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Mitoxantrone metabolism in the isolated perfused rat liver

Gerhard Ehninger, Barbara Proksch, Franz Hartmann, Hermine-Valeria Gärtner, and Klaus Wilms

Medizinische Universitätsklinik, 7400 Tübingen 1, Federal Republic of Germany

Summary. *The hepatobiliary pharmacokinetics of mitoxantrone, a new anthracenedione derivative, was studied in the isolated perfused rat liver. Mitoxantrone was administered in doses of 0.2 and 0.4 mg/kg body weight. Multiple bile samples were obtained for 4 hours. Mitoxantrone and three metabolites were separated by high-performance thin-layer chromatography (HPTLC) and measured at 610 nm.*

Following 0.2 mg mitroxantrone/kg body wt, 25.8% ± 2.6% of the administered dose was excreted in the bile during 4 h, the major metabolite M_1 accounting for 80% of this. After 0.4 mg mitoxantrone/kg body wt the amounts excreted were lower and light microscopic examination showed disseminated areas of cell necrosis.

Introduction

Mitoxantrone, a new anthracenedione dervative, has shown antitumor activity superior or equal to that of adriamycin in a number of animal tumor systems and in the tumor stem cell assay [1, 8]. Phase-I clinical trials showed dose-limiting leukopenia and thrombocytopenia. Nausea, vomiting, and diarrhea are uncommon [1]. A pharmacokinetic study demonstrated a first-order process and a terminal half-life of 1.88 h [5].

In the present study we demonstrate mitoxantrone metabolism in the isolated perfused rat liver. Previous studies have shown that after several hours of perfusion the liver maintains its viability. Microsomal cytochrome b_5, cytochrome P 450, and NADPH cytochrome C reductase are stable for 4 h of perfusion [2].

Materials and methods

Male Sprague-Dawley rats (350–450 g) were used as liver donors. The liver perfusion was performed according to the technique of Miller [4] with minor modifications. After cannulation of the portal vein, the thoracic vena cava inferior, and the common bile duct, the liver was excised and connected to the circulation medium in a perfusion apparatus. The perfusion was started at 37° C with 150 ml medium in a recycling system consisting of 0.1 g/ml bovine erythrocytes, 5% bovine albumin in Tyrode solution, and 0.2 or 0.4 mg mitoxantrone/kg body weight.

Reprint requests should be addressed to G. Ehninger

Chromatographic separations were performed on HPTLC (High-performance thin-layer chromatography) plates (Merck no. 5642). Two solvent systems were used, system I being methanol : ethylacetate 95 : 10 (v/v, 2 × 10 min) and system II, chloroform : methanol : 17% ammonia 40 : 40 : 20 (v/v/v, 8 min).

The quantification was performed by using a Shimadzu TL scanner CS 910. Metabolites are expressed in mitoxantrone equivalents. The bile was collected in 15-min samples, and 10-µl aliquots were spotted in duplicate. Light, and electron microscopic morphology of the liver were checked at the end of the perfusion.

Mitoxantrone in aqueous form, with sodium disulfite as stabilizer, was supplied by the Cyanamid Company.

Results

After automatic slope setting on the blank the smallest amount of mitoxantrone detectable was 30 ng. The recovery was 97.6% ± 3.7% (SD), obtained by measuring 10 spiked biles (2.5×10^{-7} g/ml) on different days. The calibration curve was linear from 3×10^{-8} g to 1×10^{-4} g.

Without the addition of mitoxantrone to the perfusion medium the bile flow decreased from 416 ± 55 µl/h in the 1st h to 216 ± 70 µl/h in the 4th h ($n = 6$). After the addition of 0.2 mg mitoxantrone/kg body wt the flow rate for the first hour of perfusion increased to 598 ± 32 µl/h. In subsequent hours the amounts were comparable to those seen without mitoxantrone ($n = 5$). After addition of 0.4 mg mitoxantrone/kg body wt the flow rate decreased to 286 ± 15 µl/h ($n = 3$) in the 1st h, with a further rapid decline (Fig. 1).

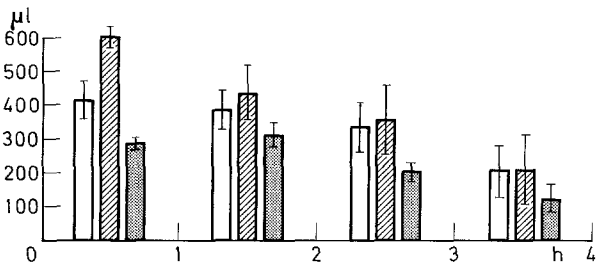

Fig. 1. Bile flow without mitoxantrone (□) and after addition of 0.2 mg mitoxantrone/kg body wt (▨) and 0.4 mg mitoxantrone/kg body wt (▦)

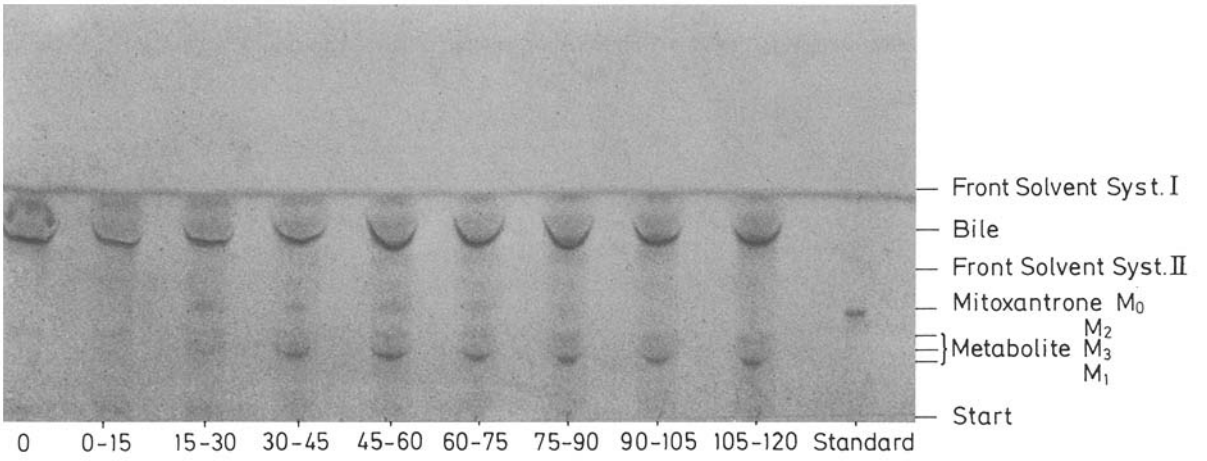

Fig. 2. Thin-layer chromatogram pattern of mitoxantrone (M_0) and its metabolites (M_1, M_2, M_3). The bile was collected in 15-min samples. Solvent system I, methanol : ethylacetate 95 : 10 (v/v, 2 × 10 min); solvent system II, chloroform : methanol : 17% ammonia 40 : 40 : 20 (v/v/v, 8 min)

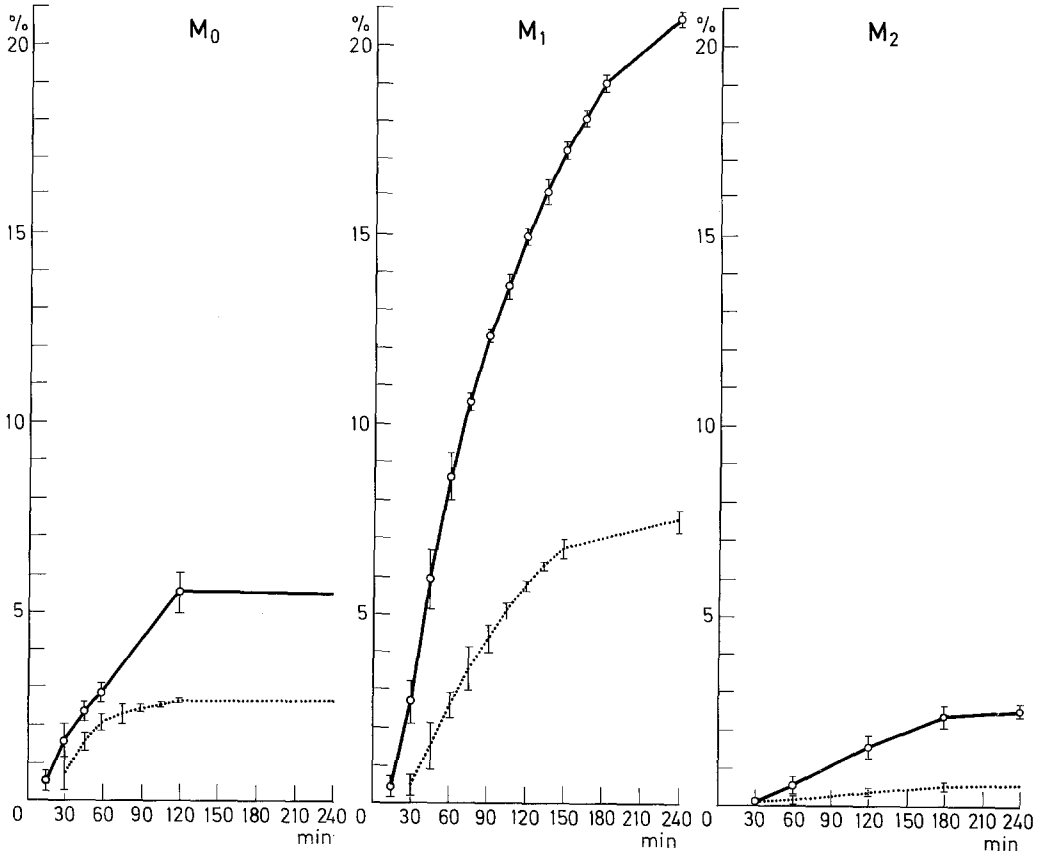

Fig. 3. Cumulative excretion of mitoxantrone (M_0) and metabolites M_1 and M_2 in bile in the isolated perfused rat liver after addition of 0.2 mg mitoxantrone/body wt (———) and 0.4 mg mitoxantrone/kg body wt (· · · · ·)

Microscopic examination of the liver perfused for 4 h without mitroxantrone showed a well-preserved structure. Occasionally single cell necrosis was observed. Livers perfused with 0.4 mg mitoxantrone/kg body wt showed disseminated areas of cell necrosis. Cells in necrotic areas had disintegrated, and the cell membrane was ruptured. The cytoplasm stained only slightly; the nuclei were pyknotic or had already disappeared. With a dose of 0.2 mg mitoxantrone/kg body wt only small areas of cell necrosis could be observed. Electron microscopic studies revealed rarefication of mitochondrial cristae and precipitates in the cytoplasm.

Figure 2 illustrates the TL plate of the bile samples. Three darkblue spots were separated from mitoxantrone. Mitoxantrone (M_0) and metabolite (M_1) were detected after 15 min of perfusion. M_1 was excreted in constant amounts, while M_0 excretion decreased. Metabolites M_2 and M_3 appeared after 30 min in small amounts.

The Rf values were: M_0, 0.82; M_1, 0.47; M_2, 0.62; and M_3, 0.55.

In the experiments in which 0.2 mg mitoxantrone/kg body wt was given, 25.8% ± 2.6% ($n = 4$) of the administered dose was excreted in the bile within 4 h, as mitoxantrone and three

metabolites. The major metabolite, M_1, accounted for 80% of the total amount recovered, while M_2 and M_3 each accounted for less than 2%.

With 0.4 mg mitoxantrone/kg body wt recovery decreased to 10.5% ± 3.1% ($n = 3$) (Fig. 3).

Discussion

In the isolated perfused rat liver mitoxantrone was extensively and rapidly metabolized. Three metabolites were separated by HPTLC. These products were only observed after liver perfusion. Incubation of mitoxantrone with rat bile alone did not lead to formation of any of the metabolites.

In clinical studies Alberts et al. [1] and Goldsmith et al. [3] did not observe any metabolite formation in plasma and urine, which might be explained by a different analytical approach.

With the HPLC system used by ourselves, the reported metabolites in isolated perfused rat liver could also be separated in human plasma, urine, and bile (unpublished results). They could be differentiated from artefacts and degradation products, as described by Reynolds [7].

The observation of 'metabolite formation' in the isolated liver perfusion needs further confirmation in in vitro settings. The substances described may finally be called metabolites after chemical characterization.

The high rate of biliary metabolism and excretion might imply the necessity of a dose reduction in patients with impaired liver function. In accordance with these results, Goldsmith et al. [3] observed rising urinary excretion from 2%–4% in patients with normal hepatic function to 8%–13% in patients with elevated bilirubin.

Dose-dependent hepatotoxicity as observed in our experiments with isolated rat liver perfusion might be of considerable importance for further phase-II and -III trials. In man a dosage of 0.3–0.5 mg/kg (14 mg/m^2) mitoxantrone is used. The venous plasma concentrations observed at the end of a 30-min infusion are 0.3–0.8 µg/ml (not published). The same level (about 0.8 µg/ml) is used in the toxic perfusate dose (0.4 mg/kg). In view of the possibility of hepatotoxicity careful observations of liver function are recommended during administration of mitoxantrone. The relevance of these observations awaits further experimental and clinical evaluation.

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