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CASE REPORT

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Fatal cerebellar haemorrhage due to phenprocoumon poisoning

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Abstract A 32-year-old patient died of a cerebellar haemorrhage and the blood coagulation analysis before death suggested defective synthesis of vitamin K-dependent clotting factors due to vitamin K deficiency. The post-mortem toxicological examination of different tissues revealed phenprocoumon poisoning as the cause of death. The differential diagnosis of vitamin K deficiency and the toxicology of hydroxycoumarins are discussed.

Key words Fatal haemorrhage · Vitamin K deficiency · Hydroxycoumarins · Warfarin · Phenprocoumon

Introduction

Criminal poisoning with medical drugs represents a serious challenge for specialists in forensic medicine. This is especially true for drugs in which the repeated administration of small doses leads to a considerable increase in toxicity and where symptoms only occur with an overdose. One such example is the group of hydroxycoumarins, which can cause serious bleeding after a few days of regular ingestion. Although these drugs are widely available and have been used in medical practice since the end of the 1940s, very few cases of hydroxycoumarin poisoning with fatal outcome have been described in the literature. In most described cases unexplained episodes of nose bleeding or gastrointestinal haemorrhages led to the discovery of the crime, preventing a fatal outcome. We describe the case of a young patient who died of a cerebellar haemorrhage and in whom phenprocoumon poisoning was only detected after death.

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Case report

A 32-year-old man collapsed into unconsciousness at work and after resuscitation he was transferred to an intensive care unit. Physical examination showed a comatose patient with multiple bruises on the left arm and both calves. The pupils were dilated and unreactive, there were no reactions to pain or other stimuli and brain stem reflexes were absent. A computer tomography scan of the head revealed a large haemorrhage in the left cerebellum and hypodensity of the brain stem, suggesting ischaemia of this area. Laboratory results showed a normal platelet count of 309/nl. A thromboelastogram was normal. Prothrombin time was 10% (normal 70–100%), while activated partial thromboplastin and thrombin times were normal. Detailed analysis of the clotting factors (normal 70–100%) showed factor II 25%, factor VII 20%, factor VIII 160%, factor V 96% and factor X 25%. Liver function tests were normal with AST 12 U/l (normal < 18 U/l), ALT 12 U/l (normal < 18 U/l), γ-GT 11 U/l (normal < 20 U/l), AP 115 U/l (normal < 150 U/l), bilirubin 0.5 mg/dl (normal < 0.8 mg/dl) and a cholinesterase level of 6.7 kU/l (normal 4.5–6.8 kU/l). According to information obtained from the patient's wife he had recently suffered from recurrent nose bleeds. A clotting test performed 1 year before admission had been normal. There was no history of high blood pressure, liver disease or alcohol abuse. He had occasionally taken Aspirin because of headaches, but he did not take oral anticoagulants.

Two days after admission brain death was diagnosed and life-sustaining therapy was stopped. A post-mortem examination showed superficial haemorrhages to the left arm and both legs. A large haemorrhage had destroyed the entire left cerebellar hemisphere and had ruptured into the subdural and subarachnoideal spaces. The liver weight was 840 g. Histological examination of the liver showed steatosis hepatis and a number of von Meyenburg complexes. Lobe architecture was normal but portal tracts were slightly enlarged. There were no signs of cirrhosis.

Post-mortem specimens of stomach content and venous blood, heart blood and tissues (liver, kidney, lungs) were investigated for anticoagulants and other drugs by means of thin-layer chromatography (TLC), high-pressure liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS).

Materials and methods

Materials

Acetonitrile and water were HPLC grade, all other chemicals and reagents were analytical grade and purchased from Merck (Darmstadt, Germany). Phenprocoumon was kindly donated from Hoffmann-La Roche (Grenzach-Wyhlen, Germany).

Instrumentation

High-pressure liquid chromatography

The chromatographic determinations were carried out using a HP 1050 series liquid chromatograph (Hewlett Packard. Waldbronn, Germany) with quaterny pump, autosampler, and diode array detector. A Vectra Workstation with HPChemstation software and a Lichrospher 60 RP-Select B ECOCART column (125 × 3 mm i.d.) (Merck, Darmstadt, Germany) was used. Drug screening was performed using an eluent consisting of acetonitrile and 0.05 M KH_2PO_4, pH 2.3 buffer (31:69, v/v) with a flow rate of 0.5 ml/min. The software used for identification was equipped with a special library containing the UV spectra and retention times of 285 different substances (benzodiazepines, barbiturates, antidepressants, anticoagulants, analgesics, β-blockers and others). For phenoprocoumon quantification the column was eluted in an isocratic mode with acetonitrile-water, 48.5:51.5 (v/v), at a flow rate of 0.5 ml/min and a temperature of 35° C. The wavelength of the maximum with highest absorbance (285 nm) was used for quantification. Under these operating conditions the limit of detection for phenprocoumon was about 100 ng/ml.

Gas chromatography/mass spectrometry

GC/MS analysis was performed with an 8600 Perkin Elmer gas chromatograph (Perkin Elmer, Überlingen, Germany) equipped with a Permaphase DMS (25 m × 0.32 mm i.d.) capillary column and an ion trap detector (ITD 800), operated in the electron-impact mode (70 eV) and full scan mode. The flow of carrier gas (helium) was 2 ml/min. the injection temperature was 280° C and the gas chromatograph was temperature-programmed from 100° C (2 min hold) to 280° C at 30° C/min (11 min hold). Drug screening was done by visual and computerized comparison of the peak underlying full mass spectra with reference spectra (Fig. 1).

General drug screening

Aliquots of heart blood and stomach contents were extracted using Extrelut columns (Merck, Darmstadt, Germany) and standard routine procedures. Acid and neutral compounds were separated from basic compounds by subsequent elution at pH 2 and pH 10 with diethylether and dichloromethane/isopropanol (85:15). The organic phases were evaporated to dryness and reconstituted with 0.5 ml methanol. Aliquots of the extracts were analysed by means of TLC, HPLC and GC/MS.

Phenprocoumon quantification

Tissue samples (20 g) were homogenized using a blade type homogenizer (Ultra turrax) after addition of 20 ml water. Aliquots of 1 ml of blood, tissue homogenates and stomach contents were mixed with 5 ml 2 M HCl and 14 ml water. Phenprocoumon was extracted with 50 ml diethylether using Extrelut columns. The eluates were evaporated to dryness and reconstituted with 0.5 ml of the HPLC eluent (acetonitrile-water, 48.5:51.5). From each of these extracts 0.01 ml was injected into the HPLC. Quantification was achieved for each sample by means of external standard calibration with spiked blood samples. The extraction recovery for phenprocoumon under these operating conditions was about 60%. The reproducibility in terms of coefficients of variations was about 7% for blood and about 20% for tissue samples.

Results and discussion

Fatal cerebral haemorrhages in young patients most frequently occur because of ruptured berry aneurysms. However, in this case the atypical location of the haemorrhage (aneurysms rarely occur in the area supplied by the basilar artery and its branches) and the clotting analysis suggested a coagulation defect as the underlying disease. The extremely decreased prothrombin time of 10% was suggestive of defective clotting factors II, V, VII or X or the presence of an inhibitor of these factors. The detailed analysis of the clotting factors could exclude severe liver disease with factor V as the most sensitive parameter of liver synthesis function being 96%. In contrast vitamin K-dependent clotting factors II, VII and X were markedly decreased.

Table 1 Phenprocoumon concentrations in various specimens from the patient

Specimen	Phenprocoumon (mg/kg)
Venous blood	1.8
Heart blood	2.8
Stomach contents	0.7
Lung	3.2
Liver	3.5
Kidney	1.5

There are four major causes of vitamin K deficiency in adults (Shearer 1995): inadequate dietary intake; intestinal malabsorption; loss of storage sites due to hepatocellular disease, and ingestion of vitamin K antagonists (e.g. hydroxycoumarins, such as phenprocoumon or warfarin).

Vitamin K is widely distributed in nature and contained in variable quantities in such different foods as green leafy vegetables, cereals, fish and meat (Shearer 1995). A considerable contribution to body vitamin K stores is probably made by microfloral synthesis, especially in the terminal ileum (Shearer 1995). For these reasons vitamin K deficiency in adults has only been observed in patients with malabsorption of bile salts and patients undergoing broad-spectrum antibiotic therapy. In the case described there was no history of intestinal malabsorption or any antibiotic treatment. As he had been healthy before the incident and liver function tests were normal, ingestion of vitamin K antagonists was the most likely diagnosis. Postmortem toxicological analysis revealed the following phenprocoumon concentrations (Table 1).

Specimens were analysed 7 days after the admission of the patient to the intensive care unit and 4 days after death. During the 3 days in hospital he had not received any hydroxycoumarins, which suggests that on the day of admission the phenprocoumon concentrations had probably been considerably higher. In clinical practice anticoagulation with hydroxycoumarins is monitored by measuring the prothrombin time (PT). The patient's PT of 10% on the day of admission suggested an overdose, as therapeutic inhibition of coagulation (PT 25–35%) is usually achieved with phenprocoumon concentrations of 1–3 mg/kg).

Since the introduction of hydroxycoumarins into antithrombotic therapy at the end of the 1940s and the extension of their application to use as rodenticides in the

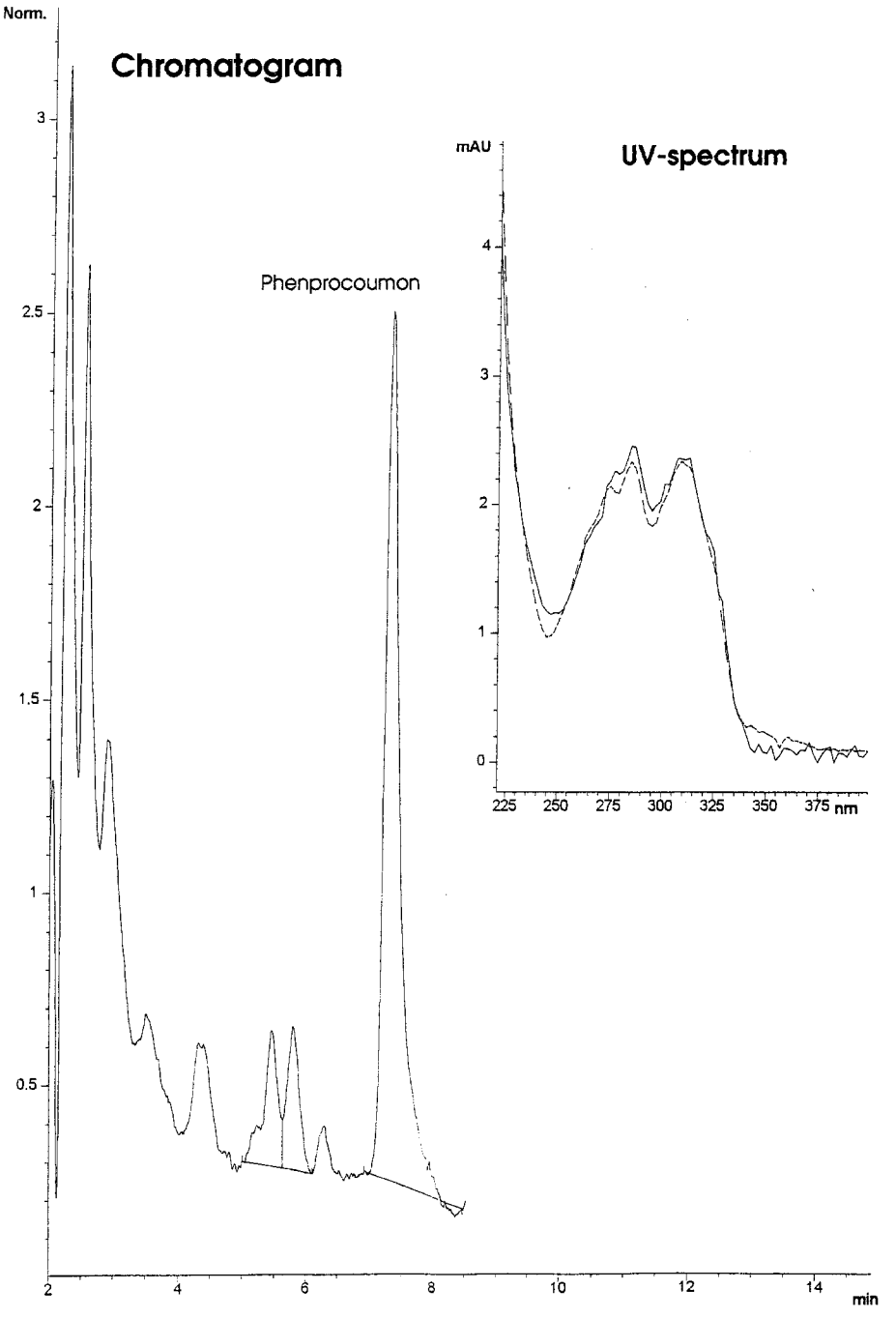

Fig. 1 Chromatogram (HPLC) of venous blood with UV spectrum of phenprocoumon. - - - - patient —— phenprocoumon reference sample

early 1950s , a number of papers have described accidental intoxications (Stark et al. 1971; Held et al. 1980; Bodemann et al. 1988), suicide attempts (Stafne and Moe 1951; Holmes and Love 1952; Richert et al. 1984; Blume 1990) and criminal poisoning (Ikkala et al. 1964; Pribilla 1966; Schneider et al. 1975) with these drugs. Coumarin anticoagulants prevent the reduction of vitamin K epoxides in the liver microsomes and induce a state analogous to vitamin K deficiency. In very rare cases they may induce hepatitis (Höhler et al. 1994) or even liver failure (Stark et al. 1971).

Phenprocoumon is a colourless and tasteless white powder, as are other hydroxycoumarins. It is almost completely absorbed after oral administration (Moffat et al. 1986). The protein binding in the plasma is more than 99% and plasma half-life 3–9 days (mean 5 days) (Moffat et al. 1986). The volume of distribution is 0.1–0.2 l/kg (Moffat et al. 1986). In humans large single ingestions rarely lead to haemorrhages (Brewer and Hagerty 1957; Goulding 1968). The acute oral single-dose LD_{50} for warfarin in male rats is 100 mg/kg, with the highest mortality rates between 4 and 10 days after treatment (Back et al. 1978). Correspondingly, an average adult male of 70 kg would have to ingest 7 g of warfarin, corresponding to 1400 tablets (@ 5 mg) or 1400 g of a rodenticide containing 0.5% of warfarin, making hydroxycoumarins unsuit-

able for successful suicide attempts. However, in clinical practice effective anticoagulation is usually achieved after 1 week with as little as 5–10 mg/day of warfarin or equivalent (Handin 1991), demonstrating that a substantial increase in effect is achieved due to repeated administration. This effect has been used by a number of persons coming mostly from a medically trained environment, in attempts at murder (Schneider et al. 1975).

Poisoning initially is without symptoms in the victims. Only haemorrhages may indicate inhibited coagulation, but as in the described case symptoms such as repeated nose bleeding may not be taken seriously. Aspirin, which the patient in the described case had been taking for headaches before the incident, probably increased the phenprocoumon effect due to its inhibitory action on platelet aggregation leading to the cerebellar haemorhage.

Our case report underlines the high degree of suspicion necessary in people who die of haemorrhages of unclear origin. If there are any doubts on clinical grounds, modern toxicology and HPLC make the identidication of hydroxycoumarins in blood or tissues a relatively easy task.

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