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Short communication

Metabolism of epidoxorubicin in animals: absence of glucuronidation*

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Summary. Metabolism of epidoxorubicin was studied in plasma of seven different animal species at 2 h after administration of 4 mg/kg. None of the animals showed significant glucuronidation of epidoxorubicin, although small amounts of the glucuronides could be detected in the rabbit. However, large differences in formation of epidoxorubicinol and 7-deoxy (7d) doxorubicinol aglycone were observed between the species. These phenomena may be relevant for interspecies differences with regard to anthracycline-induced histomorphological changes in for example, heart tissues and cardiotoxicity in relation to formation of 7d aglycones.

Introduction

Doxorubicin (A) and epidoxorubicin (E), its 4'-epimer (Fig. 1), are both well-known cytostatic drugs with good antitumor properties. Glucuronidation of E (at the sugar moiety) in humans is an important metabolic pathway. High amounts of glucuronides are found in plasma (45%) as well as in urine (37%) [9]. In contrast, glucuronidation of A at the sugar moiety is not observed at all [9]. Formation of 4'epidoxorubicin glucuronide (E-glu) and 4'-epidoxorubicinol glucuronide (Eol-glu) (Fig. 1) has been related to the lower cardiotoxicity of E than of A. In order to study this relationship in animals, an animal species is required which has an ability similar to that of humans to glucuronidate E. The purpose of this study was to find such an animal by screening a number of animal species for their formation of the glucuronides Eol-glu and E-glu.

Materials and methods

Animals. The following animals entered the study: 16 mice of 25 g, eight rats of 300 g, eight hamsters of 100 g, four guinea pigs of 600 g and two rabbits of 2200 g. Plasma was collected 2 h after administration of 4 mg E/kg. Sodium heparinate (Thromboliquine, Organon, Boxtel, The Netherlands) was used as anticoagulant. Plasma of the same animal species were pooled. Prior to injection, "blank" plasma was collected.

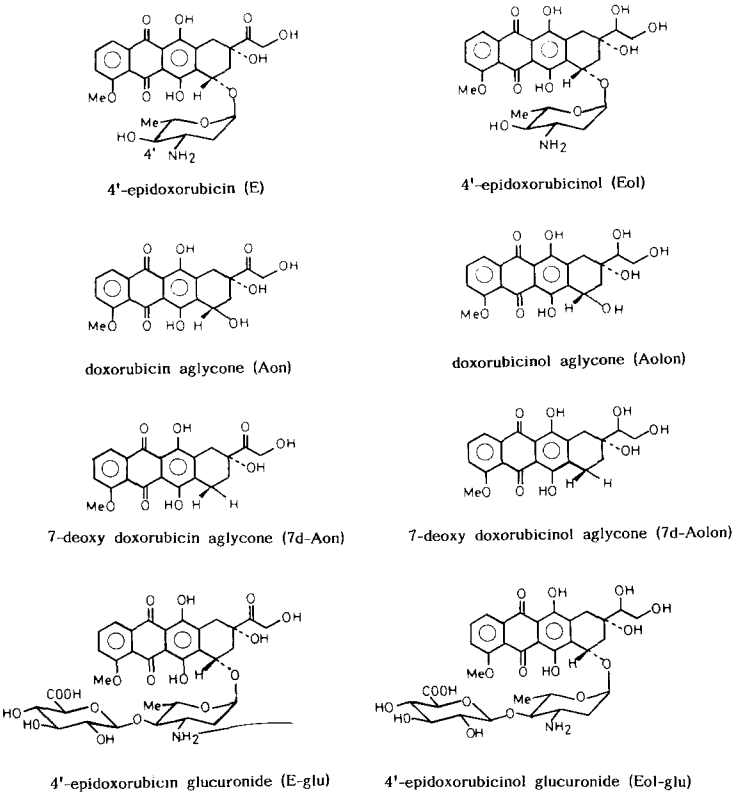

Fig. 1. Structure of epidoxorubicin and its metabolites

In preliminary experiments one pig of 25 kg and two chickens of 550 g were included in the series.

Chemicals. Epidoxorubicin was supplied by Farmitalia Carlo Erba (Milan, Italy). A $3.4.10^{-3}M$ solution of E in saline was used to treat the animals. β-Glucuronidase was obtained from Sigma (No. G-9387, Amsterdam, The Netherlands).

Analytical assay. One milliliter of pooled plasma (spiked with 50 µl A, $5.10^{-7}M$, as internal standard) was processed in duplicate by the method described previously by our group [8]. To check the presence of glucuronides two plasma samples of each species were also treated with 1000 units β-glucuronidase (in aqua dest.) for 1 h at 37° C. The mobile phase of the high-performance liquid chromatography

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Table 1. Concentrations of epidoxorubicin (E) and its metabolites in plasma 2 h after administration of 4 mg E/kg (.10^{-9}M)

Animal	Route of administration	Eol-Glu	E-Glu	Aolon	Eol	7d-Aolon	7d-Aon	E
Mouse	i.p.	0	0	0	0.2	20	0	49
Rat	i.v.	0	0	0.1	0.4	4.2	0	56
Hamster	i.p.	0	0	0.1	3.3	21	1.3	83
Guinea pig	i.p.	0	0	0	0.2	1.7	0	39
Rabbit	i.v.	0.5	1.7	0.3	23	4.2	0	87
Man[a]	i.v.	90	460	1	15	25	14	46

[a] After a dose of 1.5 mg E/kg

(HPLC) system consisted of 0.02 *M* NaH_2PO_4, pH 4/ acetonitrile 2.6/1 (v/v). Calibration lines ($r^2 > 0.99$) of E and its metabolites in heparinized plasma were made in the concentration range $5.10^{-10}M$–$2.5.10^{-8}M$. The detection limit of the assay was $1.10^{-10}M$ for doxorubicinol aglycone (Aolon) and Eol, $2.10^{-10}M$ for 7-deoxy doxorubicinol aglycone (7d-Aolon) and the glucuronides and $3.10^{-10}M$ for E and 7-deoxy doxorubicin aglycone (7d-Aon).

Results and discussion

Previous studies in patients [10] and preliminary studies in animals indicated that in plasma the highest levels of metabolites were present at 1–2 h after bolus injection of E. Therefore, this study was performed in plasma obtained at 2 h after drug administration. Concentrations of E and its metabolites in plasma 2 h after administration of E are summarized in Table 1. No E-glu or Eol-glu ($< 2.10^{-10}M$) could be detected in mouse, rat, hamster and guinea pig. In the rabbit only small amounts were present, as determined from the peak heights at the appropriate retention times in the HPLC chromatogram and confirmed by the β-glucuronidase experiment. Apart from E and glucuronides, the following metabolites were observed: Eol, Aolon, 7d-Aon and 7d-Aolon. Their structures are shown in Fig. 1. Interestingly, large differences in the concentration of these metabolites were observed between the animal species. As noticed earlier in the case of A [3] and 4-demethoxydaunorubicin [6], the aldoketoreductase activity is prominent in rabbits, resulting in large amounts of Eol. Such high levels of Eol may be related to more pronounced toxicity as observed in rabbits after administration of A [3]. In our experiment, mouse, rat, hamster and guinea pig produced low levels of Eol. This observation is in agreement with low levels of Aol in mouse [5, 7] and rat [11] after administration of A. On the other hand, high plasma levels of 7d-Aolon are present in mouse and hamster (Table 1), consistent with high levels of 7d-Aolon in mouse plasma and serum [5, 6, 7] and hamster liver tissue [1] after administration of A. Qualitative preliminary experiments indicated that also the pig did not produce significant amounts of glucuronides, whereas chickens did not metabolize E at all.

For comparison with the animal data, the mean concentrations of E and its metabolites in plasma of seven patients 2 h after an i. v. bolus injection of 1.5 mg/kg E (own unpublished results) are given in Table 1. These values indicate that the main metabolic pathway of E in man is glucuronidation.

In this study *no* appreciable amounts of E glucuronides were found in plasma of seven animal species. Consequently, no animal model is suitable to study the effect of glucuronidation on the cardiotoxicity in humans. As reduced cardiotoxicity of E compared to A has been reported in mouse and rat [4], glucuronide formation in humans cannot be the sole cause of reduced toxicity. An alternative hypothesis for the difference in cardiotoxicity between A and E directs attention to the 7d aglycones. Semiquinone radicals of A and E, the presence of which was demonstrated in vitro [2], are supposed to play a role in the development of cardiotoxicity. Furthermore, these radicals are thought to be chemically converted into the 7d aglycones in vivo. Thus, high levels of 7d-Aolon, as found in plasma of mouse and hamster, may be correlated to higher levels of free radicals and probably more severe cardiac damage in these species. Histomorphological and electron spin resonance studies will be performed in relation to pharmacokinetics of 7d aglycones in plasma and cardiac tissue in order to investigate such a relationship.

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