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INTERSPECIES VARIABILITY IN MITOXANTRONE METABOLISM USING PRIMARY CULTURES OF HEPATOCYTES ISOLATED FROM RAT, RABBIT AND HUMANS

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Abstract—Metabolism of mitoxantrone was studied in primary cultures of hepatocytes freshly obtained from rat, rabbit and humans. Metabolic pattern was evaluated by a high performance liquid chromatographic method which specifically resolved mitoxantrone from its mono- and dicarboxylic acid derivatives. Studies were carried out over 48 hr and at [^{14}C]mitoxantrone concentrations ranging between 1 and 20 μM. In all species studied, metabolism occurred: extracellular unchanged mitoxantrone concentrations represented around 50, 25 and 20% of total extracellular radiolabel at 48 hr in rat, rabbit and humans, respectively. Although minor interspecies variability was observed in total amount of drug biotransformed by hepatocytes, large variability in the metabolic pattern occurred between the different species: hence, in rats the main derivatives were two polar compounds and only trace amounts of the mono- and dicarboxylic acid metabolites were present. In both rabbits and humans however, these polar derivatives represented minor metabolic pathways and the main metabolites were the mono- and dicarboxylic acid derivatives. While the percentage of total biotransformation was similar in these two latter species, the monocarboxylic acid derivative was the main metabolite in rabbits while the dicarboxylic acid was predominant in humans. Only small interindividual differences (N = 4) were observed in the metabolism of mitoxantrone by human hepatocytes in primary culture. These data demonstated that: (i) in all species, mitoxantrone was biotransformed into metabolites which rapidly effluxed in the extracellular compartment, (ii) there were low interspecies differences between rat, rabbit and humans in terms of total biotransformed drug, but (iii) large interspecies variability was demonstrated in the qualitative (rat versus both rabbit and human) and relative (rabbit versus man) patterns of the metabolites. Furthermore, the metabolism of mitoxantrone was linear over a wide range of concentrations (i.e. 1–20 μM). In conclusion, rabbit appears to be the animal species most closely related to humans in terms of mitoxantrone metabolism.

Mitoxantrone (Novantrone^{R}, 1,4-dihydroxy-5,8-bis[(2-[(2-hydroxyethyl)-amino]-ethyl)amino]-9,10-anthracenedione dihydrochloride) is a new anticancer drug currently used for the treatment of metastatic breast cancer and acute leukemias [1, 2]. It is an anthracenedione drug which can be compared to doxorubicin, but its toxic side-effects, particularly cardiotoxicity, are much less pronounced. Numerous pharmacokinetic studies conducted in phases I and II clinical trials [3–5] have shown that (a) plasma concentration time-curves are adequately described by an open two- or three-compartment model, (b) total distribution volume is very large, (c) urinary elimination is very low, and (d) terminal half-life values show marked interpatient variability and are much longer in case of hepatic impairment‖ [3].

Experimental studies undertaken in rat [6–8] and rabbit [9] demonstrated that the biliary route was the main elimination pathway for mitoxantrone and/or its metabolites. In agreement with these observations, fecal elimination represents 18.3% of injected dose in man [4] and urinary elimination is very low (5–10%) [3–5]. Another study conducted in a single patient showed that biliary elimination was very low (2.7% of injected dose) [3]. However in this patient, hepatic metastases induced a very large decrease in hepatic functions. Although numerous studies were performed to determine the main pathways for drug elimination, only little is known about the importance of its biotransformation. Using the isolated perfused rat liver model, Ehninger *et al.* [8] demonstrated that mitoxantrone was slowly metabolized and that main observed derivatives were more hydrophilic than the respective mono- and dicarboxylic acid derivatives. HPLC analysis of the urine or bile following mitoxantrone i.v. injection has revealed different metabolic profiles in rat [7], rabbit [9] and man [4, 5]. Indeed, using rabbit hepatocytes both in suspension and in primary culture but also bile-duct cannulated rabbit [9], we reported that the main metabolites were the carboxylic acid derivatives. Both quantitative and

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‖ B. Richard, unpublished observation.

qualitative results from this *in vitro* study were correlated with the *in vivo* situation. All of these clinical and experimental studies show large interindividual and interspecies variabilities in pharmacokinetic parameters. The major drawback of these studies is that results are difficult to compare since they were obtained on different models with various biological fluids.

This study therefore sought to investigate mitoxantrone biotransformation using primary culture of hepatocytes isolated from rat, rabbit and humans in order to better understand the role of the liver in the disposition of mitoxantrone.

MATERIALS AND METHODS

Chemicals

Mitoxantrone (M_r 517.4), the mono- and dicarboxylic acid derivatives and [^{14}C]mitoxantrone were kindly supplied by Lederle Laboratories (Pearl River, New York). Radioactive [2-hydroxyethyl-^{14}C]mitoxantrone (11.2 mCi/mmol) was 95% radiochemically pure as assessed by HPLC and was used without further purification. Type IV collagenase was purchased from the Sigma Chemical Co. (St Louis, MO). Chromatographic solvents were of analytical grade. Other chemicals and reagents were obtained from regular commercial sources.

Preparation of hepatocyte suspension

Hepatocytes were prepared from Wistar male rats (200–250 g), New Zealand male rabbits (0.6–0.9 kg) and from various human livers.

Rats and rabbits. Hepatocytes were isolated by liver perfusion with a collagenase solution (0.05%) according to previously described techniques [9–11].

Humans. Human livers were obtained from organ (kidney and heart) donors at the Marseilles Medical Centers, La Timone and Sainte Marguerite. Patient characteristics are reported in Table 1. In this table are also reported metabolic activities of the liver, obtained following preparation of the microsomal fractions and analysis of various enzyme activities.

After an intensive *in situ* washing step with sterile Eurocollins medium at 4° through the portal vein and the aorta, liver was removed and transported to the laboratory in less than 20 min. Liver was then washed with 10 L of oxygenized HEPES buffer (pH 7.4; 10 mM; containing 2.5% glucose) at 37° using a peristaltic pump (flow rate around 1–2 L/min). Liver was finally perfused with a collagenase solution (0.05% in HEPES buffer; 10 mM; pH 7.5) over 20–30 min. The surrounding Glisson's capsule was disrupted and the digested pieces of the liver were then minced gently and resuspended in 10 volumes of a buffer containing 120 mM NaCl, 6.7 mM KCl, 10 mM HEPES (pH 7.4), 0.9 mM $CaCl_2$ and 2.5% glucose.

Under these conditions a cell yield of 10–50 × 10^9 hepatocytes was routinely achieved by perfusing the whole liver. This cell yield (10–30 × 10^6cells/g wet liver) is similar to that reported by others [12]. Viable hepatocytes were obtained no longer than 90 min following liver removal. Viability of hepatocytes, assessed by Trypan blue exclusion, was 80% or higher. This technique to obtain functional

Table 1. Characteristics of kidney-transplant donors, from whom livers were obtained and hepatocytes prepared. Metabolic ability of microsomal fractions prepared from these different liver samples were evaluated by measuring specific biotransformation reactions

Patient number	Age	Sex	Cause of death	Co-medications	Cytochrome P450 content (nmol/mg)	Phenacetin O-deethylation P450IA (nmol/min/mg)	*d*-Benzphetamine N-demethylation P450IIB (nmol/min/mg)	Debrisoquine hydroxylation P450IID (nmol/min/mg)	Nifedipine oxidation P450IIIA (nmol/min/mg)
1	22	Male	Brain damage (traffic accident)	Solumedrol Dopamine Penthotal	0.28	1.10	1.02	0.012	1.41
2	21	Male	Brain damage (traffic accident)	Dopamine	0.30	1.21	1.10	0.048	1.27
3	22	Male	Brain damage (traffic accident)	Dopamine	0.43	1.19	1.87	0.019	1.81
4	41	Male	Cerebral hemorrhage	Dopamine (alcoholic)	0.39	0.75	1.90	0.031	1.68

human hepatocytes has been previously described in details [13].

Hepatocyte incubation conditions

Hepatocytes from the various species, resuspended in 1.5 mL of Ham F12 medium (0.8×10^6cells) supplemented with 10% fetal calf serum, were inoculated in six-well plastic dishes (35 mm diameter). At the end of a 6- to 12-hr exposure period during which cells attached to the plastic dishes, the medium and the dead cells were removed. The hepatocytes were then incubated in serum-free Ham F12 medium in the presence of various mitoxantrone concentrations ranging between 1 and 20 μM. Details of experiments have been reported elsewhere [9, 14–16].

Experiments were also performed in hepatocyte-free medium to check the stability of mitoxantrone under these experimental conditions.

Experiments were performed in triplicate for rat and rabbit hepatocytes and in quadruplicate for human hepatocytes. Extracellular media were recovered at the time points indicated in Fig. 2 and stored at −20° until HPLC analysis. The monolayers were washed twice with phosphate buffered saline (pH 7.4) and removed from the dishes by scraping. Proteins were precipitated with acetonitrile (20% final concentration), removed by centrifugation at 15,000 *g* for 5 min, and the supernatant fluid was stored at −20° until HPLC analysis.

Extracellular media as well as precipitated proteins were analysed by liquid scintillation counting for total radiolabel determination.

HPLC analysis

A high performance liquid chromatograph (Hewlett-Packard 1084B) was equipped with a 10 μm-particle size C_{18} μBondapak column (30 cm ×3.9 mm; Waters Millipore S.A.). Elution was carried out at 1.0 mL/min and UV absorbance was monitored at 254 nm. The mobile phases were formate buffer (pH 4.0; 1.6 M; solvent A) and acetonitrile:water (48:52; v/v; solvent B). The solvent programmer was set to deliver 45–60% of solvent B along a 15-min linear gradient. Eluent from the column was analysed by liquid scintillation counting with a radioactive flow detector (Radiomatic Instruments).

Under these HPLC conditions, mitoxantrone was baseline-separated from its various metabolites. This HPLC method has been already used to identify mitoxantrone metabolites in various biological fluids [9].

RESULTS

Intra- and extracellular kinetics of mitoxantrone metabolites in various species

Figure 1 illustrates radiochromatograms of extracellular media obtained following a 12-hr exposure of rat (A), rabbit (B) and human (C) hepatocytes in primary culture to 5 μM [^{14}C]mitoxantrone.

In all species studied, various mitoxantrone metabolites were recovered in the extracellular compartment but marked qualitative differences were observed among the various species. In rabbit and human hepatocytes, the main metabolites co-chromatographed with the mono- and dicarboxylic acid derivatives, respectively. In rats however, radiolabel was principally eluted with derivatives whose retention times were close to those of void volume of the HPLC column. These metabolites were termed "polar derivative(s)". The mono- and dicarboxylic acid derivatives represented only minor metabolites in this animal species.

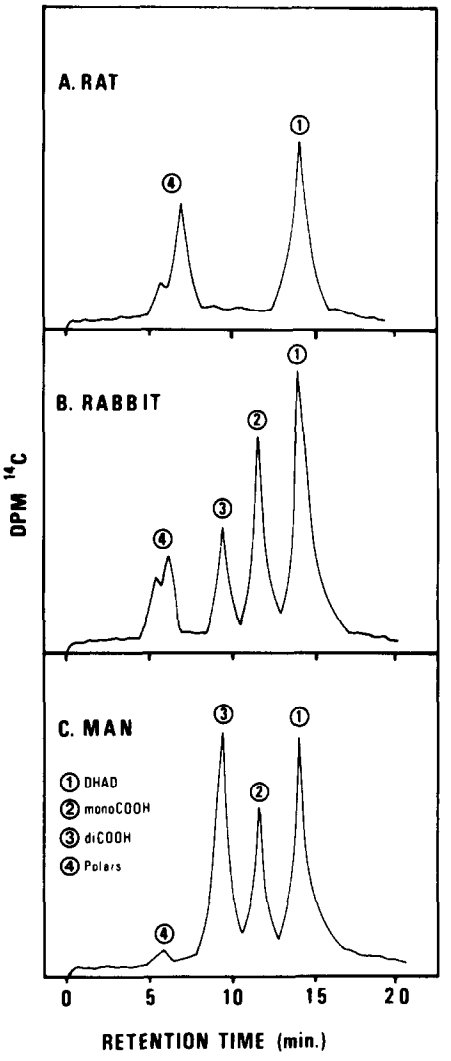

Fig. 1. HPLC analysis of [^{14}C]mitoxantrone and its metabolites in extracellular medium, obtained following a 12-hr exposure of freshly isolated rat (A), rabbit (B) and human (C) hepatocytes in primary culture to 5 μM [^{14}C]mitoxantrone (DHAD).

The intra- and extracellular behaviors of mitoxantrone and its various derivatives were studied over a 48-hr exposure of hepatocytes in primary culture to 5 μM [^{14}C]mitoxantrone. Figure 2 illustrates these extracellular kinetics in rat (N = 3), rabbit (N = 3) and humans (N = 4). In all species, unchanged mitoxantrone disappeared rapidly from the extracellular medium, reaching one-half of its initial value in less than 20–30 min. Over this period of

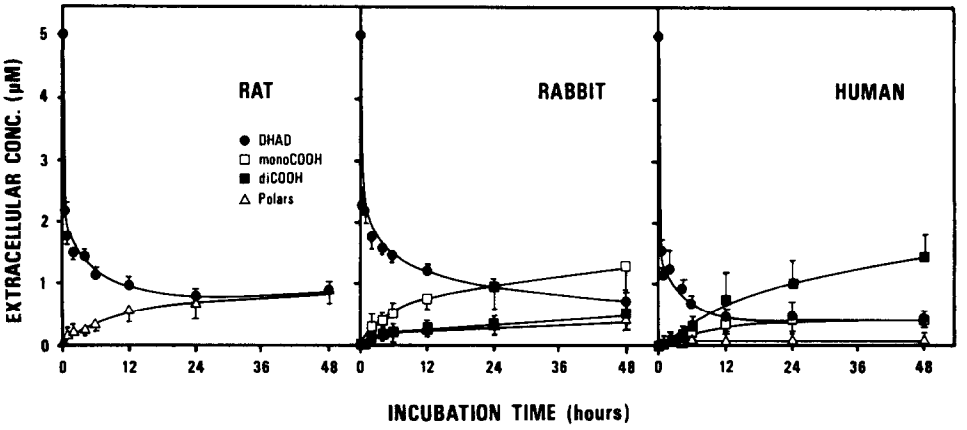

Fig. 2. Extracellular mitoxantrone and metabolites behavior after exposure of primary cultures of hepatocytes freshly isolated from rat, rabbit and humans to 5 μM [^{14}C]mitoxantrone over 48 hr. Standard deviations are expressed by bars for every time-point.

observation, no metabolite was detected in the extracellular compartment.

These results are in agreement with those of previous studies demonstrating the rapid uptake of the drug into rabbit [9] and human* hepatocytes incubated in suspension. After 30 min of exposure to the radiolabelled drug and over the entire period of incubation, metabolites appeared slowly in the extracellular compartment. This cannot be accounted for by a chemical degradation of mitoxantrone due to incubation conditions (medium nutrients, temperature, time of exposure) since no degradation of mitoxantrone occurred in hepatocyte-free medium.

In the extracellular medium, the unchanged mitoxantrone concentration achieved after a 48-hr incubation period with 5 μM [^{14}C]mitoxantrone was 0.87 ± 0.09, 0.70 ± 0.19 and 0.41 ± 0.20 μM in rat, rabbit and humans, respectively. However, large differences in the metabolic pattern of mitoxantrone were observed among the species. In rats, the main metabolites were two polar derivatives (0.85 ±0.20 μM) which did not co-elute with available reference compounds. Following a 24-hr exposure of a 48-hr extracellular medium to β-glucuronidase (0.3 M acetate buffer, pH 4.5), no hydrolysis of polar derivatives was observed, suggesting that these derivatives were not glucuronidated. The mono- and dicarboxylic acid derivatives reached only very low levels, very variable from one experiment to another. In contrast, in both rabbit and humans, the main metabolites of mitoxantrone recovered in the extracellular compartment were the mono- and the dicarboxylic acid derivatives. The monocarboxylic acid derivative (1.27 ± 0.50μM) of mitoxantrone was relatively higher than the dicarboxylic acid derivative (0.49 ±0.18 μM) in rabbits; the latter was preponderant in humans (0.37 ±0.09 and 1.42 ±0.36 μM for the mono- and dicarboxylic acid, respectively).

In order to evaluate the potential involvement of cytochrome P450 isozymes in mitoxantrone biotransformation, metabolism was studied on hepatic microsomal fractions prepared from untreated rats or rats pretreated with β-naphthoflavone (P450IA inducer), phenobarbital (P450IIB inducer) or erythromycin (P450IIIA inducer). After a 30-min incubation period with 5 μM [^{14}C]mitoxantrone, absolutely no metabolite was detected at all.

Radiolabel was also analysed in the intracellular compartment for each species. In human hepatocytes (N = 3), assuming an approximate cell monolayer volume of 10 μL $\times 10^6$ cells^{-1}, total intracellular drug (unchanged drug ±metabolites) concentrations were 183.6 ± 11.5 and 252.0 ±64.0 μM for extracellular concentrations of 1.0 μM (at the 2nd hr) and 1.6 μM (at the 12th hr), respectively; initial extracellular mitoxantrone concentration was 5 μM. The partition ratio was independent of the extracellular concentration as previously reported [9]. Total radiolabel was predominantly accounted for by unchanged mitoxantrone (more than 75%) as assessed by HPLC analysis of intracellular radiolabel. This suggested a specific distribution of mitoxantrone metabolites in the extracellular compartment, and a selective retention of mitoxantrone inside the cells. These data are in agreement with pharmacokinetic observations which showed a very large distribution volume (around 1000–4000 L) following i.v. mitoxantrone injection [3–5].

In Table 2 the distribution of total radiolabel between intra- and extracellular compartments following a 12-hr exposure to mitoxantrone concentrations ranging between 1 and 20 μM is reported. This table demonstrates that, whichever the species studied, more than 90% of total radiolabel was recovered in both intra- and extracellular compartments following incubation of hepatocytes with mitoxantrone concentrations higher than 2 μM. A loss of 10–25% of radiolabel, probably bound to plastic dishes, was observed at the lower mitoxantrone concentration, i.e. 1 μM.

This table also specifies the total intracellular distribution for the different species. It reached

* B. Richard, unpublished observations.

Table 2. Distribution of total radiolabel between intra- and extracellular compartments following a 12-hr exposure to increasing mitoxantrone concentrations up to 20 μM

Incubation concentrations* μM (nmol/dish)	Extracellular compartment†	Intracellular compartment			Recovery (%)	Intracellular incorporation (%)
		ACN soluble†	Total†	% ACN soluble/total		
Rat						
1 (1.5 nmol)	0.37 ± 0.08	0.33‡	0.95	34.7	90.2	72.0
2 (3.0 nmol)	0.78 ± 0.10	0.86	2.01	42.8	91.7	72.0
5 (7.5 nmol)	2.22 ± 0.31	2.45	4.81	50.9	90.9	68.4
10 (15.0 nmol)	4.38 ± 0.37	5.39	10.88	49.5	99.7	71.3
20 (30.0 nmol)	7.93 ± 1.50	9.70	20.27	47.8	97.5	71.9
Rabbit						
1 (1.5 nmol)	0.66 ± 0.09	0.26 ± 0.04	0.61 ± 0.06	43.1	84.7	48.0
5 (7.5 nmol)	3.62 ± 0.40	1.41 ± 0.30	3.24 ± 0.60	43.4	91.5	47.2
20 (30.0 nmol)	17.29 ± 1.96	5.97 ± 1.41	12.56 ± 1.56	47.5	99.5	42.1
Humans						
1 (1.5 nmol)	0.41 ± 0.13	0.34 ± 0.11	0.85 ± 0.27	40.0	75.0	67.5
2 (3.0 nmol)	1.04 ± 0.16	0.80 ± 0.25	1.89 ± 0.52	42.3	97.7	64.5
5 (7.5 nmol)	2.60 ± 0.42	2.02 ± 0.51	4.88 ± 1.01	41.4	99.7	65.2
10 (15.0 nmol)	5.27 ± 0.94	4.47 ± 1.06	10.24 ± 1.73	43.6	103.4	66.0
20 (30.0 nmol)	10.89 ± 2.33	8.92 ± 1.21	20.63 ± 3.10	43.2	105.1	65.4

* Hepatocytes were incubated in a volume of 1.5 mL which signifies that they were exposed to 1.5, 3.0, 7.5, 15.0 and 30.0 nmoles of mitoxantrone.

† Results are expressed in nmoles per dish.

‡ N = 1 for rat, N = 3 for rabbit and N = 4 for humans.

approximately 70, 50 and 65% in rat, rabbit and humans, respectively. However, this percentage is similar over a large range of concentration in a specified species.

Among intracellular radiolabel, most of the radioactivity was recovered in the acetonitrile-insoluble fraction which suggests a high affinity of mitoxantrone and/or its metabolites for cellular proteins. The percentage of drug bound to proteins is linear between 1 and 20 μM and is similar from one species to another (approximately 50–60% of intracellular drug).

Time- and dose-dependent relationship of mitoxantrone metabolism

The metabolism of mitoxantrone was studied over a wide range of concentrations: 1.0, 2.0, 5.0, 10.0 and 20.0 μM in all animal species.

It has to be noted that mitoxantrone concentrations up to 3.0 μM are usually achieved in cancer patients treated with 12 mg/m^2 mitoxantrone [17]. Higher concentrations (around 5 μM) have been occasionally observed in patients with impaired hepatic functions or in patients treated with larger doses of mitoxantrone.*

The time course of metabolite accumulation in the extracellular compartment following incubation with increasing mitoxantrone concentrations is illustrated in the upper panel of Fig. 3. The rate of metabolite accumulation increased faster between the 1st and the 12th hr than between the 12th and the 48th hr. This difference could be due to a decrease in unchanged drug available for further metabolism. Although a dedifferentiation of hepatocytes during the primary culture cannot be excluded, it is now well recognized that dedifferentiation occurs slowly in human hepatocytes, most of enzyme activities being preserved over 24 hr, at least [12].

The lower panel of Fig. 3 reports the amounts of each metabolite following a 48-hr (24-hr for humans) incubation of hepatocytes with mitoxantrone concentrations up to 20 μM. In each species, metabolite formation was time-dependent and the accumulation of each metabolite in the extracellular compartment exhibited a linear relationship with initial extracellular concentrations.

Mitoxantrone metabolism was studied in hepatocytes obtained from various human liver samples (Fig. 4, Table 1). Mitoxantrone metabolism was studied over 24 hr at an extracellular concentration of 5 μM. Results for each human hepatocyte preparation are presented in Fig. 4. Whichever human hepatocyte preparation was used, extracellular mitoxantrone concentration decreased very rapidly as a result of its rapid initial uptake. Mitoxantrone metabolites which were formed in the intracellular compartment, appeared in the extracellular compartment as early as 1 to 2 hr from the beginning of the incubation.

As illustrated in Fig. 4, interindividual variability between these various human hepatocyte preparations was slight. In all preparations, the dicarboxylic acid derivative of mitoxantrone was the main extracellular metabolite at 24 hr and its extracellular concentration was 0.62, 0.96, 1.53 and 0.79 μM for the individual hepatocyte preparations, respectively. On the other hand, values for

* B. Richard, unpublished observation.

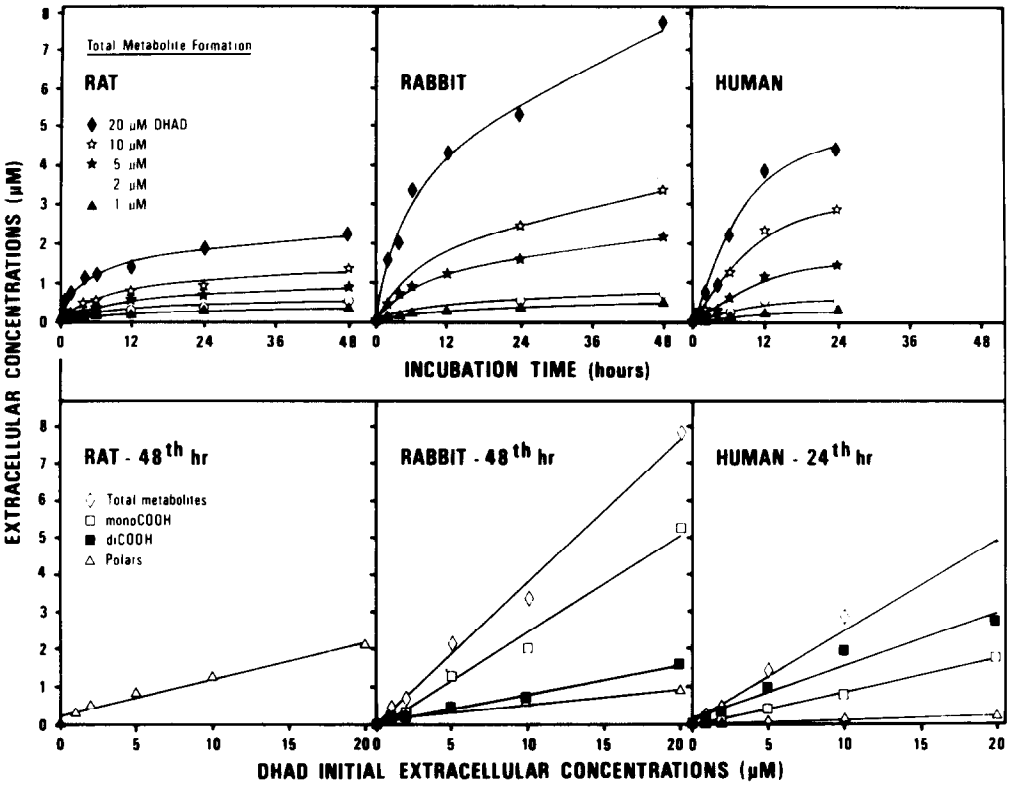

Fig. 3. Upper panel: Time-dependency relationship of mitoxantrone metabolism. Total metabolite accumulation in extracellular medium over 48 hr (rat, rabbit) and 24 hr (humans) following exposure of hepatocytes in primary culture to increasing [^{14}C]mitoxantrone concentrations. Lower panel: Dose-dependency relationship of mitoxantrone metabolism. Metabolite levels in extracellular medium were evaluated after a 48-hr (rat, rabbit), or a 24-hr (humans) incubation of mitoxantrone with hepatocytes in primary culture.

unmetabolized mitoxantrone concentration were 0.47, 0.80, 0.22 and 0.41 μM, respectively.

DISCUSSION

In the present study we have investigated the metabolism of mitoxantrone in various species, rat, rabbit and humans, by using hepatocytes in primary culture.

Hepatocytes isolated from various species have been routinely and increasingly used over the last decade for pharmacological and toxicological studies. Since hepatocytes express most of the functional activities of the intact liver, they represent a suitable model for drug metabolism studies. The major drawback of this approach is that most of these studies were carried out with rodents and that results had to be extrapolated to humans.

We have demonstrated that mitoxantrone is metabolized at a relatively slow rate by the various species. While no important difference in unchanged mitoxantrone concentrations was observed among species after a 24-hr period of incubation, large qualitative differences were found in the various biotransformation pathways: in rats, the main derivatives recovered in the extracellular compartment were highly polar compounds. The presence of these derivatives has already been reported by others [6–8]. Using the isolated perfused rat liver model, Ehninger *et al.* [8] demonstrated that the mono- and dicarboxylic acid derivatives represented only a very low percentage of the metabolites excreted in bile. These data are in complete agreement with those obtained in our study with rat hepatocytes in primary culture. In a more detailed study, Wolf *et al.* [18], using both rat liver microsomal fractions and hepatocyte homogenates, reported that the main mitoxantrone metabolites were the glutathione- and glucurono-conjugates of mitoxantrone. In both rabbit and human hepatocytes, however, the main derivatives co-eluted with authentic mono- and/or dicarboxylic acid derivatives. In a previous study investigated both *in vivo* and *in vitro* in New Zealand rabbits, we reported that large quantities of these derivatives were recovered in the bile of bile-duct cannulated rabbits [9]. Little information is available on humans. Different authors [5, 19, 20] have demonstrated the presence of these two metabolites in human urine following i.v. injection of 12–14 mg/m^2 in cancer patients. Although the same metabolites are recovered in both rabbit and humans, some quantitative differences occurred. Hence in rabbits, the main derivative was the monocarboxylic acid, while in

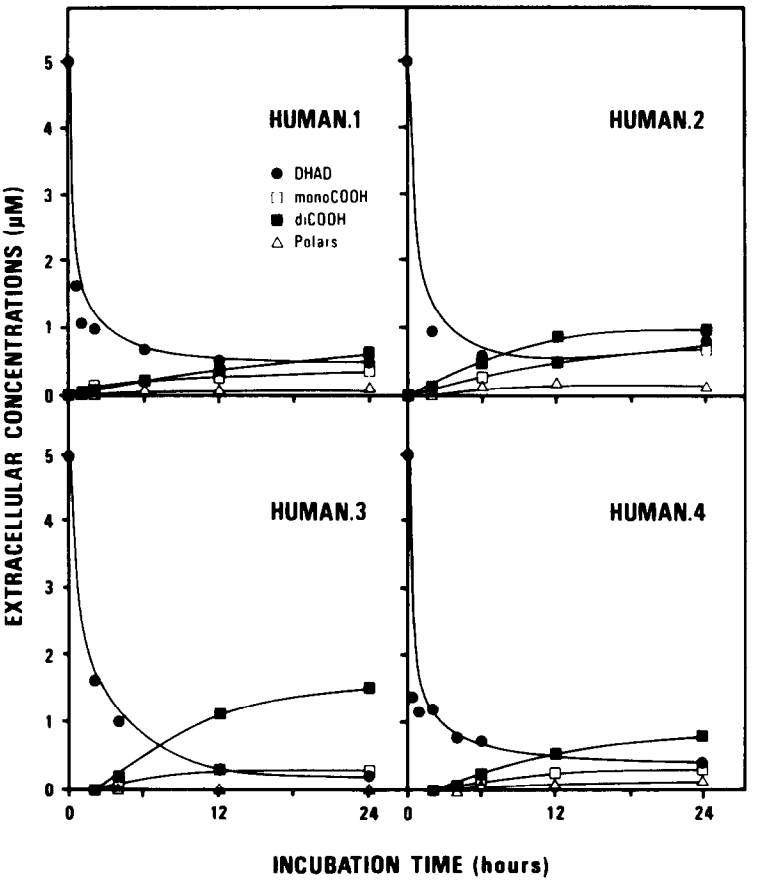

Fig. 4. Interindividual variability of mitoxantrone metabolism in humans. Extracellular mitoxantrone and metabolites behavior were analysed after a 24-hr exposure of primary cultures of human hepatocytes freshly isolated from four different organ donors to 5 μM [^{14}C]mitoxantrone.

humans it was the dicarboxylic acid. On the basis of both *in vivo* reported data [5–9, 19, 20] and this study, it appears that the main biotransformation pathways exhibit large interspecies differences between rat and the other species, i.e. rabbit and humans. Rabbits appear to be the animal species the most closely related to humans, at least at the metabolic level.

It is now well known that large interindividual variabilities [16] occur in the metabolism of various drugs, such as cyclosporine A [21], midazolam [13] or vinca alkaloids [14] which are metabolized (at least in part) by cytochrome P450 monooxygenases. Hence it is of particular interest to demonstrate, by using human hepatocytes in primary culture, that only low intersubject variability occurs in mitoxantrone metabolism. This could be accounted for by the following observations: (a) a rapid and intense intracellular uptake within the cells, which can be associated to a large distribution and (b) a slow rate of metabolism as well as the non-involvement of cytochrome P450 monooxygenases in mitoxantrone metabolism [18].

Since the liver plays a crucial role in mitoxantrone behavior (intra-hepatic distribution, metabolism and biliary excretion), its administration has to be carefully monitored in patients with liver impairment to avoid hematological toxicity associated with high mitoxantrone plasma levels [22, 23].

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REFERENCES

1. Smith IE, Mitoxantrone (Novantrone): a review of experimental and early clinical studies. *Cancer Treat Rev* **10**: 103–115, 1983.
2. Shenkenberg TD and Von Hoff, Mitoxantrone: a new anticancer drug with significant activity. *Ann Med Int* **105**: 67–81, 1986.
3. Savaraj N, Lu K, Valdiviesco M and Loo TL, Pharmacology of mitoxantrone in cancer patients. *Cancer Chemother Pharmacol* **8**: 113–117, 1982.
4. Alberts DS, Peng YM, Leigh S, Davis TP and Woodward DL, Disposition of mitoxantrone in cancer patients. *Cancer Res* **45**: 1879–1884, 1985.
5. Ehninger G, Proksch B, Heinzel G and Woodward DL, Clinical pharmacology of mitoxantrone. *Cancer Treat Rep* **70**: 1373–1378, 1986.

6. Avramis V, Pharmacokinetics of dihydroxyanthracenedione (DHAD) and its metabolite in rat. *Pharmacologist* **24**: 241, Abstract 783, 1982.
7. Chiccarelli FS, Morrison JA and Gautam SR, Biliary pharmacokinetic of ^{14}C-mitoxantrone in the rat following different intravenous doses and characteristics of drug related material. *Fed Proc* **43**: 345, Abstract 354, 1984.
8. Ehninger G, Proksch B, Hartmann F, Gärtner HV and Wilms K, Mitoxantrone metabolism in the isolated perfused rat liver. *Cancer Chemother Pharmacol* **12**: 50–52, 1984.
9. Richard B, Fabre G, Fabre I and Cano JP, Excretion and metabolism of mitoxantrone in rabbits. *Cancer Res* **49**: 833–837, 1989.
10. Berry MN and Friend DS, High-yield preparation of isolated rat liver parenchymal cells. *J Cell Biol* **43**: 506–520, 1969.
11. Fabre G, Bertault-Peres P, Fabre I, Maurel P, Just S and Cano JP, Metabolism of cyclosporin A: I. Study of freshly isolated rabbit hepatocytes. *Drug Met Dispos* **15**: 384–390, 1987.
12. Guguen-Guillouzo C and Guillouzo A, Methods for preparation of adult and fetal hepatocytes. In: *Research in Isolated and Cultured Hepatocytes* (Eds. Guillouzo A and Guguen-Guillouzo C), pp. 1–12. John Libbey Eurotext Ltd/INSERM, Paris, 1986.
13. Fabre G, Rahmani R, Placidi M, Combalbert J, Covo J, Cano JP, Coulange C, Ducros M and Rampal M, Characterization of midazolam metabolism using human hepatic microsomal fractions and hepatocytes in suspension obtained by perfusing whole human livers. *Biochem Pharmacol* **37**: 4389–4397, 1988.
14. Rahmani R, Richard B, Fabre G and Cano JP, Extrapolation of preclinical pharmacokinetic data to therapeutical drug use. *Xenobiotica* **18**: 71–78, 1988.
15. Cano JP, Rahmani R, Lacarelle B, Fabre G, Boré P, Richard B, De Sousa G, Bertault-Peres P, Sengwald I and Placidi M, Human hepatocytes as an alternative method to the use of animal in experiments. In: *Pharmacologie Clinique: Actualités et Perspectives II* (Eds. Strauch G and Morselli PL), pp. 33–58. John Libbey Eurotext Ltd/INSERM, Paris, 1988.
16. Cano JP, Fabre G, Maurel P, Bichet N, Berger Y and Vic P, Inter-individual variability and induction of cytochromes P-450 and UDP-glucuronosyl transferases in human liver microsomes and primary cultures of human hepatocytes. In: *Cellular and Molecular Aspects of Glucuronidation* (Eds Siest G, Magdalou J and Burchel B), pp. 249–260. John Libbey Eurotext Ltd/INSERM, Paris, 1988.
17. Richard B, Maraninchi D, Tubiana-Mathieu N, Lejeune C, Launay MC, Payet B, Just S, Cano JP and Carcassonne Y, Modalités d'administration de la Novantrone^{R}. Etudes cliniques. Etudes métaboliques *in vitro*. *Cancer Commun* **2**: 29–37, 1988.
18. Wolf CR, Macpherson JS and Smyth JF, Evidence for the metabolism of mitozantrone by microsomal glutathione transferases and 3-methylcholanthrene-inducible glucuronosyl transferase. *Biochem Pharmacol* **35**: 1577–1581, 1986.
19. Chiccarelli FS, Morrison JA, Cosulich DB, Perkinson NA, Ridge DN, Sum FW, Murdock KC, Woodward DL and Arnold ET, Identification of human urinary mitoxantrone metabolites. *Cancer Res* **46**: 4858–4861, 1986.
20. Payet B, Arnoux P, Catalin J and Cano JP, Direct determination of mitoxantrone and its mono- and dicarboxylic acid metabolites in plasma and urine by high-performance liquid chromatography. *J Chromatogr* **424**: 337–345, 1988.
21. Combalbert J, Fabre I, Fabre G, Dalet-Beluche I, Devancourt J, Cano JP and Maurel P, Metabolism of Cyclosporin A: IV. Purification and identification of the rifampicin-inducible human liver cytochrome P-450 (cyclosporin A oxidase) as a product of P450IIIA gene subfamily. *Drug Metab Dispos* **17**: 197–207, 1989.
22. Wynert WR, Harvey HA, Lipton A, Schweitzer J and White DS, Phase I study of a 5-day schedule of mitoxantrone (dihydroxyanthracenedione). *Cancer Treat Rep* **66**: 1303–1306, 1982.
23. Chlebowski RT, Tong M and Woodward DL, Mitoxantrone administration in patients with hepatic dysfunction. In: *Advances in Cancer Chemotherapy, The Current Status of Novantrone^{R}* (Ed. Coltman CA Jr), pp. 23–28, 1985.