% and therefore four-day quadruple treatment may be attractive.

To compare the results of a seven-day with a four-day treatment in a single centre, we conducted a prospective, open, nonrandomised study testing the efficacy and adverse effect profile of first a seven-day and then a four-day pantoprazole-based quadruple therapy. In contrast to the previous four-day lansoprazole- or omeprazole-based quadruple treatment studies, in the present study pantoprazole was used in the quadruple regimen.

**P A T I E N T S A N D M E T H O D S**

The patients who participated in this study were consecutive outpatients referred for gastrointestinal investigation to Slingeland Hospital, Doetinchem, the Netherlands. This is a nonacademic community hospital situated in a rural area in the east of the Netherlands. Patients with nonulcer dyspepsia as well as patients with proven ulcer disease in whom endoscopic biopsies had confirmed the presence of *H. pylori* and in whom antibiotic therapy was indicated participated in this study. Before entering the study, patients were informed about the study design and gave their oral informed consent. The study protocol was not submitted to the Medical Ethics Committee of Slingeland Hospital, since the authors felt that four-day as well as seven-day quadruple treatment were standard and accepted anti-*Helicobacter* treatment schedules.

**H. pylori status**

Before inclusion and at least six weeks after cessation of treatment, *H. pylori* infection was proven with endoscopy. Six biopsies were taken. First, a antral biopsy was taken for a rapid urease test prepared by the hospital pharmacist.\(^5\)\(^\text{b}\) Then, a antral biopsy was taken for culture with an antibiogram for metronidazole and clarithromycin by means of an agar dilution method. Metronidazole resistance was defined as a mean inhibitory concentration (MIC) value \(\leq 8\) \(\mu\)g/ml and clarithromycin resistance was defined as a MIC value \(\leq 2\) \(\mu\)g/ml. Finally two biopsies from the antrum and two from the corpus were taken for histology using a modified Giemsa stain. At the pretrial endoscopy 115 patients were biopsied according to the protocol. In five patients no *H. pylori* culture was performed. At the posttrial endoscopy a patient was only considered to be cured if all biopsy-based tests were negative for the presence of *H. pylori*. Pretreatment use of PPIs, defined as the use of a PPI for three or more days prior to the first endoscopy and the start of the anti-*H. pylori* treatment, and concomitant use of nonsteroidal anti-inflammatory drugs (NSAIDs) were recorded in all patients.

**Treatment**

The first consecutive 56 patients were treated with seven-day quadruple therapy to judge the efficacy of pantoprazole-based quadruple therapy. The next 59 consecutive patients were treated with four-day quadruple therapy. Patients were prescribed the following treatment for seven or four days: pantoprazole (Pantozol, Altana Pharma, Zwannenburg, the Netherlands) 40 mg bd before breakfast and before the evening meal, bismuth (tripotassium dicitrato bismuthate, De-Nol, Yamanouchi Pharma, Leiderdorp, the Netherlands) 120 mg qid before the three meals and at bedtime, tetracycline hydrochloride 500 mg qid with the three meals and at bedtime, and metronidazole 500 mg tid with the three meals. In both regimens, pretreatment with a PPI was not obligatory. Patients received the medication from their own pharmacies. The use of alcohol was discouraged. Patients were instructed to take their treatment precisely as prescribed. They were informed about possible adverse effects.

**Tolerability**

Tolerability and adverse effects were assessed with an internationally accepted questionnaire in which patients were asked to judge these aspects of the treatment on a scale of A to E: category A was no adverse effects, category B slight discomfort not interfering with daily activities, category C moderate adverse effects interfering with daily activities, category D severe adverse effects, work not possible, and category E severe adverse effects, discontinuation of treatment.\(^5\)\(^\text{c}\)

**Compliance**

Compliance was assessed by counting the medication returned within one week after the medication had been taken.

**Statistical analysis**

Statistical analysis was performed with a Mann-Whitney U-Wilcoxon rank-sum W test. A p value of less than 0.05 was considered to be statistically significant.

**R E S U L T S**

A total of 115 consecutive patients (61 male, 54 female) were included between July 1997 and March 1998. Mean age was 56 years (range 22 to 87 years). Indications for treatment were nonulcer dyspepsia \((n=67, 58\%)\), gastric ulcer disease \((n=9, 8\%)\), erosive bulbitis, or first or recurrent duodenal ulcer disease \((n=39, 34\%)\). Of the 115 patients, 18 were immigrants. Characteristics of both treatment groups are shown in table 1.

Follow-up was available for all patients. In the seven-day treatment group, using intention-to-treat, 54 out of 56

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De Boer, et al. Comparison of four-day and seven-day pantoprazole-based quadruple therapy as a routine treatment for *Helicobacter pylori* infection.

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and post-treatment *Helicobacter* status was obtained with a carbon-13 breath test. Results of primary metronidazole susceptibility testing were available in 33 patients. Seven of these patients (13%) carried a metronidazole-resistant strain. Four of these patients were cured. In three patients in whom therapy failed, secondary metronidazole resistance was found post-treatment (see also table 2). Of the eight treatment failures in the four-day treatment group, four patients were not pretreated with a PPI immediately before starting the anti-*Helicobacter* treatment. The cure rates, intention-to-treat and per protocol were not significantly different between the four- and seven-day regimens (p=0.0585), but there was a trend towards higher efficacy in the seven-day group, especially in patients with a metronidazole-resistant strain.

### Compliance and adverse effects

The compliance was excellent and there were no differences for patients receiving four-day versus seven-day treatment. Only one patient, who was in the seven-day treatment group, stopped taking the drugs after four days. The overall dropout rate was therefore less than 1%.

The reported adverse effects were mild. No serious adverse events were observed. The adverse effects that were reported, such as nausea, headache, diarrhoea and general malaise, were of short duration and in all except one patient were not a reason for stopping the treatment prematurely. We used a well-known questionnaire in which patients were asked to judge the tolerability of the treatment on a scale from A to E. Of the patients, 29 (25%) chose category A, 66 (57%) category B, 22 (19%) category C, and 6 patients (5%) chose category D. One of the patients, the previously mentioned noncompliant patient, chose category E but in this patient the *Helicobacter pylori* infection was nevertheless eradicated (also see table 2). No significant differences were found in adverse effects between both treatment groups, when groups A and B, and groups C, D and E were taken together. No significant differences in adverse effects were found between the patients who were cured and those who were not.

### DISCUSSION

It is generally accepted that the combination of a PPI, bismuth, tetracycline and metronidazole is a potent drug combination for the treatment of *H. pylori*, even in areas with a high incidence of metronidazole resistance. Although we used this treatment as the first-line approach to this infection, others have suggested only using this regimen in patients in whom a previous regimen has failed. The Maastricht 2000 European consensus advises starting with a PPI- or ranitidine-bismuth-subcitrate-based triple therapy, but to use quadruple therapy in the second
line for the failures of triple therapy. The first goal of this study was to determine the effect of pantoprazole in combination with the classical triple therapy in the treatment of H. pylori. With a cure rate of more than 95% and no failures in seven patients with metronidazole-resistant strains, it seems that seven-day pantoprazole-based quadruple therapy is as effective as other seven-day PPI-based quadruple therapies. Since patient compliance, drug tolerability and adverse effects are major factors determining the success of treatment, shortening the treatment period has been tried in many studies to find an optimal balance between cure rate, adverse effects and the induction of antibiotic resistance against H. pylori. This allowed the exploration of treatments shorter than seven days. Two-day quadruple therapy was not sufficiently effective. Recently four Dutch studies showed that a four-day quadruple therapy with either omeprazole or lansoprazole as a PPI was very effective and well tolerated. In the present study we confirmed the findings of these four-day studies in a routine clinical setting. The cure rate (86%) was somewhat lower compared with the former studies (about 90%), but reaches the efficacy requirements put forward by the European Helicobacter pylori Study Group. In patients infected with metronidazole-resistant strains the cure rate was suboptimal and lower than in patients with metronidazole-sensitive strains or those treated for seven days. Three of the eight patients in whom the four-day therapy failed developed secondary resistance against metronidazole. Induction of metronidazole resistance was not found in the previous four-day quadruple therapy studies, but the number of failures is low and the clinical significance of this finding is not clear. Clinically relevant adverse effects did not differ between the regimens. The question can be raised whether the advantage of efficacy of about 10% in favour of the seven-day treatment group, together with the metronidazole-resistance induction found in the four-day treatment group, should be an argument to treat all patients for at least seven days. We have confirmed the high efficacy of seven-day therapy in the metronidazole-resistant strains, whereas with four days the results of previous studies have also shown a decrease in efficacy, mainly in the metronidazole-resistant strains. In this study most patients were not pretreated with a PPI before starting with the antibiotics. In the previous four-day quadruple studies, patients were always pretreated with a PPI three days before the treatment started, with the argument that PPI pretreatment should augment the treatment response. There are, however, no firm data to show that this hypothesis is correct. Annibale et al. found no advantage of PPI pretreatment in the anti-H. pylori treatment in peptic ulcer patients. In both our seven-day and four-day regimens the results were slightly, but not significantly, higher in the patients without PPI pretreatment (table 2). This study was not designed to study the role of pretreatment with acid inhibition, and firm conclusions cannot be drawn from this limited data.

We have demonstrated that both pantoprazole-based quadruple therapies are well tolerated and attractive for use in a routine setting to eradicate H. pylori in an area with relatively low metronidazole resistance. Only one patient had severe side effects, which led to discontinuation of treatment on the 4th day, but the H. pylori infection was nevertheless eradicated. In our hands, shortening of the treatment duration was not an instrument to improve compliance. The adverse effect profile is comparable with the findings of other authors.

Although we acknowledge the limitations of this non-randomised study, we feel that we have shown that the cure rates of the seven-day treatment group were, although not statistically significant, superior to the four-day treatment group especially in patients carrying a metronidazole-resistant strain. We have also shown that the adverse effects in both treatment groups were comparable. The authors feel that shortening of a seven-day quadruple treatment to four days carries a certain risk of lower cure rates, mainly in resistant strains, whereas we could not demonstrate any advantage of four-day therapy in terms of better compliance or adverse effect profile.

ACKNOWLEDGEMENT

The authors wish to thank Altana Pharma, the Netherlands for their financial support.

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Watch out,
De Boer’s four day battle!
Into hiding and build up resistance!

De Boer, et al. Comparison of four-day and seven-day pantoprazole-based quadruple therapy as a routine treatment for Helicobacter pylori infection.
ABSTRACT
Thrombocytopenia is a well-known side effect following intramuscular gold therapy in patients with rheumatoid arthritis. Thrombocytopenia may occur at any time and it can be irreversible and sometimes fatal despite cytotoxic or immunosuppressive therapy.

We describe two patients who presented with haemorrhagic diathesis on the day after the administration of aurothioglucose. The thrombocytopenia in these patients was caused by aurothioglucose-induced antibody-mediated platelet destruction. Both patients made an uneventful recovery and the platelet count returned to normal within several weeks without further treatment. Antibody-detecting tests were repeated five years later and could not demonstrate the presence of antibodies. Also after incubation with aurothioglucose no antibodies could be demonstrated.

INTRODUCTION
Haematological abnormalities are well-known complications following intramuscular gold therapy in patients with rheumatoid arthritis with an incidence of 1 to 3% in most series. The occurrence of thrombocytopenia during gold therapy is unpredictable and may occur at any time during treatment. Development of thrombocytopenia may be insidious or acute and the cause can be diverse and sometimes difficult to assess. Both bone marrow depression and antibody-mediated platelet destruction have been described. The course of thrombocytopenia can be irreversible and sometimes fatal despite cytotoxic or immunosuppressive therapy. Long-term follow-up after thrombocytopenia caused by antibodies directed against platelets in the presence of gold is unknown.

We studied the cause of thrombocytopenia in two patients who presented on the day after the administration of aurothioglucose with severe haemorrhagic diathesis.

CASE REPORTS

Patient 1
A 51-year-old woman was seen in our outpatient clinic because of a seropositive, erosive and nodular rheumatoid arthritis since 1991. After initial treatment with sulphasalazine, intramuscular aurothioglucose was started at 100 mg weekly until a cumulative dose of 1000 mg. While receiving a dose of 50 mg aurothioglucose once every two weeks, to a cumulative dose of 1350 mg, she mentioned that she was developing haematomas on the day after the aurothioglucose injection. During outpatient follow-up no haematomas and normal platelet counts (192–320 x 10⁹/l) were found on the day the aurothioglucose was given. Clinical examination one day after the aurothioglucose injection showed petechiae and haematomas and the platelet count had fallen from 238 x 10⁹/l to 6 x 10⁹/l in two days. Indomethacin, calcium and vitamin D were stopped and prednisone 7.5 mg daily was continued. A bone marrow biopsy showed normocellular bone marrow with normal megakaryopoiesis. Immunoglobulin G (IgG) could be demonstrated on the patient’s platelets by the direct platelet immunofluorescence test and the ether-eluate made of the patient’s own platelets reacted positively to donor platelets (table 1). Also IgG was demonstrated on donor platelets after incubating with the patient’s serum. No binding of
immunoglobulin of the IgM class was observed on the platelets. With no additional treatment her platelet count gradually returned to normal in three weeks (figure 1) and no further aurothioglucose was given.

Five years later all tests were repeated without aurothioglucose and with four different aurothioglucose concentrations (table 1). All tests showed no detectable IgG on donor and patient’s platelets.

Table 1
Tests measuring antibodies against platelets of both patients during five-year follow-up

<table>
<thead>
<tr>
<th>TESTS</th>
<th>PATIENT 1</th>
<th>PATIENT 2</th>
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<tbody>
<tr>
<td>Indirect PIFT during admission</td>
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<td>+</td>
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<tr>
<td>Direct PIFT during admission</td>
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<tr>
<td>Indirect PIFT after five years</td>
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<tr>
<td>Indirect PIFT after five years with aurothioglucose</td>
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<tr>
<td>Direct PIFT after five years</td>
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<td>Ether-eluation after five years</td>
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Patient 2
A 47-year-old woman was seen in our outpatient clinic because of a seropositive and erosive RA since 1989. After treatment with sulphasalazine, intramuscular aurothioglucose was given at a dose of 50 mg weekly until a cumulative dose of 1000 mg was reached. After a cumulative dose of 2700 mg, while receiving aurothioglucose injections of 25 mg every third week, she mentioned that haematomas had started to develop the day after the aurothioglucose injection. No skin abnormalities, however, were noticed during outpatient follow-up and the platelet counts were repeatedly normal (260-330 x 10^9/l) before the next injection. However, the clinical examination three days after the injection revealed petechiae and haematomas and the platelet count had fallen from 280 x 10^9/l to 18 x 10^9/l. The bone marrow biopsy showed normal megakaryopoiesis and normocellularity. Immunofluorescence tests demonstrated IgG on patient’s own platelets and on donor platelets after incubating with patient’s serum. An eluate made from patient’s platelets reacted positively with donor platelets (table 1). No IgM antibodies could be demonstrated. Aurothioglucose injections were stopped and without additional treatment her platelet count returned to normal (figure 2). Five years later all tests were repeated with serum alone and with four different concentrations of aurothioglucose (table 1). All tests showed no IgG on the patient’s platelets (direct immunofluorescence test). Platelets from one of three donors showed binding of IgG to platelets after incubation with patient’s serum without aurothioglucose, probably caused by HLA antibodies.

DISCUSSION
In patients with RA, thrombocytopenia can be caused by decreased thrombopoiesis, increased platelet destruction and platelet pooling. Increased platelet destruction is regularly encountered, caused by autoantibody-mediated destruction on the basis of concomitant diseases (e.g. Felty’s syndrome) or medication. Gold, as has been
described in several case reports,1 can cause thrombocytopenia by bone marrow aplasia4,5 or antibody-mediated platelet destruction.6,7 Gold-induced aplasia is often refractory to treatment. Most patients die of septic shock before or after receiving immunosuppressive or cytoreductive therapy or even bone marrow transplantation.4,5 Gold-induced antibody-mediated platelet destruction has been described by several authors in case reports or small case series.4,5 Some studies showed HLA-DR3 positivity in 68 to 100% (normal prevalence 23%) of the patients.3,16-17

The positive direct and indirect platelet immunofluorescence tests (positive tests to donor and patient platelets together with positive eluate reactions) can point to antibodies directed against platelet-specific antigens (e.g. idiopathic thrombocytopenia, Aldomet), against hidden antigens exposed in the presence of aurothioglucose (as seen in EDTA-dependent thrombocytopenia), or against aurothioglucose itself, the platelet being destructed by the antibody-antigen complex as an innocent bystander. The positive reaction of the eluate with donor platelets, however, excluded antibody-antigen complexes. The negative tests five years later with incubation of aurothioglucose made the mechanism with a hidden antigen unlikely. The tests point to a true auto-antibody character of the antibodies directed against a platelet-specific antigen, but induced by aurothioglucose. The rapid resolution of the thrombocytopenia points to a dose-dependent reaction of this autoantibody induction. Gold administration can induce acute autoantibody-mediated platelet destruction that is completely reversible without treatment. Serious attention should be paid to patients who report haemorrhagic diathesis in the days following drug administration of any kind.

NOTE
Case histories and data were presented at the HOVON continuous medical education day 2002 in Rotterdam.

REFERENCES
Serious allergic reaction to administration of epirubicin

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ABSTRACT
A 47-year old woman was admitted for adjuvant treatment with chemotherapy consisting of epirubicin and cyclophosphamide. During the second course of chemotherapy an allergic reaction occurred after administration of epirubicin. Treatment with clemastine 2 mg iv caused a quick recovery and after 24 hours there was only a slight redness of the face.

A discussion follows on allergic reactions to antracyclines and the literature is updated.

INTRODUCTION
Pharmarubicin (epirubicin) is frequently used in the treatment of breast cancer. Known adverse effects are nausea, vomiting, alopecia and bone marrow suppression. We describe a patient with a serious allergic reaction following administration of epirubicin.

CASE REPORT
A 47-year-old woman was admitted for adjuvant treatment after a modified radical mastectomy, according to the Patey-Madden procedure, for breast cancer. Histological examination showed lymph node metastases in 1 out of 17 axillary lymph nodes. Her medical history was uneventful. She had had no allergic reactions in the past. Chemotherapy consisted of cyclophosphamide 500 mg/m² and epirubicin 70 mg/m² intravenously. As antiemetic treatment, oral granisetron was given. The first course was well tolerated apart from slight nausea. After three weeks the second course was given and during administration of epirubicin the patient complained of itching in the neck region after a few minutes, followed by a generalised urticarial exanthema. There was no drop in blood pressure. After administration of clemastine 2 mg iv, the exanthema diminished. The cyclophosphamide was given after one hour without any complications. Twenty-four hours after the chemotherapy only a slight facial redness remained. During the third and fourth course of chemotherapy clemastine 2 mg iv and dexamethasone 8 mg iv were added to the oral antiemetic medication. Nevertheless the same allergic reaction occurred, which was more severe than in the second course. There were no other allergic reactions.

DISCUSSION
Allergic reactions to antracyclines are well known, though rare. In 1984, Solimando reported three patients with rash and urticaria after administration of doxorubicin hydrochloride (adriamycin). The symptoms developed immediately after the doxorubicin infusion was started; in one out of three patients angio-oedema occurred followed by a slight drop in blood pressure and a tachycardia. None of the patients became bronchospastic. In two patients antihistamines were given, which improved the allergic symptoms and adriamycin could be administered after one hour without any problems. During the next courses no antracyclines were given. In one of the three patients treatment was continued and during the following five courses of doxorubicin, antihistamines were given orally.
During each course comparable allergic reactions occurred. Turtle described three patients out of 17 treated for breast cancer with a regimen containing epirubicin.\(^2\) Besides a slight raise in temperature in one of these three patients, no abnormalities occurred during the first course. In two out of the three patients a raise in temperature to 40°C developed during the second course and in one of three patients during the third course after administration of chemotherapy, accompanied by a raise in body weight and a skin reaction. A severe drop in blood pressure occurred accompanied by severe hypoxaemia. Antihistamines were not given.

Wilson reported a patient with local induration and fading of the skin considered to be extravasation.\(^3\) Three weeks later epirubicin is administered in the other arm and two weeks later pain and swelling developed in the region of the former extravasation. Plastic surgery followed within 24 hours. The histological examination of the removed tissue showed lymphocytic infiltration. Finally, Cassidy reported a patient with extravasation during the second infusion of epirubicin.\(^4\) Urticarial reaction developed during the following infusion three weeks later in the other arm. The infusion of epirubicin was discontinued and the skin reaction resolved within 20 minutes. Three weeks later the urticarial lesions situated on bifurcations of veins appeared to become ulcers. Recovery occurred within three months. Antracyclines are no longer administered.

**CONCLUSION**

The literature describes various types of reactions after administration of antracyclines. High doses of epirubicin can cause high fever, hypertension and hypoxia within 24 hours of administration, in addition to skin reactions. Other symptoms seem to be related to extravasation and the concentration of epirubicin locally within the veins. In our patient a type I allergic reaction occurred. If precautions are taken, this kind of allergic reaction does not need to prevent further administration of effective chemotherapy.

**REFERENCES**

Late-onset cardiotoxicity of chemotherapy and radiotherapy

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ABSTRACT

An increasing number of patients with malignant lymphoma are becoming long-term survivors following treatment with chemotherapy (CT) and/or radiotherapy (RT). Therefore, late therapy-related complications are becoming increasingly clear.

We present three young patients to illustrate the dire consequences of late-onset cardiotoxicity as sequel to potentially curative treatment.

We advise yearly life-long follow-up of CT/RT patients treated with curative intent.

Echocardiography should be performed when cardiac murmurs arise. Prevention of further cardiac damage by reducing other cardiac risk factors as well as endocarditis prophylaxis when indicated is recommended.

INTRODUCTION

With increasing successes in chemotherapy (CT) and/or radiotherapy (RT) many patients with malignant lymphomas have become long-term survivors of cancer. Therefore, an increasing number of potentially cured patients suffer from several therapy-related long-term effects, diminishing their quality of survival. We present three young patients successfully treated for their lymphoma to illustrate the impact of late-onset cardiotoxicity. We review the relevant literature and discuss possible guidelines to prevent potentially fatal cardiotoxic complications.

PATIENTS

Patient A

A 31-year old woman was diagnosed with large-cell anaplastic lymphoma stage IVb, localised in the right os ileum, para-aortic lymph nodes and subpleurally. She received eight cycles of CHOP chemotherapy (cyclophosphamide, adriamycin (cumulative dose 400 mg/m²) and prednisone) resulting in a partial remission. Subsequently she received two cycles of IMVP16 (iposphamide, methotrexate, VP16), to which a complete remission was documented.

Consolidation followed with high-dose chemotherapy according to the BAM schedule (BCNU, Ara C, melphalan) in combination with a G-CSF stimulated whole-blood stem cell transplant.$ She was admitted to hospital three years later because of progressive dyspnoea of effort, palpitations and oedema for two weeks. Risk factors for coronary heart disease were smoking (10 cigarettes daily). On physical examination we saw a fatigued, breathless woman, with a blood pressure of 100/60 mmHg, arterial pulse of 112 beats/min. and an elevated central venous pressure. A holosystolic murmur was auscultated at the apex cordis as well as basal crackles over both lungs. An X-ray of the chest confirmed the diagnosis of congestive heart failure.

The ECG showed sinus tachycardia without signs of ischaemia or myocardial infarction. Continuous electrocardiographic monitoring revealed short asymptomatic ventricular tachycardias. Laboratory examinations showed a pattern consistent with a small myocardial infarction. Echocardiography showed diminished left and right ventricular function, bialtrial dilatation, and serious mitral and aortic regurgitation. She was treated with diuretics, an ACE inhibitor, digoxin and a β-blocker. Following discharge a strongly diminished exercise-tolerance persisted.

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Additional workup showed a very high ferritin level (1115 μg/l) although HFE mutation analysis did not reveal the common genetic mutation for haemochromatosis.

Patient B
A 27-year old woman was diagnosed with nodular sclerosing Hodgkin’s disease stage IIa, localised to left supraclavicular and mediastinal lymph nodes. Mantle field irradiation was given (4000 cGy in 20 fractions) to which she achieved a clinical complete remission. Six years later Hodgkin’s disease recurred with localisations in mediastinal and right axillary lymph nodes. She was treated with MOPP/ABV chemotherapy (mitoxane, vincristine, procarbazine, prednisone/ adriamycin (cumulative dose 215 mg/m²), bleomycin, vincristine, prednisone, lomustine, mitomycin). After four cycles a clinical complete remission was attained which was consolidated with two additional cycles. During the next two years she developed hypothyroidism as well as premature onset of menopause. Five years after chemotherapy she was admitted to the hospital because of dyspnoea of effort, orthopnoea and palpitations. There were no risk factors for coronary heart disease. On physical examination the blood pressure was 150/80 mmHg, arterial pulse 105 beats/min. On auscultation of the heart, a holosystolic murmur grade II/VI was noted. Transoesophageal echocardiography showed a good left ventricular function with marked mitral and aortic regurgitation, without valvular vegetations or papillar dysfunction, and some pericardal effusion. After treatment with diuretics her symptoms disappeared and never recurred. A tentative diagnosis of viral pleuropericarditis was made with possible diastolic myocardial dysfunction. Six years after this combined modality treatment she was admitted to the coronary care unit because of chest pain irradiating to the left arm. Risk factors for coronary heart disease were smoking and a positive cardiovascular family history. On physical examination the blood pressure was 140/90 mmHg, arterial pulse 105 beats/min. On auscultation of the heart, a holosystolic murmur grade II/VI at the apex and a systolic murmur grade II/VI were noted at the left and right second intercostal space irradiating to the carotid arteries. The ECG showed sinus tachycardia with ST-segment depression in leads II, III, aVF as well as the precordial leads. After treatment with nitroglycerin the chest pain dissipated and the ECG normalised. Laboratory examination showed a troponin of 0.48 μg/l (normal < 0.2 μg/l), suggesting myocardial ischaemia. Transoesophageal echocardiography showed diminished left ventricular function and aortic regurgitation.

Coronary angiography showed a significant stenosis of the ramus CX for which she underwent a PTCA with coronary stenting. This procedure had to be repeated four months later because of an in-stent stenosis. Three months after the second PTCA procedure an aortic valve replacement and coronary artery bypass grafting (aorta-Mo/Cx) were performed.

DISCUSSION

These three case histories illustrate the serious late cardiac sequelae of chemotherapy and radiotherapy that can occur even years after initial treatment. Anthracyclines, a group of potent chemotherapeutic agents widely used in the treatment of many different tumours, are known to cause acute and chronic cardiotoxicity.1 They cause a cumulative, dose-related, progressive, irreversible, and destructive cardiomyopathy. Pathological-anatomical examination shows dilatation of sarcomplasmatic reticulum with disappearance of myofibrils finally culminating in myocardial fibrosis. The pathophysiology is thought to consist of binding of anthracycline metabolites to cellular iron, forming toxic free oxygen radicals that cause myocardial fibrosis.6 Acute cardiotoxicity can present as nonspecific ST/T-segment changes in the ECG, hypotension, tachycardias, arrhythmias and pericarditis during treatment with chemotherapy. Chronic cardiotoxicity presents as congestive heart failure, mostly (>80%) within one year after treatment, however in some cases even after a latency period of many years.7,8 The most widely used anthracycline is adriamycin (ADM). A cumulative dose of 400 mg/m² results in an incidence of heart failure of 0.4%, reaching 1 to 4% incidence at 500 mg/m², 7% incidence at 550 mg/m² and increasing to more than 20% at doses above 700 mg/m².9
Bolus infusions are more cardiotoxic than continuous infusions.\textsuperscript{56} Combining anthracyclines with high doses of cyclophosphamide or mediastinal irradiation can cause heart failure at lower cumulative doses.\textsuperscript{5,6} Known additional risk factors are very young and old age (>70 years), female gender and pre-existent cardiovascular disease.\textsuperscript{10} In our first patient we observed serious cardiomyopathy after a cumulative ADM dose of 400 mg/m\textsuperscript{2}. It is possible that pre-existent iron overload could have been a contributory factor.\textsuperscript{10,15} A general recommendation is to measure sequentially the left ventricular ejection fraction (LVEF) at rest by radionuclide angiocardiography (RAG) at different cumulative doses of ADM (0, 300, 450 mg/m\textsuperscript{2} and every 50 mg/m\textsuperscript{2} thereafter) and to stop ADM therapy when a significant reduction (>10\%) and/or a subnormal value (LVEF <50\%) is found.\textsuperscript{9,14,15} Additional RAG monitoring should be performed when continuous sinus tachycardia is observed without an evident cause and should be considered at a cumulative dose of 400 mg/m\textsuperscript{2} when other cardiac risk factors are present. Subnormal baseline values (LVEF 30-50\%) do not exclude ADM therapy provided that serial RAG monitoring is performed in these patients\textsuperscript{16} prompting cessation of anthracyline administration if the LVEF drops >10\% and/or reaches a value <30\%.

The value of RAG monitoring in the subgroup of patients less than 40 years of age receiving ADM up to a cumulative dose of 350 mg/m\textsuperscript{2} has been questioned because heart injury in these patients nearly always remains subclinical.\textsuperscript{17,19} In addition, economic analysis has shown pretreatment RAG screening not to be cost-effective in this subgroup.\textsuperscript{20}

Moreover, it is questionable if RAG monitoring during treatment can predict late-onset cardiotoxicity as echocardiographic findings have been shown to worsen in the course of many years.\textsuperscript{21} Confounding this issue is the observation that only a modest agreement exists between echocardiographic methods and RAG.\textsuperscript{22} As our first patient demonstrates, congestive heart failure can occur unexpectedly after several years. Recently it was demonstrated that clinically manifest ADM cardiotoxicity can be predicted with good sensitivity early during treatment if the RAG ejection fraction drops 5\% or more after receiving 200 mg/m\textsuperscript{2} ADM.\textsuperscript{23} This observation argues for continued use of baseline RAG monitoring but raises the issue whether RAG should be performed at this rather modest cumulative dose when administering potentially curative treatment. In the future, magnetic resonance imaging may provide a new tool in detecting anthracycline cardiotoxicity.\textsuperscript{24} When treating lymphoma patients with curative intent, practical advice could be to monitor LVEF at baseline and at cumulative ADM doses of 300 mg/m\textsuperscript{2} corresponding to six cycles of CHOP therapy. A promising development in the prevention of anthracycline-induced cardiomyopathy is the cardioprotectant dexrazoxane (ICRF-187).\textsuperscript{25} A meta-analysis of six randomised trials with disseminated breast cancer patients showed that the risk of developing clinical cardiotoxicity was significantly reduced when dexrazoxane was administered prior to ADM starting at a cumulative dose of 300 mg/m\textsuperscript{2}.\textsuperscript{26} Whether dexrazoxane protects against late-onset cardiotoxicity has to be established. Randomised clinical trials have to be performed in lymphoma patients to establish if dexrazoxane can antagonise potentially fatal anthracycline-induced cardiomyopathy without loss of effectivity in potentially curative treatments. Anthracycline-induced congestive cardiomyopathy is treated with diuretics, digitalis, selective \beta-blockers and ACE inhibitors.\textsuperscript{27} In younger patients heart transplants have been performed successfully.\textsuperscript{28,29}

Mediastinal irradiation is cardiotoxic as well. It can cause pericarditis, myocardial fibrosis and premature coronary heart disease.\textsuperscript{30} The relative risk of cardiac death after mediastinal irradiation is increased threefold.\textsuperscript{30,31} Known additional risk factors are old age, pre-existent cardiovascular disease, female gender\textsuperscript{32} and radiation dose (starting at 30 Gy). In about 25\% of patients treated for Hodgkin’s disease with mediastinal irradiation, a combined aortic and mitral valve regurgitation is found,\textsuperscript{33} being caused by valvular fibrosis.\textsuperscript{34} Two of our patients had such combined valvular disease. An important consequence for patients with valvular insufficiency is the need for endocarditis prophylaxis.

We conclude that it is increasingly clear that potentially curative cancer treatments can cause late-onset cardiac injury. In the long-term follow-up of potentially cured patients we advise evaluating cardiac function clinically at least every year to detect late-onset cardiotoxicity and to determine if endocarditis prophylaxis is necessary. The threshold for additional echocardiographic evaluation should be low. Prevention strategies to reduce other cardiac risk factors, such as high blood pressure, cholesterol, diabetes and smoking, seem indicated in these patients.

\textbf{REFERENCES}


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