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REVIEW

Hepatic veno-occlusive disease associated with toxicity of pyrrolizidine alkaloids in herbal preparations

Zhe Chen, Ji-Rong Huo*

Department of Digestive Disease, The Second Xiang-Ya Hospital of Central South University, Changsha, Hunan Province, China, *corresponding author: tel.: +86-731-85295035, e-mail: HJR198@hotmail.com

ABSTRACT

Hepatic veno-occlusive disease (VOD) is frequently linked to stem cell transplantation (SCT), mainly related to the conditioning regime, and contributes to considerable morbidity and mortality. However, pyrrolizidine alkaloid (PA)-induced VOD has long been overlooked. The pathogenesis of VOD remains poorly understood; studies suggest that endothelial cell injury, cytokines and haemostatic derangement are all involved in the pathogenesis of VOD. Until recently, treatment options have been limited and no uniformly effective therapy has been established. Thus, treatment is largely supportive and symptomatic. Ongoing work, including development of new animal models and clinical studies, is needed to help us fully understand the pathogenesis of VOD and enable us to devise effective solutions. Furthermore, it is strongly advised that supervisory measures be taken to standardise the use of herbal medication.

KEYWORDS

Hepatic veno-occlusive disease; pyrrolizidine alkaloids; coagulation parameters

INTRODUCTION

Hepatic veno-occlusive disease (VOD), first described in 1920 by Willmot and Robertson,[1] is a clinical syndrome characterised by hepatomegaly, ascites, weight gain and jaundice.[2-4] It is due to several factors. In Western countries, VOD is now recognised as a complication most commonly associated with high-dose chemotherapy and stem cell transplantation (SCT).[5,6] The incidence of VOD following SCT ranges from 5 to 70% in different reports,[7-9] depending on the variations in patients' characteristics, diverse criteria for diagnosis, sample size, variable distribution of risk factors, differences in conditioning therapy used and the capacity to diagnose early and mild VOD.[5,10] VOD has also been reported after solid organ transplantation, especially kidney transplantation, mainly related to the toxicity of azathioprine.[11,12] Many cases of VOD after kidney transplantation had been reported, and in one report, VOD occurred in up to five of the 200 patients who underwent kidney transplantation.[13-15] In addition, VOD has been described in association with other agents, such as oral contraceptives, chemotherapeutic drugs (actinomycin D, mithramycin, dacarbazine, cytosine arabinoside, 6-thioguanines, cyclophosphamide, gemtuzumab ozogamicin),[5] alcohol and radiation injury.

While hepatic impairment resulting from conventional pharmaceutical drugs is widely acknowledged, the potential hepatotoxicity of herbal preparations and other botanicals has been underestimated due to public misconception that they are harmless. They are commonly used for self-medication without supervision. Several species of pyrrolizidine alkaloid (PA)-containing plants can cause VOD and have been associated with epidemics in developing countries such as Jamaica, India, Egypt, Iraq and South Africa.[9,16,17] Most of them are results of food contamination or when PA-containing plants are misused for medical purposes. Nowadays, there is growing concern even in the developed countries over the use of PA-containing herbal remedies in the treatment of arthritis, thrombophlebitis, gout and diarrhoea. This review outlines the pathogenesis of PA-associated VOD, with an emphasis on endothelial cell injury and coagulation parameters. The current status and future directions of treatment are also discussed.

PYRROLIZIDINE ALKALOIDS

Pyrrolizidine alkaloids are a group of more than 350 natural toxins sharing a basic structure derived from esters of three necine bases: platynecine, retronecine or otonecine. They are named for their inclusion of a pyrrolizidine nucleus (a pair of linked pyrrole rings). Each pyrrole can be diagrammed as a five-sided structure with four carbons and one nitrogen forming the ring, and pyrroles are incorporated into the chlorophyll molecule.[18] It is assumed that more than 6000 plant species belonging to the families of *Compositae*, *Boraginaceae* and *Leguminosae* contain PAs at different levels and in different patterns. In turn it has been estimated that about 3% of the world's flowering plants contain one or more of the toxic PAs.[19] Among them, the *Senecio, Crotalaria, Cynoglossum, Heliotropeum, Echium* and *Symphytum* species are of particular importance due to their toxicity to livestock and humans (*table 1*).[19-21] Acute intoxications caused by PAs are characterised by hepatotoxicity and haemorrhagic liver necrosis. Long-term exposure caused hepatic megalocytosis, veno-occlusive disease in liver and to a lesser extent in the lungs, proliferation of the biliary tract epithelium, fatty liver degeneration and liver cirrhosis. Moreover, many PAs are genotoxic and carcinogenic in rodents. In humans, PAs cause primarily hepatic veno-occlusive disease.[22]

Although PAs can be found in all plant organs, they are usually concentrated more in roots than in leaves.[23,24] Research done by Couet *et al.*[23] revealed that the roots of comfrey had a range of 1400 to 8300 ppm PAs content while the leaves had only 15 to 55 ppm. It is also suggested that small, younger leaves contain more PAs than large, older leaves.[25] A necine base is the main structure of PAs.[26] A pyrrolizidine alkaloid should contain at least one 1,2 unsaturated necine base, which is usually esterified to necic acid for the induction of hepatoxicity. Different structural characteristics of PAs determine different degrees of toxicity. Its toxicity will increase if the hydroxyl groups are esterified in positions 7 and 9, if the necic acids have branched chains or are unsaturated, or if the necic acid forms a cyclic diester ring, as observed in senecionine and monocrotaline (*figure 1*).[22]

The PAs, which have minimal toxicity in their original form, are metabolised in the liver through a CYP (P450 cytochrome)

Figure 1. *Structure of two representative PAs, senecionine and monocrotaline*

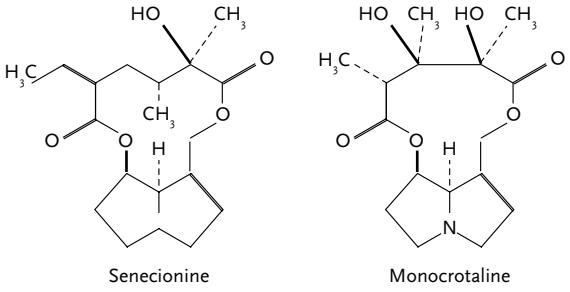


Table 1. *Common plants containing PAs*

Family	Species	Usage, constituents and toxicity
***Boraginaceae* family**	*Anchusa officinalis*	Used as an expectorant and a diuretic, also used to treat skin diseases. Total alkaloid content is about 0.12%
	Borago officinalis	It is used in Western herbs to treat inflammatory diseases and cough, also used as a diuretic. Total alkaloid content is less than 0.001%
	Cynoglossum officinale	Used to treat diarrhoea and topically for bruises. Total alkaloid content is 0.7 to 1.5%
	Helitropium arborescens	It is commonly used in Africa as medicinal herb. Contains heliotrine and indicine. Total alkaloid content is about 0.01%
	Lithospermum officinale	Used to treat gout, kidney stones, diarrhoea, and also as for contraceptive purposes. Contains lithosenine. Total alkaloid content is about 0.003%
	Myosotis scorpioides	Used as a sedative and externally as an eye wash. Contains myoscorpine, scropioidine, and symphytine. Total alkaloid content is 0.08%
	Symphytum officinale	Commonly called comfrey. It is widely used as an 'alternative'. Contains intermedine, lycopsamine, symphytine, echimidine and symglandine. Total content of PAs is about 0.5%
***Asteracea* family**	*Emilia sonchifolia*	Used to treat influenza, cough and bronchitis. Contains senkirkine and doronine. Total alkaloid content is 0.2%
	Eupatorium cannabinum	Used as an antithermic, also a diuretic. Contains supinine, rinderine, echinatine and lycopsamine
	Petasites hybridus	It is used in Europe for numerous diseases, especially abdominal pain. Contains, senecionine, integerrimine, retrosine, seneciphylline, jacobine, et cetera. Total content is about 0.01%
	Senecio aureus	Used as a treatment for injuries, also a diaphoretic and diuretic. Contains senecionine, riddelline, retrorsine, monocrotaline and otosenine
	Tussilago farfara	It is widely used in Europe to treat lung disorders and gastrointestinal disorders. Contains senkirkine and senecionine
***Fabacaea* family**	*Cortalaria spp.*	Contaminated cereal crops blamed for human poisoning. Contains crotananine, monocrotaline and cronaburmine

3A-mediated transformation to N-oxides and conjugated dienic pyrroles. Pyrroles are alkylating compounds that are highly reactive with proteins and nucleic acids. The complex of pyrroles with proteins and nucleic acids may persist in tissues and generate chronic injury, whereas N-oxides may be transformed into epoxides and toxic necines.[23,27] CYP3A inducers could enhance PAs' toxicity, while CYP3A inhibitors could decrease it. PAs can decrease glutathione (GSH) in sinusoidal endothelial cells and oxidative stress plays an important role in PA-induced VOD. It has been demonstrated that intraportal infusion of GSH can prevent VOD in monocrotaline-induced rat models; a possible reason is that GSH could conjugate with dehydromonocrotaline to form GSDHP, a compound of much lower toxicity that is released in high concentration into bile.[28,29] The enhanced oxidative stress can also affect collagen α1 transcription directly and/or through the activation of hepatic stellate cells, thus, ultimately leading to VOD.[30] Moreover, PAs can inhibit the proliferation of hepatocytes; decrease the levels of the antiapoptotic protein Bcl-x, while increasing the expression of the proapoptotic protein Bax. The latter leads to the release of cytochrome C from mitochondria and activates the intrinsic apoptotic pathway.[22]

Not everyone taking PAs-containing food or plants shows signs of VOD, and a strict dose-dependency may be absent. As we know, the most important drug-metabolising enzyme system regarding the biotransformation of exogenous compounds is cytochrome P450s, and more than 150 different cytochrome P450s have been detected. Thus, differences in enzyme activities lead to individual variabilities in handling PAs.[31] Besides, the external use of PAs is safer than oral or systemic administration given that the hepatic bioavailability is minimal. The systemic bioavailability after external use is about 20- to 50-fold lower than that after oral ingestion,[22] but the absorption of PAs will increase when inflammation or lesions are present on the skin.

HISTOPATHOLOGY AND PATHOGENESIS

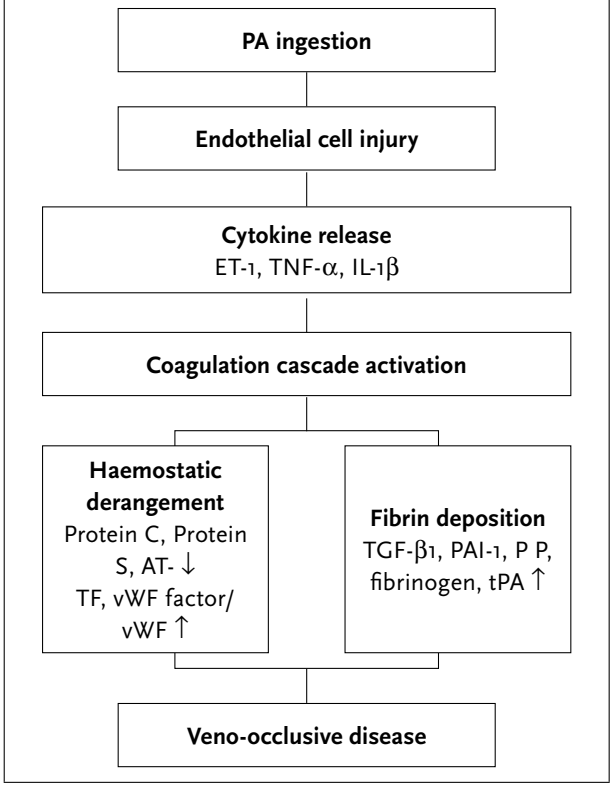
Injury to sinusoidal endothelial cells and hepatocytes in zone 3 of the liver acinus is considered to be the initial event in the development of VOD.[32-34] The reason may be that zone 3 is rich in cytochrome P450 and GST enzymes, but contains lower levels of GSH than other zones.[35-37] In fact, sinusoidal endothelial cells are more sensitive to damage than hepatocytes. This is supported by the following evidence.[37-39] First, the concentration of GSH in sinusoidal endothelial cells, which is required for the detoxification of PAs, is less than half that in hepatocytes. Second, when precursor amino acids are added to culture media, hepatocytes can synthesise GSH while sinusoidal endothelial cells cannot. Third, portal hypertension is a presenting feature of VOD rather than a late event secondary to progressive parenchymal failure. Fourth, sinusoidal endothelial cells possess the CD14 surface antigen, a receptor for lipopolysaccharide (LPS), and this makes them the first cells to be exposed to toxins and bacteria in portal blood.

Many animal models have been established to study the histological features of VOD.[40-43] Injury to the hepatic venules, which is characterised by subendothelial oedema, red cell exudation, deposition of fibrin and factor α/ von-Willebrand factor (VWF) within venular walls,[32,44] is believed to be the first histological change. In the early stage of VOD, damage to the endothelium of central venule (CV), subendothelium and sinusoidal haemorrhage, varying degrees of coagulative necrosis in the centrilobular and concentric narrowing of terminal hepatic venules and sinusoids are present, but fibrosis is mild or absent. Late VOD is characterised by subendothelial and adventitial fibrosis, while damage to CV and haemorrhage persist throughout. All these lesions distribute unevenly in the liver, and cirrhosis may ultimately appear.[9,10,42]

COAGULATION PATHWAYS

The role of coagulation pathways in the pathogenesis of VOD is still debatable (*figure 2*). Although VOD is considered a nonthrombotic vascular disease, sufficient

Figure 2. *The potential role of coagulation pathways in the pathogenesis of VOD*



evidence suggests that haemostatic derangement may be relevant to the occurrence of VOD.[5,45,46] The endothelial injury caused by PAs triggers the coagulation cascade and induces a hypercoagulable state.[10,33,34] In animal models, monocytes are recruited to the lobule and venous endothelium at an early stage.[42] VWF, thrombomodulin and several cytokines, including tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), endothelin-1 (ET-1), P-selection and E-selection, are released by monocytes and (or) endothelial cells[32,37,47,48] as responses to the toxicity of PAs. Both TNF-α and IL-1β are procoagulant. The expression of several coagulation factors, such as tissue factor (TF)[49,50] and plasminogen activation inhibitor-1 (PAI-1), is increased by the stimulation of TNF-α and IL-1β.[51,52] Furthermore, platelets are activated, and the expression of fibrosis markers, such as transforming growth factor beta-1 (TGFβ1)[53,54] and N-terminal propeptide for type III procollagen (PIIIP),[37,55] is increased, and these eventually lead to fibrous obliteration of the affected venules.

Decrease of natural anticoagulants, as well as changes in fibrinolytic activity, have also been found in many clinical cases of VOD. A study about changes of coagulation parameters in 44 consecutive patients who underwent allogeneic SCT suggested that PIIIP, tissue plasminogen activator (t-PA), and protein C were predictive markers for VOD ($p<0.0001$).[56] David *et al.*[57] reported that reduction of antithrombin, protein C, protein S and elevation of D-dimer, prothrombin fragment F1+2 could be detected in patients with VOD, and the elevation of PAI-1 and VWF were characteristic markers of VOD. In addition, Park *et al.*[58] suggested that increase in t-PA, PAI-1 and decrease in antithrombin-III (ATIII) might be useful markers for VOD. A consecutive study revealed that ADAMTS 13 (a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13), a metalloproteinase produced specifically in hepatic stellate cells that cleaves unusually large VWF multimers (UL-VWFMS),[59] was involved in the pathogenesis of VOD. Activity of ADAMTS 13 was significantly reduced in VOD patients, and could be a predictor for the occurrence of VOD.[60,61]

Among all the coagulation parameters, PAI-1 and protein C are thought to be of great importance. PAI-1 is considered to be not only an independent predictor of VOD, but also associated with the severity of disease, clinical course and response to treatment.[62-64] Protein C is a sensitive and early marker of liver dysfunction due to its short plasma half-life, and in a multivariate analysis, it was the only variable that could distinguish VOD from non-VOD patients independently.[65-67]

In summary, coagulation pathways play an important part in VOD. It is, however, unclear whether these changes are involved in the pathogenesis or are merely consequences of the disease.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The diagnosis of VOD is based on the classical triad of weight gain, painful hepatomegaly and jaundice. Symptoms usually appear after one to two months of continuous exposure to PAs.[42] Clinically, the diagnosis is usually made in accordance with the criteria put forth by the Seattle[1] or Baltimore[68] group (*table 2*). The specificity of these two criteria is about 92%, but the sensitivity is rather low.[32] None of the signs are specific for the diagnosis. Moreover, a history of ingestion is usually difficult to obtain because patients are reluctant to divulge the use of herbal preparations, even on repeated questioning, either assuming their safety or fearing not being taken seriously for using herbs.

Histological biopsy of liver is the gold standard for the diagnosis of VOD. This procedure is usually delayed because of thrombocytopenia, clotting abnormalities and extensive ascites, which are contraindications to liver biopsy.[37,64] As an alternative, a catheter-based percutaneous transjugular approach is being used. This approach allows the measurement of hepatic venous pressure gradient (HVPG) and a liver biopsy at the same time. Studies show that an HVPG greater than 10 mmHg is correlated with VOD, the specificity, positive predictive value and sensitivity are 91%, 86% and 52%, respectively.[32,70]

Ultrasonography may also be helpful for the diagnosis by showing ascites, hepatomegaly, attenuated hepatic flow, hepatic vein dilation and thickening of gallbladder wall. These non-specific findings can be used to exclude other diseases that mimic VOD, such as extrahepatic biliary obstruction and malignant infiltration of the liver. Pulse Doppler ultrasound usually shows a decreased or

Table 2. *Diagnostic criteria for veno-occlusive disease (VOD)*

1) **Seattle criteria**[1]
Presence of at least 2 of the following 3 clinical features within 30 days after transplantation:
   1. Jaundice
   2. Hepatomegaly with right upper quadrant pain
   3. Ascites and /or unexplained weight gain

2) **Baltimore criteria**[64]
Presence of hyperbilirubinaemia (total serum bilirubin >2 mg/dl) within 21 days after transplantation and at least 2 of the following features:
   1. Hepatomegaly (usually painful)
   2. Weight gain >5% from baseline
   3. Ascites

3) **Modified Seattle criteria**[7]
Presence of 2 of the following features within 20 days after transplantation:
   1. Hyperbilirubinaemia (total serum bilirubin >2 mg/dl)
   2. Hepatomegaly or right upper quadrant pain of liver origin
   3. Unexplained weight gain (>2% of baseline body weight) because of fluid accumulation

inverted portal blood flow, which is a relatively late finding in patients with VOD.[71] Thus, it may be of prognostic value.[72] Magnetic resonance imaging findings, include hepatomegaly, hepatic vein narrowing, periportal cuffing, gallbladder wall thickening, ascites, and reduced portal venous flow velocity,[73] can be used as a method for differential diagnosis.

PROGNOSIS

The reported mortality of VOD varies from 20 to 50%. While there is a gradual resolution of symptoms in mild and moderate patients, the mortality of severe patients approaches 100%, often involving multiple organ failure (MOF). Patients can be classified into three groups[5,9,10] (mild, moderate and severe) according to the severity of disease and the outcome (*table 3*). Unfortunately, this classification is a retrospective assessment of the disease and is not useful for its management. A regression model proposed by Bearman *et al.*[74] suggests that patients who develop hyperbilirubinaemia and significant fluid retention earlier and worsen faster are at high risk of severe VOD. Research also finds that the severity of clinical features is associated with the number of histological changes rather than the occlusive of small hepatic venules.[75] Moreover, an HVPG greater than 20 mmHg indicates a poor prognosis.[74]

Table 3. *Classification of veno-occlusive disease (VOD) according to its severity*

Mild VOD
1. No adverse effects of liver disease are present
2. No treatment of VOD is needed
3. The illness is self-limited

Moderate VOD
1. Presence of an adverse effect of liver disease
2. Treatment of VOD is needed (such as diuretics for fluid retention or medication to relieve pain from hepatomegaly)

Severe VOD
1. Signs and symptoms of VOD do not resolve by day 100
2. Patients die of complications of VOD

PREVENTION

Human exposure originates from PA-containing herbs, teas and dietary supplements. Possible routes of human dietary exposure are accidental or intentional ingestion of PA-containing plants, consumption of PA-contaminated honey, and exposure through products of animal origin such as milk and eggs. Exposure may also result from the intentional consumption of herbal medicinal products. A study showed that 60% of people using herbal remedies took them along with conventional medicines and the remedies are often made by simple processes with no brand name and neither written recommendations nor warnings.[76] In most cases of PA-associated VOD, the daily intake ranges from several milligrams to hundreds of milligrams.[18] Thus, supervision and instruction of PA content in food and herb medicine are crucial.

European Food Safety Authority (EFSA) has recommended to obtain more data on carry over of PAs into milk as infants may have high exposure via this pathway. Also highlighted was the need for quantitative assessment of the contribution of honey to human exposure, as honey is found to contain residual amounts of PA metabolites.[77] Some countries and organisations have set standards for the use of PA-containing herb preparations. In the USA and many European countries, herbal supplements must be approved by national public health institutions before sale. Many herbs known to contain toxic PAs and to be potentially damaging, such as Senecio, are not allowed as ingredients of herbal supplements. Regulatory efforts have been taken by many countries to prevent these injuries: the German government has set a limit for daily exposure to PAs of no more than 0.1 µg for less than six weeks per year;[19] in Belgium the limit for PAs in herbs is 1 ppm (1 µg per gram of herb); the American Herb Products Association (AHPA) recommends that all products with botanical ingredients which contain toxic pyrrolizidine alkaloids bear the following cautionary statement on the label: For external use only. Do not apply to broken or abraded skin. Do not use when nursing.[18] In addition, extract manufactures are seeking methods of removing the PAs from their finished products. In China, the government should support timely risk management actions as real and potential risks to the health of consumers occur. The Chinese traditional herbal medicine (*table 4*) should meet specific and appropriate standards of safety and quality and the herbal products should be accompanied by necessary information for safe use.

TREATMENT

The success of treatment for VOD depends on early diagnosis and early intervention. Various analytical techniques, particularly chromatography methods in conjunction with mass spectrometry, can be used to detect PAs in plants or plant-derived products. It is of great importance to avoid further contact with the suspicious toxin as soon as possible once the diagnosis is confirmed or symptoms appear. So far, there is no uniformly effective treatment for VOD, and supportive care in established VOD is the cornerstone of management. The purpose of supportive care is to maintain intravascular volume and renal perfusion without causing extravascular fluid accumulation.[2] General measures include restriction of water and sodium supply together with the usage of

Table 4. *Commonly used Chinese herbs that are currently known to contain pyrrolizidine alkaloid*

Chinese herbs	Plant species	Effectiveness and constituents
Herba crotalariae asssamicae	*Crotalaria asamica Benth.*	Mainly used to treat cough, swelling and toothache. Contains monocrotaline. Alkaloid content is 2-3% in stems and leaves
Herba crotalariae sessiliflorae	*Crotalaria sessiliflora L.*	Used in the treatment of bruises, also as an anticancer agent. Contains monocrotaline, reteonecine and platynecic acid
Radix cynoglossi officinalis	*Cynoglossum officinale L.*	Used to clear heat and expectoration, also useful for the treatment of cough, aphonia and rhinorrhagia. Contains heliosupine, lasiocarpine, heliotrine and platyphylline. The concentration is less than 1%
Gynura root	*Gynura segetum (lour.) Merr.*	Used extensively in Chinese folk medicine to promote microcirculation, relieve pain and cure injury. It contains at least five kinds of PAs, such as senecionine, seneciphylline and integerrimine. Many cases of Gynura root-related VOD have been reported
Senecio scandens	*Senecio scandens Buch.-Ham.*	It can clear away the heat-evil and expel superficial evils. In traditional Chinese medicine, it is widely used to treat acute inflammatory diseases, such as bacterial diarrhoea, enteritis. It contains nine hepatotoxic PA (senecionine, seneciphylline, et al.) with a content of 6.95–7.19 mg/g
Flos farfarae	*Tussilago farfara L.*	Used to relieve cough and sputum. Contains tussilagine, isotussilagine, senkirkine and senecionine
Herba tephroseritis kirilowii	*Senecio kirilowii Turcz. ex.*	Used as an antipyretic-detoxicate drug, also a diuretic. Contains senecionine, seneciphylline and integerrimine
Liparis japonica	*DC.* *Liparis japonicus (Miq.) Maxim.*	Used to promote blood circulation to restore menstrual flow, also as a cardiac tonic and a sedative

loop diuretics and spironolactone. Avoidance of other hepatotoxic drugs is as important as the application of drugs that have protective effects on the liver. The role of albumin or other colloids is debatable: it can help maintain the intravascular volume in patients with severe hypoalbuminaemia, but will eventually accumulate in extravascular spaces. Low-dose dopamine is recommended if renal dysfunction appears. Paracentesis can be used to reduce the ascites and relieve symptoms such as abdominal distention and dyspnoea. When fluid overload or renal failure is present, haemodialysis is needed. Mechanical ventilation may be helpful to relieve respiratory failure.[5,9,32,78,79] Transjugular intrahepatic portosystemic shunt (TIPS) has been used to relieve refractory ascites and portal hypertension, but the outcome is poor in many reports.[80,81] The recent review on clinical practice guidelines for TIPS did not recommend TIPS for VOD.[82] Finally, if hepatic failure is imminent, orthotopic liver transplantation is necessary,[83] but a suitable liver donor is seldom readily available.

Since cytokine activation may be involved in the pathogenesis of VOD, inhibitors of cytokines may be useful. As a potent inhibitor of cytokine production, the effect of methylprednisolone is promising. In a report, Khoury *et al.*[49] used high-dose methylprednisolone (a dose of 500 mg/m^2 intravenously every 12 hours for a total of six doses) to treat 20 patients with VOD, where the response rate was 60%. In another report, 48 patients with VOD were treated with methylprednisolone (a dose of 0.5 mg/kg *iv* every 12 hours for a total of 14 doses and then discontinued without taper), 30 patients (63%) responded with a reduction in bilirubin of 50% or more.[84] The regimen was well tolerated with minimal side effects. Despite these encouraging results, randomised controlled trials are required to determine the exact effect of methylprednisolone for the treatment of VOD. Pentoxifylline, a modulator of TNF-α which inhibits the transcription of TNF messenger RNA, was reported to have a protective effect against VOD with no significant adverse effects.[85] Further studies are needed to prove the therapeutic effect of pentoxifylline.

Based upon the histological findings of fibrin deposition and intense factor VIII/VWF staining in VOD, as well as the coagulation abnormalities observed, thrombolytic therapy with or without anticoagulation has been developed. Several reports about the use of t-PA have been published, but most of them are case reports or research studies including no more than ten patients.[86-88] In the largest study so far, 42 cases of VOD were treated with the combination of recombinant human tissue plasminogen activator (rh-tPA) and heparin. The response rate was 29%. Major bleeding occurred in 24% of the patients and 7% of the patients died of it.[89] The data suggested that the therapeutic effect of t-PA was better with earlier application, but once multiorgan dysfunction developed, it was unlikely to be successful. The efficacy of ATIII is controversial: it failed to be effective in a number of studies,[32] while in a trial of 48 patients being treated with ATIII, the overall mortality was decreased with no overt bleeding or thrombosis complications.[90]

One of the most promising agents being used is defibrotide (DF). DF is a large, single-stranded polydeoxyribonucleotide derived from porcine mucosa. It is identified to have antithrombotic, anti-ischaemic, anti-inflammation

and thrombolytic properties without significant systemic anticoagulant effects.[91] The efficacy of DF may due to its ability to increase levels of endogenous prostaglandins (PGI_2 and E_2), stimulate the expression of thrombomodulin, and increase the function of t-PA while decreasing the activity of PAI-1.[92-95] It is well tolerated: adverse events, such as flushing, nausea, and gastrointestinal disturbances, are slight (incidence ranging from 1 to 9%). Chopra *et al.*[96] reported a European multicentre programme of using DF to treat 40 patients with VOD. Overall, 17 out of 40 patients showed complete response to DF (42.5%) with no significant toxicity. Treatment of severe VOD with DF is also encouraging. In a report, 19 patients with severe VOD were treated with DF. The dose ranged from 5 to 60 mg/kg/day, and the improvement of symptoms was seen in eight patients (42%). Six of the eight responders survived 100 days after transplantation, contrasted with the 2% survival reported in comparable patients.[97] A multicentre, phase II trial was conducted to confirm the effect and safety of DF. In the trial, 150 patients with severe VOD and MOF were randomised to 25 mg/kg/day or 40 mg/kg/day of DF. A complete response rate of 46% was observed with 41% survived at day 100 after transplantation and 25 mg/kg/day was the preferred dose.[98] Although many therapeutic modalities with intriguing results have been reported, further multicentre randomised-controlled trials either as individual therapy or in combination with others are needed.

So far, literature about PA-associated VOD is only in the form of case reports, and the available reports do not provide sufficiently reliable data to be used in establishing a health-based guidance. The majority of the large-scale clinical trials on treatment are about VOD after SCT. Nevertheless, similar pathogenesis and limited case reports suggest that these methods may be equally useful for VOD caused by PAs.

CONCLUSIONS

Hepatoxicity of PAs has perplexed human beings for more than 80 years, since senecio poisoning was first described in South Africa.[4] Many studies on animal models and patients with VOD have been done. The use of biological markers may allow an earlier diagnosis while the exact pathogenesis is still unknown. Recent studies suggest that coagulation pathways may play an important role in the pathogenesis of VOD. Clinically, thrombolytic therapy, with or without anticoagulant agents, has been tried with unfavourable results. The bleeding complication is sometimes fatal. DF is a promising therapy, but further studies are needed to determine who to treat, when to treat them and how much to treat them with. As no uniformly effective treatment is present, prevention of this formidable toxicity of PAs is of great importance. And a better understanding of the pathogenesis of VOD is essential if we are to improve the survival rate.

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