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Comparative metabolism and pharmacokinetics of doxorubicin and 4'-epidoxorubicin in plasma, heart and tumor of tumor-bearing mice

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Summary. Epidoxorubicin, a stereoisomer of doxorubicin, shows comparable antitumor activity but diminished cardiotoxicity compared with the latter. To find a pharmacokinetic basis for the observed difference in cardiotoxicity between the drugs, concentrations of doxorubicin, epidoxorubicin and all known metabolites were measured in the plasma, heart and tumor tissue of BALB/c mice bearing colon-26 tumors. Both drugs were injected at the same dose (10 mg/kg) as an i. v. bolus. Plasma, heart and tumor samples were obtained from two mice sacrificed at regular intervals over 48 h. Plasma and tissue extracts were analyzed by HPLC with fluorescence detection. The parent compounds and the two 7d-aglycones were present in all three compartments, whereas doxorubicinol (Aol) and epidoxorubicinol (Eol) could only be detected in the plasma and heart. Half-lives and AUCs of doxorubicin and its metabolites were higher than the corresponding values for epidoxorubicin and its metabolites in all three compartments.

Introduction

Among anthracyclines, doxorubicin is the most active cytotoxic drug currently available [14]. However, its clinical use is hampered by unfavorable side effects such as cardiotoxicity. With the introduction of 4'-epidoxorubicin, a stereoisomer with comparable anti-tumor activity but lower cardiotoxicity [16] became available. Pharmacokinetics and metabolism studied in plasma during clinical trials have revealed a lower half-life of elimination for epidoxorubicin than for doxorubicin, which could be explained by the unique glucuronidation of the former [11, 18]. This difference in pharmacokinetic and metabolic behavior has also been used to explain the reduced cardiotoxicity of epidoxorubicin. However, glucuronidation of the latter did not take place in animals [8], yet epidoxorubicin appeared to be less cardiotoxic than doxorubicin in these species [2, 7].

Rather than in glucuronidation, an explanation might be found in pharmacokinetic and metabolic differences in the target organ, the heart. In the past, a comparative study [1] of the pharmacokinetics of these two drugs in plasma and various organs, including the heart and tumor, was carried out in rats by using radiolabeled drugs and thin-layer chromatography to separate the metabolites. However, this procedure did not enable a reliable quantification of the metabolites. Therefore, the aim of our study was to compare the pharmacokinetics and metabolism of doxorubicin, epidoxorubicin and all their known metabolites in the plasma, heart and tumor of tumor-bearing mice, using sensitive and selective HPLC procedures [9, 10].

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Materials and methods

Healthy female BALB/c mice 8 weeks of age and weighing 20 g were used for the study. Mice (Centraal Proefdieren Bedrijf TNO; Zeist, the Netherlands) had access to food and water ad libitum. Colon-26 tumors were implanted s. c. in both flanks of 47 mice under ether anesthesia. At 12 days after implantation, 2 groups of 22 mice were injected (i. v., 10 mg/kg in saline) with doxorubicin and epidoxorubicin, respectively. After 0, 5, 15 and 30 min and 1, 1.5, 2, 4, 8, 24 and 48 h, two mice in each group were sacrificed by cervical dislocation.

At each time point the blood, heart and tumor were collected. Hearts were immediately submerged in 1 ml solution containing glucose and glucaric acid 1,4-lactone to prevent decomposition of any glucuronide formed [10]. They were subsequently rinsed with 0.9% NaCl solution. Blood was centrifuged for 5 min at 3,000 rpm and plasma was removed. Plasma, hearts and tumor tissues taken at each time point were pooled. Plasma and hearts were stored at −20° C. Three mice were not treated with anthracyclines; their plasma and tissues (blanks) were also processed as described above.

Tissues were homogenized by dismembration for 1 min at 77 K and immediately suspended (10-min vortex) in the glucose/lactone solution, for a concentration of 120 mg tissue (wet wt.)/ml solution. Aliquots of 1 ml were stored at −20° C until analysis.

Plasma and tissue homogenates were extracted and purified using Sep-Pak C-18 cartridges as previously described [9, 10]. The extracts were analyzed in duplicate by HPLC with fluorescence detection within 2 months after sampling, using individual calibration lines for the parent compounds and their metabolites.

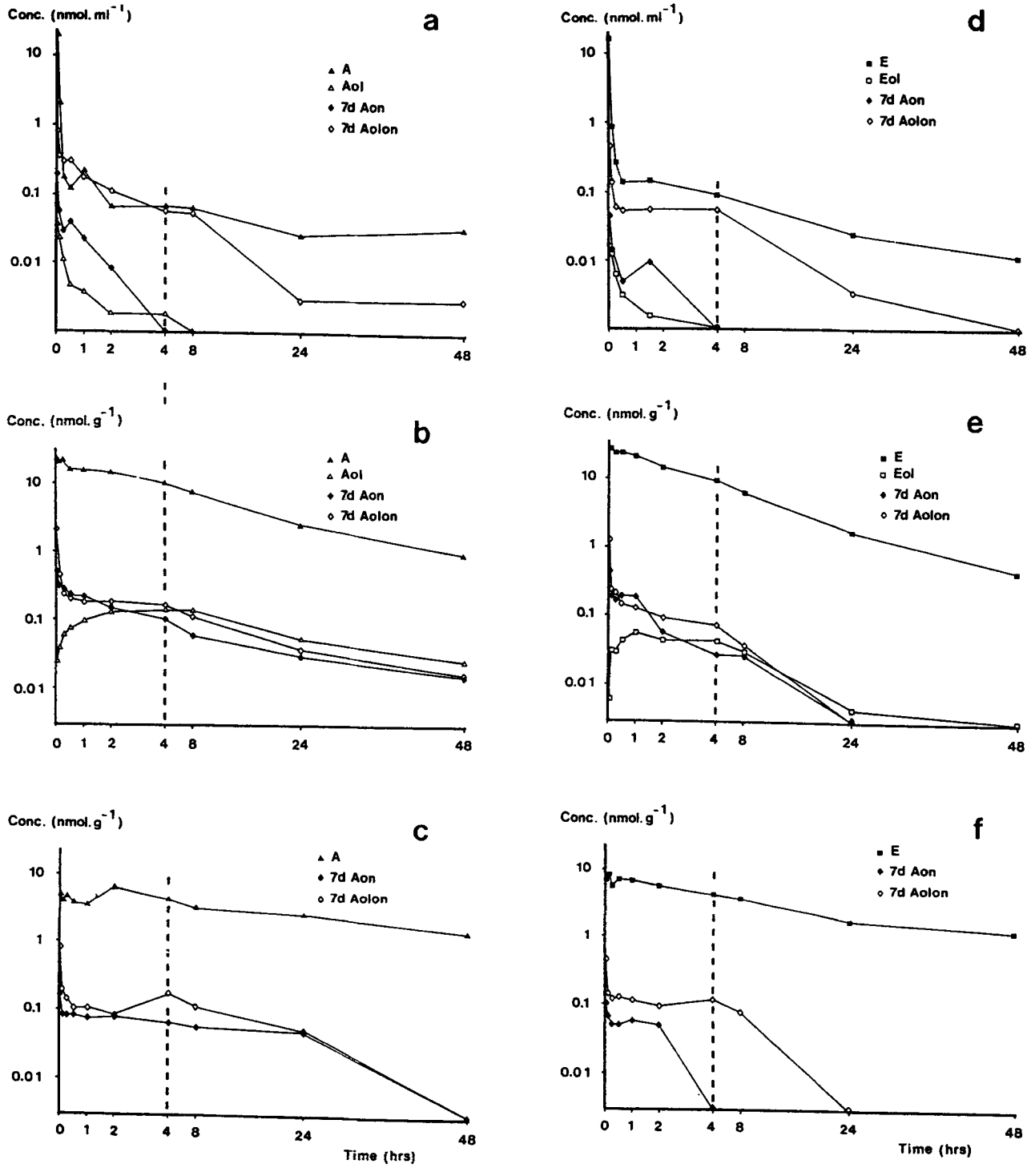

Fig. 1. Concentration-time curves for doxorubicin (*A*) and epidoxorubicin (*E*), including their metabolites, in **a, d** plasma, **b, e** heart and **c, f** tumor

Results and discussion

Concentration-time curves for doxorubicin and epidoxorubicin including their metabolites, as determined in plasma, heart and tumor tissues are shown in Fig. 1. The parent drugs were abundantly present in all compartments during the 48-h study. In plasma a rapid distribution ($t_{1/2d}$, 1 min) was followed by an intermediate and a slow elimination phase, whereas in tissues a more gradual decline of the concentration of the parent drugs was observed. High concentrations of the parent drugs in tissues found immediately after injection are in agreement with the short initial half-life of the drugs in plasma and their large volume of distribution.

Final half-lives of elimination calculated from 8 h onwards by means of the least-squares method were 49.7 and 15.6 h in plasma, 13.2 and 10.6 h in heart and 32.1 and 24.7 h in tumor for doxorubicin and epidoxorubicin, respectively. Parent drugs were slowly eliminated from tumor tissues, which is in accordance with findings of previous studies [4]. Half-lives of elimination of epidoxorubicin were smaller than those of doxorubicin in all com-

Table 1. AUC values (0–48 h) for doxorubicin, epidoxorubicin and their metabolites in plasma, heart and tumor

Compound	Plasma (nmol·min·ml^{-1})	Heart (nmol·min·g^{-1})	Tumor (nmol·min·g^{-1})
A	204.9	14,064	8,697
Aol	1.7	228	0
7dAon	4.1	152	135
7dAolon	83.3	218	204
E	179.0	12,787	7,868
Eol	0.5	46	0
7dAon	1.6	49	11
7dAolon	53.9	66	99

A, doxorubicin; E, epidoxorubicin

partments studied. A small difference between the half-lives of elimination of the drugs has also been found in the plasma of humans [11], which was explained by the unique glucuronidation of epidoxorubicin. The smaller final half-lives of the latter compared with doxorubicin in mice cannot be explained by glucuronidation of epidoxorubicin as glucuronidation does not take place in these species [10], but are in agreement with the higher biliary excretion of epidoxorubicin previously observed in rats [3, 15].

As metabolites, two 7d-aglycones (7dAon and 7dAolon) were present in plasma, heart and tumor tissue, whereas the 13-carbinol metabolites could only be observed in plasma and heart tissues. No aglycones could be detected in any of the compartments studied. These findings extend those of previous studies [4, 15] dealing with fewer metabolites in less tissues. In plasma, the concentration-time curves of the three metabolites showed, like those of the parent compounds, a rapid initial decay followed by one or two slower elimination phases.

The occurrence of peak concentrations for each of the metabolites immediately after drug administration indicates a very rapid conversion of the parent compound into these metabolites. 7dAolon was formed in the highest quantities, whereas the β-carbinol metabolites were formed to the slightest extent. The same picture was obtained in the tissues, with the exception of the β-carbinol compounds in heart tissue, which reached maximal levels 1–4 h after administration. As β-carbinol compounds are amongst the most polar metabolites, their distribution to other compartments is lower than that of the parent compounds or 7d-aglycones. This is in agreement with the relatively small volume of distribution of Aol in dogs, about 150 l [13], and its limited uptake in heart tissue in vitro [12]. Therefore, it seems plausible that the concentration-time curves of the β-carbinol compounds in heart tissue mainly originated from the production of these compounds in the tissues [5], which corresponds with the results of in vitro experiments [12]. The tissue levels of Eol were lower than those of Aol, which might be explained by epidoxorubicin being a poorer substrate than doxorubicin for conversion to its β-carbinol metabolite by aldo-keto reductase enzymes, as previously measured in crude rat-liver homogenate preparations [2]. Final half-lives of elimination of the β-carbinol metabolites in the heart corresponded with those of the parent drugs and were 16.5 and 7.0 h for Aol and Eol, respectively.

To omit possible production of 7d-aglycones by reductive cleavage of the daunosamine moiety of doxorubicin, Aol, epidoxorubicin or Eol, as previously observed in injured and dead tissues [6, 17], we handled our samples at 0° C. Moreover, we treated corresponding samples of the doxorubicin and epidoxorubicin series similarly to enable a comparison of the amounts of 7d-metabolites between the series.

AUC values as calculated by the trapezoidal rule from 0 to 48 h are shown in Table 1. Figure 1 and Table 1 clearly show that AUC values in both tissues (expressed as nmol.min.g^{-1}) were much higher than those in plasma (expressed as nmol.min.ml^{-1}). The high AUC values for the parent compounds in tissue compared with those obtained in plasma illustrate their large volume of distribution (90 l/kg). The AUCs of doxorubicin in plasma and tissues were slightly higher than those of epidoxorubicin (10%). Metabolism of the two drugs in plasma of mice, as assessed by comparing the percentual AUCs of the metabolites, was less extensive than human metabolism [11]. Furthermore, it appeared that the AUC values for Aol were higher than those for Eol in plasma of both mice and humans [11].

The AUC values are a measure of the exposure of the tissues to the parent drugs and their metabolites. The consequences of the differences in the measured AUCs for the difference in cardiotoxicity and antitumor effect between doxorubicin and epidoxorubicin can be established only if the individual antitumor and cardiotoxic effects of epidoxorubicin and all of its metabolites are known in comparison with those of doxorubicin. A recent study [12] has indicated that Aol is more cardiotoxic than doxorubicin. If such a relationship also exists between Eol and epidoxorubicin, then the relatively large difference between the small AUC values of the β-carbinol compounds might be of importance in explaining differences in the cardiotoxicity and antitumor effects of doxorubicin and epidoxorubicin.

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