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A Case of Fatal Poisoning by Rifampicin

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Zusammenfassung. Es wird über die Ergebnisse der Obduktion und der toxikologischen Untersuchung einer tödlichen Vergiftung mit Rifampicin berichtet. Die Konzentration von Rifampicin und seinen beiden Hauptmetaboliten 25-Desacetylrifampicin und 3-Formylrifamycin wurde mittels Hochdruckflüssigchromatographie in Blut, Harn, Gallenflüssigkeit und Leber bestimmt.

Der Tod war etwa 10 Std nach Einnahme von 14–15 g Rifampicin eingetreten. Die Ergebnisse der toxikologischen Analysen werden mit den Ergebnissen in anderen tödlichen und nichttödlichen Vergiftungen sowie mit den Ergebnissen nach therapeutischer Applikation des Arzneimittels verglichen. Für den tödlichen Ausgang wird eine mögliche Erklärung gegeben.

Abstract. The toxicologic findings of a fatal poisoning with rifampicin (Rimactan) are presented. The concentration of rifampicin and its two major metabolites 25-desacetylrifampicin and 3-formylrifamycin in post-mortem blood, urine, bile and liver at about 10 h after ingestion of 14–15 g was determined using a high-performance liquid chromatographic method. The results of the toxicological analyses were compared with findings in fatal and non-fatal intoxications and after therapeutic administration of the drug. Possible explanation for the fatal outcome is given.

Key words: Rifampicin – Intoxication – Rifampicin and metabolites in post mortem material

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Introduction

Rifampicin is a semisynthetic broad-spectrum antibiotic belonging to the group of rifamycins. It is widely used for the treatment of pulmonary tuberculosis and in the elimination of meningococci from carriers. Furthermore the antibiotic is of therapeutic value in the treatment of leprosy and gonorrhoea (Goodman and Gilman 1975; Walter and Heilmeyer 1975; Wade 1977). The drug is administered orally in daily doses varying from 300–1,200 mg. After oral administration rifampicin is readily absorbed from the gastrointestinal tract and maximum serum concentrations of about 9.8 μg/ml have been reported 2 h after a dose of 450 mg and 16.1 μg/ml after a dose of 900 mg (Walter and Heilmeyer 1975). About 75–80% of the antibiotic is bound to serum proteins. It is distributed throughout the body and is present in effective concentrations in many organs and body fluids, including the cerebrospinal fluid. Rifampicin is mainly metabolized to active desacetylrifampicin and is excreted in the bile and to a lesser extent in the urine (Walter and Heilmeyer 1975). It has a biological half-life varying from 1.5–5 h (Goodman and Gilman 1975; Walter and Heilmeyer 1975). The antibiotic is usually well tolerated and does not cause untoward effects with great frequency. Adverse effects attributed to rifampicin include gastrointestinal disturbances, hypersensitivity reactions, liver function abnormalities, influenza-like syndrome, hematological disorders and neurotoxic symptoms (Walter and Heilmeyer 1975; Wade 1977). Overdosage with rifampicin is an unusual form of self-poisoning. So far five suicidal intoxications, including two cases with a fatal outcome have been reported (Konietzko and Burkhardt 1971; Newton and Forrest 1975; Broadwell et al. 1978; Jack et al. 1978; Meisel and Brower 1980). In this paper for the first time after fatal poisoning with rifampicin the concentration of the compound and its major metabolites in post mortem blood, urine, bile and liver will be presented.

Materials and Methods

Case History

A 28-year-old labourer, known to be depressed and to be drinking heavily, was found dead at 11.20 a.m. in bed. He already had tried to commit suicide several times during the previous years. The decendent had last been seen alive at 6.00 a.m. that same morning by his roommate.

Between 4.00 and 6.00 a.m. he had been vomiting several times and his companion already observed a faint yellow discolouration of the skin, but at that moment he did not pay any attention. A called physician estimated the time of death to have been between 9.00 and 11.00 a.m. This physician also observed an orange-yellow discolouration of the skin, staining the sheets with sweat and urine. Because of this icterus an acute infectious or toxipathic hepatitis was suspected. However, examination of the room, disclosed that of the roommates medicines 24 or 25 of 600 mg Rimactan tablets were missing, implying that the victim had obviously ingested about 14–15 g of rifampicin. According to his companion the ingestion must have taken place at about 1.00 a.m., so the presumed time interval between the intake of the drug and the death of the patient was minimal 8 h and maximal 10 h. Autopsy was performed the next day at 18.00 p.m.

Autopsy

The decendent was a 28-year-old male, 193 cm tall, weighing 70 kg. Examination of the external surface of the body revealed all over the body an intense orange discolouration of the skin and no marks of trauma. In addition, a marked orange-yellow discolouration of the mucous membranes, especially of the gastrointestinal tract, internal organs and body fluids was observed. The autopsy failed to reveal any gross abnormalities in the organs.

Microscopic pathological examination of the lungs showed a severe acute toxic edema, which was the cause of death of the victim. Brains and kidneys were congested and edematously swollen. Histologic examination of the liver parenchym showed some orange discolouration of the content of the cells indicating cristallization of some rifampicin within the liver cells. Apart from this the liver showed blood congestion and a massive cloudy swelling with liver cells containing little fatty droplets. Blood, urine, bile and liver were collected for toxicological analysis. The concentration of alcohol in blood and urine was 1.1 g/l and 1.4 g/l respectively.

Toxicological Analysis

The concentration of rifampicin and its metabolites 25-desacetylrifampicin and 3-formylrifamycin SV in post mortem blood, urine, bile and liver was determined by a modification of the high-performance liquid chromatographic method as described by Lecaillon et al. (1978). To 0.50 ml of blood, 0.10 ml of urine or bile diluted with 0.4 ml of distilled water, in a 15-ml glass centrifuge tube were added 50 µl of methanol, 1 ml of a buffer solution consisting of 2 g ascorbic acid and 10 g anhydrous sodium sulphate in 50 ml concentrated Titrisol buffer pH 6 (Merck Cat. No. 9886) and 1 ml of isooctane-dichloromethane (3 : 2, v/v). The tube was stoppered and thoroughly mixed for 15 min on a Vortex mixer and then centrifuged for 5 min at 2,000 *g*. From the supernatant organic phase 25 µl were injected into the chromatographic system. Liver tissue was finely minced, dried between Kleenex tissues, and portions of about 250 mg of the dried minced tissue were grinded in small mortars with 0.5 g of anhydrous sodium sulphate.

The consecutive mixtures were transferred to glass centrifuge tubes and then processed like blood, urine and bile. All samples were assayed in triplicate and the concentration of rifampicin and its two metabolites in the biological specimens were determined by separate analyses. Chromatography was performed on a Waters Associates chromatographic system consisted of a Model 6000 A solvent delivery pump, a U6K valve injector, a radial compression separation unit and a model 450 variable wavelength detector. The radial compression separation unit consisted of a 10 cm × 8 mm I.D. Radial-PAK B (10 µm silica) cartridge and a Model RCM-100 Module for compressing of the cartridges. The mobile phase, dichloromethane-isooctane-ethanol-0.12% acetic acid (36.6 : 45 : 16.8 : 1.4) was pumped at a constant flow-rate of 3.5 ml/min under a pressure of about 1,000 psi at room temperature.

Rifampicin, 25-desacetylrifampicin and 3-formylrifamycin were monitored at 254 nm and under the described conditions they showed retention times of about 4.5 min, 11.1 min, and 2.4 min respectively. Calibration samples were prepared by measuring volumes ranging from 100 µl to 1.0 ml of a 100 µg/ml dichloromethane solution of rifampicin, desacetylrifampicin and formylrifamycin into 15-ml glass tubes. For the calibration of the latter drug in urine and bile volumes ranging from 10–50 µl were used. Dichloromethane was evaporated under nitrogen, the compounds were redissolved in 50 µl of methanol and drug-free blood (0.50 ml), urine (0.10 ml), bile (0.10 ml) and liver tissue (250 mg) were added. Subsequent analyses were carried out as described above. Calibration curves were constructed by plotting the peak area of the standard versus the concentration and were used to calculate the amount of rifampicin and its two metabolites in the unknown samples. For the analysis of rifampicin in blood 3-formylrifamycin (40 µg) was used as internal standard.

Results

The post-mortem blood, urine, bile and liver levels of rifampicin, 25-desacetylrifampicin and 3-formylrifamycin found 8–10 h after oral ingestion of about

Table 1. Post-mortem concentration of rifampicin, 25-desacetylrifampicin and 3-formylrifamycin in blood, urine, bile, and liver in a fatal case of rifampicin overdosage

	Blood (μg/ml)	Urine (μg/ml)	Bile (μg/ml)	Liver (μg/g)
Rifampicin	55 ± 2	475 ± 22	313 ± 2	373 ± 75
3-Formylrifamycin SV	–	31 ± 4	13 ± 3	225 ± 60
25-Desacetylrifampicin	–	796 ± 29	–	268 ± 18

(–) No drug detected

15 g of rifampicin are given in Table 1. Rifampicin was present in high concentrations in all biological specimens analyzed. The highest level was observed in urine, followed by liver and bile. The urine/blood, bile/blood and liver/blood concentration ratios were 8.6, 5.7, and 6.8 respectively.

Desacetylrifampicin, the main metabolite of rifampicin (Maggi et al. 1969), was found in considerable amounts in urine and liver, however, in bile it could not be detected. Formylrifamycin, a second major metabolite, (Winsel et al. 1976) was present in low concentrations in urine and bile and showed relatively high levels in the liver. Other metabolites known, such as 3-formyl-25-desacetylrifamycin and N-desmethylrifampicin (Winsel et al. 1976), were not detectable in the biological specimens investigated. Of the total amount of rifampicin found in urine 35% was present as the parent compound, 62% as the desacetyl metabolite and 3% as the formyl metabolite. In bile 95% was present as the parent compound and 5% as the formyl metabolite, while in liver 41% was present as rifampicin, 28% as the formyl metabolite and 31% as the desacetyl metabolite.

Discussion

The methods usually applied for the determination of rifampicin in biological material are microbiological assays, spectrophotometric procedures and high-performance liquid chromatography (Furesz et al. 1969; Lecaillon et al. 1978; Maggi et al. 1969; Murray et al. 1975). The major disadvantage of the microbiological and most of the spectrophotometrical methodology is that they do not permit the separate determination of rifampicin and its metabolites. For the simultaneous and specific determination of rifampicin and its metabolites a high-performance liquid chromatographic method is most suitable. Using the described method rifampicin and its major metabolites were detectable down to 0.1 μg/ml of serum, urine or bile and 0.2 μg/g of organ. Overdosage of rifampicin is an unusual form of self-poisoning. Up to now, two fatal and three non-fatal cases of rifampicin poisoning have been reported. In 1971, Konietzko and Burkhardt, described an unsuccessful suicidal attempt with 9 g rifampicin of a patient treated for pulmonary tuberculosis for 3 months with isoniazid and rifampicin. In 1975 Newton and Forrest reported a non-fatal case of self-poisoning with 12 g rifampicin, involving a tuberculous patient receiving

during the previous year a treatment with rifampicin, ethambutol and isoniazid. In 1980, Meisel and Brower reported a case with a non-fatal outcome of a 15-year-old girl who had ingested an unknown quantity of rifampicin in a suicidal gesture. In all cases an orange to reddish discolouration of the skin ("red man syndrome"), the mucous membranes, the scleras, the urine, plasma and feces were observed. Other toxic symptoms reported were generalized pruritis, facial edema and cholestasis.

In addition, some liver function tests (plasma bilirubin, alkaline phosphatase, SGOT) were elevated within the first 48 h after ingestion, but these values returned to normal over the next 2 days.

In the case reported by Newton and Forrest (1975) also the total concentration of rifampicin and its active metabolites in plasma and urine were measured using a microbiological assay. The level in plasma at 12 h and in urine at 30 h after ingestion was 400 μg/ml and 313 μg/ml respectively. Fatal rifampicin intoxications were reported by Broadwell et al. (1978) and Jack et al. (1978). The first authors reported a case of deliberate ingestion of 60 g of rifampicin by a mentally retarded man who had a 10 year history of moderate alcohol abuse. The patient developed the above-mentioned symptoms of rifampicin intoxication and about 48 h after ingestion he suddenly died after a brief, generalized seizure attack. The fatal rifampicin-ethambutol overdosage as reported by Jack et al. (1978) involved a tuberculous patient who took an unknown amount of rifampicin and ethambutol and died shortly thereafter. Post-mortem blood levels of rifampicin and ethambutol were 182 μg/ml and 84 μg/ml respectively. In urine levels of 3,300 μg/ml and 6,800 μg/ml were observed for the respective drugs. In addition low blood and urine alcohol levels (0.2 g/l) were found. The case described here involved an acute fatal poisoning with rifampicin of a man who was known to be a heavy drinker. He had no history of pulmonary tuberculosis and never received any rifampicin medication. The observed blood level of 55 μg/ml at about 10 h after intake (Table 1) is about seven times lower than the concentration found at 12 h after ingestion of 12 g rifampicin (Newton and Forrest 1975). This marked difference may be explained by the fact that in the latter case the total concentration of rifampicin and its metabolites was measured in a patient on long-term treatment and by large variation in individual blood levels after administration of a single dose (Walter and Heilmeyer 1975). After administration of a single oral dose of 900 mg to healthy volunteers blood levels at 8 h and 12 h ranged from 7.6–24 μg/ml and from 1.7–12.4 μg/ml respectively. Compared to blood levels obtained after a therapeutic dosage of 600 mg our reported blood level at 10 h was a factor 10–24 higher than the mean rifampicin concentration at 10 h of 2.3 μg/ml as found by Lecaillon et al. (1978) and of 5.5 μg/ml as described by Pawlowska and Pniewski (1979). The latter authors also found that a doubling of the dose resulted in an increase of the plasma concentration at 6 h of 92% and at 12 h of 75%. From these data one may conclude that our observed blood level is approximately consistent with the absorption of the ingested dose of 14–15 g of rifampicin.

The observed total urinary level of rifampicin and its two metabolites at 10 h was 1,351 μg/ml, including 62% of the desacetyl metabolite and 3% of the formylmetabolite (Table 1). A total level of 313 μg/ml was found by Newton and

Forrest (1975) at 30 h after ingestion of 12 g, while Jack et al. (1978) reported a urine concentration of 3,300 μg/ml after ingestion of an unknown amount. Mean urinary concentrations in 50 patients at 6 h, 12 h and 24 h after oral administration of 1,200 mg were reported by Pawlowska and Pniewski (1979) and showed values of 180 μg/ml, 140 μg/ml, and 20 μg/ml respectively.

Extrapolation to the ingested dose of rifampicin in our case (14–15 g) and also in the case as reported by Newton and Forrest showed that the observed urinary rifampicin levels are in fair agreement with the urine concentrations as reported by Pawlowska and Pniewski (1979), especially when taken into account the observed large variation in individual urine concentrations at these times, with a factor 7–9 between the lowest and highest measured concentrations.

The observed urinary concentration of desacetylrifampicin and formylrifamycin at 10 h was 796 μg/ml and 31 μg/ml respectively and accounted for 62% and 3% of the total rifampicin concentration (Table 1).

Data on the concentration of these two metabolites in urine are scarce. Maggi et al. (1969) reported a urine level of 19.6 μg/ml desacetylrifampicin at 8–12 h after oral administration of 150 mg rifampicin, accounting for 82% of a total rifampicin concentration of 24 μg/ml. No data are available for the formylrifamycin urine level in the literature. The percentage of the desacetylated form found by Maggi et al. (1969) is higher than the values reported in this study, which may be explained by a reduced metabolic capacity of the liver. The observed total biliary concentration of rifampicin and its metabolites was 328 μg/ml, including 95% rifampicin and 5% of the formyl metabolite. No desacetylrifampicin was discernable in the bile. Maggi et al. (1969) found at 8–12 h after oral administration of 150 mg bile levels varying from 55–77 μg/ml from which concentration more than 95% was present in the desacetylform. Comparing these findings with our data the relatively low total bile level and the total lack of the desacetyl metabolite in the bile are most striking. This low bile level may be explained by the occurrence of cholestasis in rifampicin intoxications and by the fact that in cases of increasing dosages or overdosage the biliary excretion of rifampicin is strongly reduced and then as a compensatory mechanism the drug becomes predominantly eliminated by the kidneys (Walter and Heilmeyer 1975). The absence of desacetylrifampicin in the bile is most probably caused by the limited enzyme capacity of the liver especially occurring in those persons who do not use rifampicin regularly ("no enzyme induction") or have hepatic diseases. In these instances resulting in a strong decrease in the degree of desacetylation of rifampicin.

The total liver tissue concentration of rifampicin and its metabolites at 10 h observed in our study was 914 μg/g and the simultaneous blood concentration was 55 μg/ml. The calculated liver tissue/blood concentration ratio showed a value of 16.6, indicating an excellent penetration of rifampicin in the liver tissue. This good diffusibility of the antibiotic is primarily based on its good lipid solubility and its low degree of ionization (25%) at the blood pH value (Walter and Heilmeyer 1975). A similar high penetration of rifampicin was reported by Furesz et al. (1969), who found in patients undergoing surgery at 3–5 h after ingestion of 150 mg a mean serum and liver tissue level of 1.4 μg/ml and 28.3 μg/g with a ratio of 20.7. Six hours after 450 mg respective levels of 7.5 μg/ml and 36.0

μg/g with a ratio of 4.8 were found. No comparative liver tissue concentration data are available for the two metabolites. However, our data show, that besides rifampicin they are both present in considerable amounts of about 30% of the total liver concentration at 10 h after ingestion. The fatal rifampicin overdosage reported in this study and the five rifampicin intoxications described in the literature so far indicate that normally rifampicin is well tolerated by man even in very high dosages and intoxications with a fatal outcome are exceptional. The fatalities are mostly associated with a bad liver function or liver disease, a frequent use or abuse of alcohol and lack of previous rifampicin medication.

This relation may be explained by the fact that the above-mentioned conditions have in common that the enzyme capacity of the liver is inadequate for complete metabolization of rifampicin to its desacetylform in cases of acute overdosage. This results in an accumulation in the body of unchanged rifampicin, which is apparently more toxic than its desacetyl metabolite and ultimately causes the fatal poisoning.

Compared to rifampicin, the lower toxicity of the desacetyl metabolite can be explained by the larger aqueous solubility resulting in a better elimination in urine and bile (Maggi et al. 1969). Finally this study clearly demonstrate that rifampicin may cause a fatal intoxication especially in those cases associated with a bad liver function or liver disease, alcohol abuse and lack of previous rifampicin treatment.

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