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Reversible hepatic veno-occlusive disease in an infant after consumption of pyrrolizidine-containing herbal tea

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Abstract Veno-occlusive disease was diagnosed in an 18-month-old boy who had regularly consumed a herbal tea mixture since the 3rd month of life. The boy developed portal hypertension with severe ascites. Histology of the liver showed centrilobular sinusoidal congestion with perivenular bleeding and parenchymal necrosis without cirrhosis. The tea contained peppermint and what the mother thought was coltsfoot (*Tussilago farfara*). The parents believed the tea aided the healthy development of their child. Pharmacological analysis of the tea compounds revealed high amounts of pyrrolizidine alkaloids. Senecionphylline and the corresponding N-oxide were identified as the major components by thin-layer chromatography, mass spectrometry and NMR spectroscopy. We calculated that the child had consumed at least 60 µg/kg body weight per day of the toxic pyrrolizidine alkaloid mixture over 15 months. Macroscopic and microscopic analysis of the leaf material indicated that *Adenostyles alliariae* (Alpendost) had been erroneously gathered by the parents in place of coltsfoot. The two plants can easily be confused especially after the flowering period. The child was given conservative treatment only and recovered completely within 2 months.

Conclusion In all cases of veno-occlusive disease pyrrolizidine alkaloids ingestion should be excluded. The identity of collected plant material should be verified by pharmaceutically trained experts and information of composition, dosage and mode of administration should be included in guidelines for herbal preparations.

Key words Veno-occlusive disease
Pyrrolizidine alkaloids · Herbal tea

Abbreviations *VOD*-veno-occlusive disease · *FABMS* fast atom bombardment mass spectrometry *NMR* nuclear magnetic resonance

Introduction

Veno-occlusive disease (VOD) of the liver is characterized by portal hypertension with severe ascites due to obliteration of centrilobular or sublobular hepatic veins. It is the most frequent cause of hepatic vein obstruction in children. Hepatic VOD in infants may be caused by hepatic irradiation, chemotherapeutic drugs and bone marrow transplantation; in underdeveloped countries, the most common cause is ingestion of plants that contain hepatotoxic pyrrolizidine alkaloids. Epidemics of pyrrolizidine alkaloid intoxication have been reported from India, Afghanistan and Jamaica [1], whereas only sporadic cases are known from the United States of America, United Kingdom and Europe [4, 14, 18]. In the latter, comfrey products have led to an increased awareness of intoxication due to their widespread use in alternative medicine [4, 14, 15, 23].

The incidence of pyrrolizidine alkaloid poisoning may be grossly underestimated. No method is yet available to measure metabolites in body fluids. Diagnosis can be established in patients with hepatic failure only by demonstration of histological changes pathognomonic for VOD and an exact analysis of the herbal preparation that the patient has been exposed to.

Here we report on an 18-month-old boy from the Southern Tyrol with reversible hepatic VOD as a result of long-term consumption of herbal tea which contained large amounts of seneciphylline. Leaves from *Adenostyles alliariae)* (Alpendost) were mistakenly taken for *Tussilago farfara* (coltsfoot).

Case report

The patient was an 18-month-old, normally developed boy who suffered suddenly from vomiting, diarrhoea, subfebrile temperature and abdominal pain. After 3 days, he was admitted to our hospital in an apathic condition and with a distended abdomen. The liver was palpable 3 cm below the costal margin. The spleen was not enlarged. Laboratory data on admission showed hyponatraemia (128 mval/l), thrombocytopenia (15000), leukocytosis (17700 with normal differentiation), elevated transaminases (ASAT 269, $n = < 23$, ALAT 124, $n = < 28$) which increased until the 5th day after admission (ASAT 1079 mU, ALAT 923 mU), hypoproteinaemia (4.14 mg/dl, $n = 5.8–8.6$ mg/dl), diminished coagulation parameters (PT 55%, n = 80–100%, fibrinogen 55 mg/dl, n = 150–500 mg/dl) and hyperbilirubinaemia (total 2.78 mg/dl, n = 0.3–1.0, direct 1.48, $n = 0.1$). Glucose, urea, creatinine, ammonia, α_1-antitrypsin, caeruloplasmin, CRP and serological markers for viral hepatitis or other infections were all normal.

Ultrasound studies including Doppler sonography revealed severe ascites and a swollen liver with slit-like hepatic veins and compression of the vena cava by the enlarged caudate lobe. Hepatic and portal vein flow velocities were greatly reduced but normal in direction.

MRI showed a patchy inhomogeneous pattern of the liver parenchyma in T1-weighted spin echo sequences which was even more pronounced on T2 weighting. The patency of the inferior vena cava was proven by a positive flow void sign. The markedly narrowed intrahepatic veins also showed a positive flow void sign which could be traced up to the periphery of the vessels (Fig. 1a). The portal vein was of normal calibre and patency in its extrahepatic portion and could be followed up to the intrahepatic bifurcation. Both main branches and second order branches were markedly enlarged in the periportal area with low signal intensity as compared to the surrounding liver tissue (Fig. 1b). These periportal signal reductions had a hyperintense appearance in T2-weighted sequences and thus might represent areas of oedematous parenchymal changes.

Histological examination of a thin-needle biopsy specimen revealed a preserved lobular architecture of the liver parenchyma. A small number of portal tracts was innocuous with an intact parenchymal limiting plate. The liver cell plates, however, were severely distorted by massive haemorrhagic congestion affecting zones II and III of the liver acinus in some places almost extending to zone I (Fig. 2). Central veins were no longer discernible and reticulin staining could not display occlusion of the terminal hepatic vessels. Despite the lack of a clear-cut vascular occlusion within the small biopsy, the diagnosis of acute VOD was established on the basis of the severe parenchymal changes.

Therapy consisted of sodium and fluid restriction combined with high doses of spironolactone and furosemide. Paracentesis was performed three times, removing about 500 ml each time. The ascites fluid was amber coloured, clear and contained 3 mg/dl protein and leukocytes < 300. Cultures were negative. The ascites gradually disappeared under therapy. After 6 weeks of therapy, spontaneous remission of the symptoms and signs of portal hypertension occurred. The child subsequently made a complete recovery, both clinically and in terms of ultrasound findings.

After the diagnosis of VOD had been established, in-depth investigation of the nutritional habits of the family revealed that the child had been exposed to significant amounts of pyrrolizidine alkaloids. Since the 3rd month of life, the boy had drunk up to 500

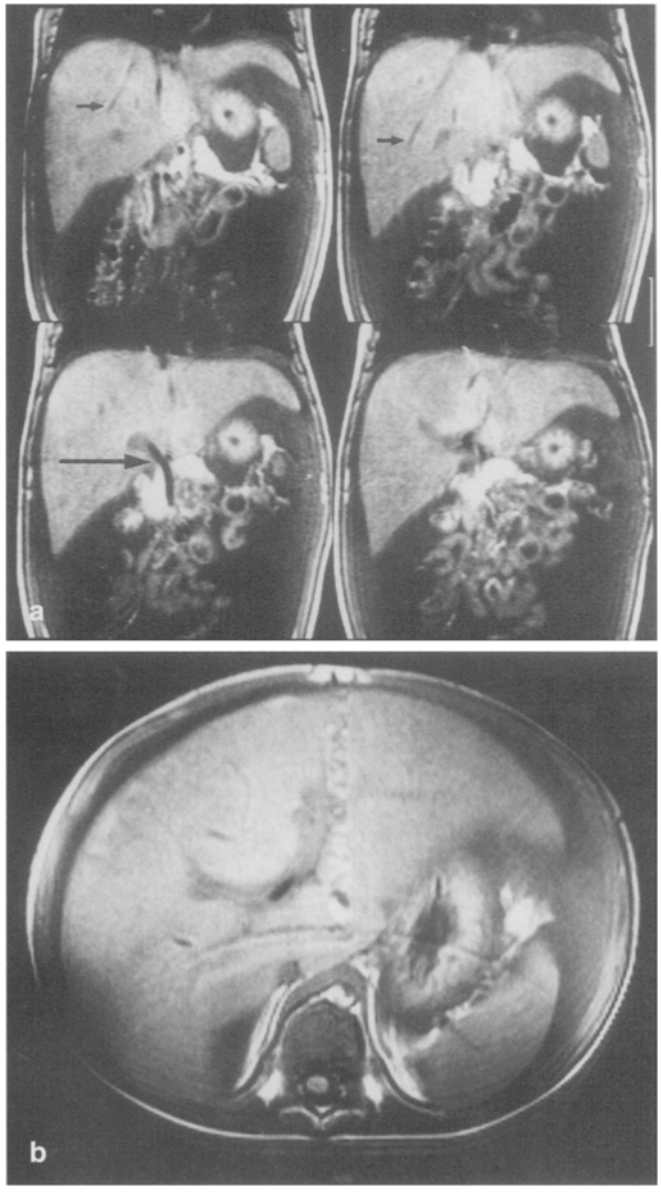

Fig. 1 **a** Coronal T1-weighted MRI (TR = 650, TE = 15) with 5 mm slice thickness: marked inhomogeneity of liver parenchyma. Liver veins *(short arrow)*, mesenteric vein and portal vein *(long arrow)* show signal void indicating blood flow. The hepatic veins are narrowed. A large amount of ascites is present. **b** Axial T1-weighted MRI (TR = 650, TE = 15) with 8 mm slice thickness: enlargement of the periportal area indicating perivascular oedema

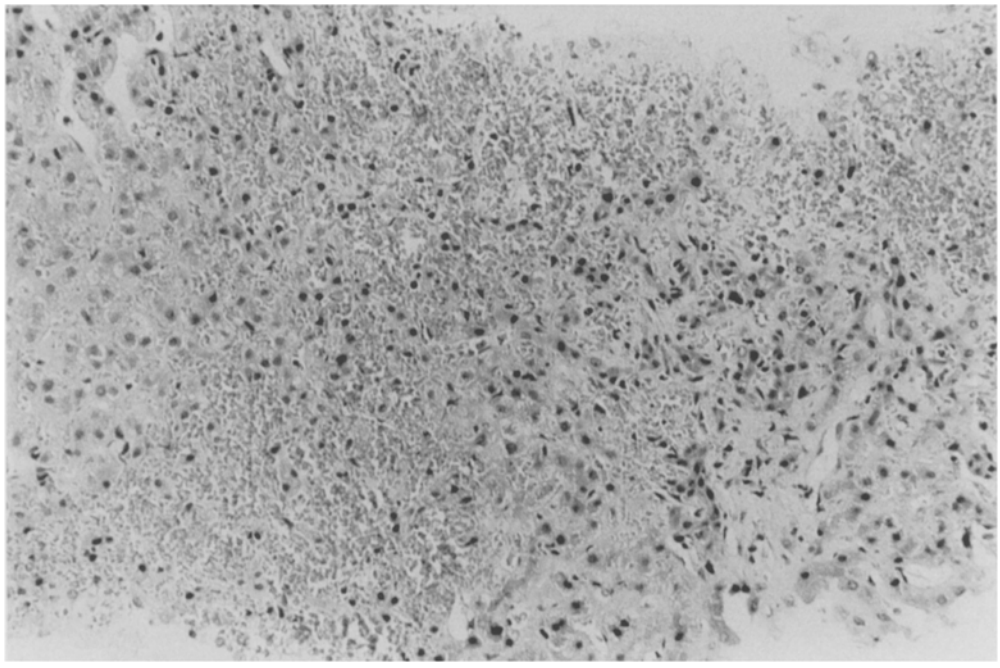

Fig. 2 Liver biopsy specimen with partial destruction of acinar zone II and III and atrophy of hepatocytes. There are only a few unaffected remnants of liver tissue in the left upper corner and on the right bottom side close to the edge of a portal tract. (Haematoxylin & Eosin, 100×)

ml of a home-made herbal tea daily. The tea was prepared by his mother from plants grown in her own garden or gathered in the local mountains. She used a pinch of peppermint leaves and what she thought was Tussilago farfara, boiling them for 5–10 min in 500 ml water.

Materials and methods

Leaf material was collected at the end of June 1990 and 1993 in Villnößtal (South Tyrol). The air-dried powdered plant material (55 g) was extracted in a Soxhlet apparatus with methanol. The obtained extract was subjected to alkaloid enrichment performed according to Lüthy et al. [6]. The crude alkaloid fraction was subjected to silica gel column chromatography using mixtures of dichloromethane and methanol as mobile phases. Fractions containing seneciphylline and the corresponding N-oxide, respectively, were combined and the solvent evaporated. The structures of seneciphylline and the N-oxide were elucidated by fast atom bombardment mass spectrometry (Kratos MS RFA) and NMR spectroscopy (Bruker AM-300). In additon, seneciphylline was identified by gas chromatography/mass spectrometry (Hewlett Packard) as well as by co-chromatography (thin-layer and gas chromatography) with an authentic sample. Thin-layer chromatography was performed on silica gel plates using dichloromethane/methanol/NH_3 (85:14:2) as mobile phase. Dragendorff and Dan-Mattocks reagents were used for alkaloid detection [7].

Results

Macroscopic and microscopic analyses of the herbs used for the tea preparation revealed the presence of *Adenostyles alliariae* (Alpendost). Methanolic extraction of the corresponding leaves, followed by alkaloid enrichment yielded 300 mg of a crude alkaloid mixture. Thin-layer chromatography (Fig. 3) of the extract obtained showed

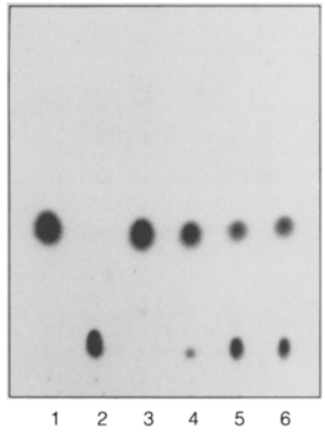

Fig. 3 Thin-layer chromatography of pyrrolizidine alkaloid mixture *(lane 5 and 6)* from *Adenostyles alliariae* compared to seneciphylline *(lane 1 and 3)*, the corresponding N-oxide *(lane 2)* and an alkaloid mixture obtained from a simple tea preparation *(lane 4)*

reactions with both Dragendorff and Dann-Mattocks reagents, suggesting the presence of pyrrolizidine alkaloids. Column chromatography of the alkaloid fraction yielded two major compounds which were identified as senecipylline (0.16%) and the corresponding N-oxide (0.33%) by mass and NMR spectroscopy (Fig. 4).

Discussion

After diagnosis of VOD had been established and the common causes of VOD in children excluded, careful evaluation of nutritional habits of the family revealed that the child had consumed large amounts of herbal tea. To our knowledge, this is the first proven case of pyrrolizidine alkaloid intoxication caused by long-term herbal tea consumption involving confusion of *Tussilago*

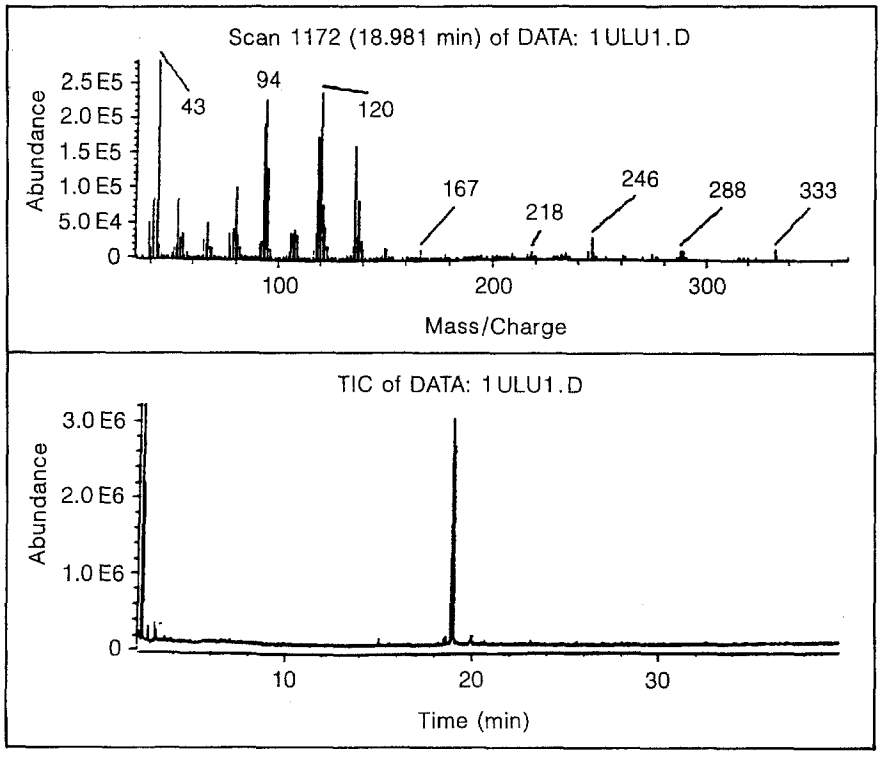

Fig. 4 GC-MS analysis of seneciphylline from the leaves of Adenostyles alliariae (Conditions: column 30 m RTx1 0.25 mm ID, 0.25 µ; T-Progr.: 180°, 1 min; 3°/min → 280° isotherm; transferline: 280°; acquisition: scan 33–400 amu 1.17 scans/s; 2 µl Inj)

farfara (coltsfoot) with *Adenostyles alliariae*. Since nearly quantitative extraction of the leaves during tea preparation can be assumed [5], (0.2 g of the drug in 500 ml water, boiled for 15 min) the child could have ingested at least 0.6 mg of the toxic alkaloid mixture daily (60 µg/kg/day), an amount far in excess of the dose considered to be safe [9, 15, 22]. In Western countries and especially in Central Europe, coltsfoot is widely recommended in natural medicine as a herbal remedy against cough. It contains pyrrolizidine alkaloids, although to a much smaller extent than *Adenostyles alliariae*. The possibility of adulteration of coltsfoot has been discussed intensively [13, 16, 18, 20, 21] because identification of leaf material after the flowering period can be difficult, even for experts [16]. A case of VOD in a newborn infant was recently reported from Switzerland [18]. The mother had consumed herbal tea containing *Tussilago farfara* during pregnancy. In a comment responding to this article, Röder assumed [16] that the herb containing pyrrolizidine alkaloids was not tussilago but petasites, because the typical leading alkaloid of tussilago, senkirkin, was not found by the authors. He argued that leaves of petasites had most probably been confused with tussilago because both plants grow wild in alpine regions and can be easily confused when gathered after the blossom period. This was confirmed later by Spang [21]. In our case, *Adenostyles alliariae*, another plant with similar leaves and a distribution within the alpine region comparable to coltsfoot, has been erroneously used as a herbal remedy. In the traditional folk medicine of the alpine region, leaves from this plant, which is also named *"Folia Cacaliae alpinae"* (Alpendost), were recommended as a remedy for cough [19]. The alkaloid composition of this plant has only recently been identified by Röder et al. [17]. Our finding of high amounts of seneciphylline and the corresponding N-oxide in *Adenostyles alliariae* is in agreement with their results.

There has been an increasing trend towards natural healing methods in Western countries in the last few decades, and a growing number of cases of pyrrolizidine alkaloid intoxication can be expected to occur. At least 26 herbal teas are known to contain toxic ingredients [12]. The tea mixture taken by our patient was considered by the parents to be harmless and was believed to support the healthy development of the child. Unfortunately the boy became very used to it and consumed quite high amounts daily. Only the exact identification of pyrrolizidine alkaloids in the herbal tea as the cause of VOD allowed us to avoid further exposure of the child to the toxic tea components. This was crucial for the long-term outcome of the patient. Other family members were not affected because they were only consuming the tea in smaller amounts and not on a regular basis.

The pathogenesis of VOD due to alkaloid intoxication and the reason for the predominance of children remains unclear [4, 18, 22]. There are at least three conditions essential for hepatotoxicity of pyrrolizidine alkaloids [8]: the double bond of the 1,2-unsaturated necine base, esterification of the hydroxy group in positions 9 and 7, and a branched carbon chain in at least one of the ester side chains. Hepatic mixed-functional oxidases transform pyr-

rolizidines to pyrrole derivatives which are highly reactive alkylating agents and form covalent linkages to proteins, RNA and DNA. The majority of pyrrole intermediates are trapped in the liver shortly after their formation [2, 22]. Of patients with pyrrolizidine ingestion and VOD, 50% undergo spontaneous remission [10], as also observed in our patient.

We conclude that a thorough history must be taken in all cases of VOD in order to exclude the possibility of pyrrolizidine alkaloid ingestion. Because of the risk of intoxication or adverse effects, the composition, dosage and mode of administration of herbal products should be monitored strictly by pharmaceutically trained experts [3, 4, 11]. This information should be included in guidelines for herbal preparations.

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