Paclitaxel induces significant changes in epidoxorubicin distribution in mice

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Summary

Background: Inhibition of Pgp can affect the distribution and the pharmacokinetics of anthracyclines, causing marked changes in their toxicity. Since both paclitaxel and cremophor are substrates of Pgp, it was hypothesized that they could modify the pharmacokinetics of anthracyclines in a similar fashion.

Purpose of the study: To evaluate whether pretreatment of mice with cremophor or paclitaxel dissolved in cremophor could induce changes in the distribution of epidoxorubicin (EpiDx).

Materials and methods: Male CDF1 mice were treated with ethanol or cremophor or paclitaxel and 30 min later with EpiDx (15 mg/kg i.v.). EpiDx serum and tissue levels were determined at several time points after EpiDx treatment by high pressure liquid chromatography (HPLC) assay coupled with fluorimetric detection.

Results: Pretreatment with paclitaxel dissolved in cremophor induced a highly significant increase in EpiDx levels in all tissues examined including heart. At 8 h heart levels in mice treated with EpiDx alone, EpiDx and cremophor and EpiDx and paclitaxel were 8.3 μg/g, 10.9 μg/g and 16.7 μg/g (P < 0.01), respectively. Cremophor alone induced a similar increase in spleen EpiDx levels but had only a moderate effect on heart and lung EpiDx levels. Levels of doxorubicin (Dx) aglycone in kidney and liver of mice treated with paclitaxel and EpiDx were higher than those in mice treated with EpiDx alone.

Conclusions: A pharmacokinetic interaction between paclitaxel and EpiDx was clearly demonstrated in mice. The much higher tissue levels of EpiDx after paclitaxel pretreatment may be the reason for the increased toxicity of EpiDx when administered soon after paclitaxel.

Key words: epidoxorubicin, interactions, paclitaxel, pharmacokinetics

Introduction

Several recent preclinical and clinical reports have demonstrated that Pgp inhibitors can modify the pharmacokinetics of anthracyclines, vinca alkaloids or epipodophyllotoxins [1-3]. This interaction involves mechanisms of altered distribution, protein binding, metabolism and elimination [4, 5]. Recently detailed pharmacokinetic studies performed in mice indicate that the major mechanism of interaction between Pgp inhibitors and anthracyclines involves an increased drug retention; for example, either pretreatment with cyclosporin A or with its analogue, PSC 833, causes a significant increase in doxorubicin levels in liver, kidney, adrenals and heart [6, 7], probably due to a reduced efflux rate of the drug from these tissues. These data suggest that combinations of two antitumor natural products which are substrates of Pgp, such as anthracyclines and taxanes, could cause similar interactions. To verify this hypothesis was of particular interest because the combination of paclitaxel and anthracyclines appears clinically promising [8-10], even though some concern about its cardiotoxicity has been raised [11-14]. Since paclitaxel is formulated in cremophor, which has also been reported to be an inhibitor of Pgp [15-18], it is possible that both paclitaxel and its solvent could affect the tissue distribution of anthracyclines when administered in combination. In the present study we have investigated the effect of cremophor or paclitaxel dissolved in cremophor on the pharmacokinetics and distribution of epidoxorubicin (EpiDx) in mice. EpiDx was selected because of the great clinical interest of its use in combination with taxanes.

Materials and methods

Drugs
Paclitaxel, kindly provided by Pharmacia-Upjohn, was freshly dissolved in cremophor:ethanol:5% glucose in water (1/1/8; v/v/v). EpiDx, kindly provided by Pharmacia-Upjohn, Milan, Italy, was freshly dissolved in distilled water.

In vivo experiments
CDF1 male mice (20 ± 2 g body weight) obtained from Charles River Italia, Calco, Italy, were used for these experiments. Procedures involving animals and their care are conducted in conformity

For pharmacokinetic studies paclitaxel was injected i.v. at a dose of 25 mg/kg. This relatively low dose was selected to avoid toxic effects that might confound the interpretation of the results. At this dose paclitaxel can be easily dissolved in the solvent above indicated. EpiDx was injected i.v. at the dose of 15 mg/kg 30 min after paclitaxel. This time interval was chosen on the basis of previous studies [6, 7]. Five min, 30 min and 1, 2, 4, 8, 12, 24, 48, 72, 96, 120 hours after EpiDx, five mice per time point were exsanguinated under light ether anesthesia, and serum and tissues (heart, lung, liver, kidney and spleen) were removed and frozen at −20 °C until use.

Analytical assay

EpiDx and metabolites were quantified by high-performance liquid chromatography (HPLC) with fluorimetric detection according to a previously described technique [19], with minor modifications. After homogenization in water, tissue samples, with daunorubicin added as internal standard, were deproteinized with AgNO₃ (33%), extracted with 8 ml of propanol and centrifuged at 3000 rpm; the organic phase was evaporated to dryness under vacuum. Extracts were injected into the HPLC with fluorescence detection at an excitation wavelength of 475 nm and an emission of 580 nm. Separation was achieved with an isocratic solvent system of water:acetonitrile:0.1 M phosphoric acid using a 30-cm u.Bondapak C18 (10-µm) column. Recovery of EpiDx extraction after the addition of a known amount of drug to cell or tissue homogenate was 85%-90% and the sensitivity was 10 ng/ml for cells and 20 ng/g for tissue.

Pharmacokinetic and statistical analysis

The area under the curve of drug concentration as a function of time (AUC, µg/ml or g x h) was calculated by the trapezoidal method up to 120 hours. Statistical significance was assessed by Duncan’s test.

Results

Figure 1 shows disappearance curves of EpiDx from serum and tissues of mice receiving either EpiDx alone or EpiDx in combination with cremophor or with paclitaxel dissolved in cremophor. EpiDx serum levels were similar in all three experimental groups. In all tissues examined paclitaxel pretreatment caused an increase of EpiDx levels at several time points. Table 1 shows EpiDx levels at 30 min and EpiDx AUC levels. As it can be seen in Figure 1, the most striking differences were observed in liver where after some time points, EpiDx levels were 2–3 times higher in the group of mice pretreated with paclitaxel. EpiDx AUC values in liver were 84% higher in mice treated with paclitaxel and EpiDx than in mice treated with EpiDx alone (Table 1).

In the other tissues EpiDx levels were also higher in mice pretreated with paclitaxel, although the differences were less marked. In heart, EpiDx levels were higher in mice pretreated with paclitaxel, with the highest difference at 8 hours, when EpiDx heart levels were 2 times higher in mice treated with paclitaxel and EpiDx than in those treated with EpiDx alone.

In lung, EpiDx levels were significantly higher in mice treated with paclitaxel at all time points except at 5 minutes. In both kidney and spleen, EpiDx levels were consistently higher in mice pretreated with paclitaxel, not only in the first hours but also at 24 and 48 hours.

EpiDx AUC values in heart, lung and spleen of mice pretreated with cremophor were markedly higher than those in mice treated with EpiDx alone. In all other tissues except spleen the differences were lower than those observed with paclitaxel pretreatment. In spleen cremophor caused an increase in EpiDx levels identical to the one seen with paclitaxel.

Figure 2 shows that both cremophor and paclitaxel pretreatment caused an increase in liver and kidney levels of doxorubicin (Dx) aglycone.

Discussion

The present study shows that the tissue distribution of EpiDx in mice is significantly modified by 30-min ad-

Figure 1. Disappearance curves of EpiDx in serum and tissues of mice treated with EpiDx alone, 15 mg/kg i.v. (●—●), EpiDx and cremophor (○--○), EpiDx and paclitaxel, 25 mg/kg i.v. (△--△).
The same authors reported that no increase in Dx heart activity is also suggested by recent clinical reports [21-23].

Concentration was found when the interval between paclitaxel and Dx was 18 hours. That the interval between received paclitaxel 4 hours before or concomitantly.

This finding suggests that the cardiotoxicity of anthracyclines could be increased when these drugs are combined with paclitaxel. Gianni et al. [12] recently reported also in another study with the same combination.

The mechanism by which paclitaxel induces an increase in anthracycline tissue distribution has not yet been investigated. One possibility is that paclitaxel inhibits Pgp, thus reducing the efflux of anthracyclines from tissues. This explanation is supported by the recent report that in knockout mice in which mdr-1a has been disrupted the concentrations of drugs which are substrates of Pgp in several tissues, including heart, were much higher than in control mice which expressed a functional Pgp protein [25, 26].

In our study an interaction between the two drugs, with an increase in EpiDx heart levels is particularly evident in the first hours after treatment, when the concentrations of EpiDx are the highest. Whether this has a corresponding lower cardiotoxic impact than Dx needs to be confirmed in the clinical setting.

However, Conte et al. [24] have reported, in metastatic breast cancer patients previously treated with anthracyclines and subsequently treated with an EpiDx and Taxol combination, very little cardiotoxicity.

The dose of paclitaxel selected for our studies was on purpose very low to avoid any possible acute toxicity which might influence the pharmacokinetics of EpiDx. It is therefore possible that at higher doses paclitaxel could induce an even more marked change in EpiDx distribution.

Our results are in accord with those recently reported in abstract form by Solis Recéndez et al. [20] who found an increase in Dx heart levels in mice that had received paclitaxel 4 hours before or concomitantly. The same authors reported that no increase in Dx heart concentration was found if the interval between paclitaxel and Dx was 18 hours. That the interval between paclitaxel and anthracyclines is crucial for cardiotoxicity is also suggested by recent clinical reports [21-23].

### Table 1. EpiDx mean levels (± s.e.) at 30 min, 8 h, 48 h and mean AUC values determined in male CDF1 mice given EpiDx alone or in combination with cremophor or paclitaxel.

<table>
<thead>
<tr>
<th>EpiDx alone</th>
<th>EpiDx and cremophor</th>
<th>EpiDx and paclitaxel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(AUC up to 24 h)</td>
<td>±0.07 ±0.01</td>
<td>±0.04 ±0.01</td>
</tr>
<tr>
<td>Heart</td>
<td>38.92 8.29 0.92 309.30</td>
<td>43.04 10.88* 0.95 347.57</td>
</tr>
<tr>
<td>Lung</td>
<td>60.19 17.92 5.21 896.15</td>
<td>73.13 26.14* 5.64 1074.00</td>
</tr>
<tr>
<td>Liver</td>
<td>21.84 5.27 0.64 180.53</td>
<td>24.65 7.40 0.49 198.25</td>
</tr>
<tr>
<td>Kidney</td>
<td>84.45 40.39 9.50 1640.84</td>
<td>89.25 38.11 15.17b 1736.05</td>
</tr>
<tr>
<td>Spleen</td>
<td>26.56 41.54 25.43 2854.89</td>
<td>36.41 54.62b 38.65b 3593.13</td>
</tr>
<tr>
<td>(AUC up to 48 h)</td>
<td>±2.54 ±0.89 ±0.13</td>
<td>±2.98 ±0.67 ±0.31</td>
</tr>
</tbody>
</table>

* P < 0.05 vs. EpiDx alone; † P < 0.01 vs. EpiDx alone; ‡ P < 0.05 vs. cremophor; § P < 0.01 vs. cremophor, Duncan's test.

EpiDx, dissolved in H2O was injected at the dose of 15 mg/kg i.v. 30 min after cremophor or after paclitaxel (25 mg/kg i.v.). Five mice per time point were studied.

- **Figure 2.** Dx-aglycone levels in liver and kidney of mice treated with EpiDx alone, 15 mg/kg (●—●), EpiDx and cremophor (○—○), EpiDx and paclitaxel, 25 mg/kg i.v. (Δ—Δ).
in a detectable amount under our conditions was Dx-aglycone. This metabolite was found at higher levels in both liver and kidney of mice treated with paclitaxel. This finding excludes the possibility that the higher tissue levels of EpiDx in mice treated with paclitaxel were due to an inhibition of the drug metabolism. The higher tissue levels of Dx-aglycone may indicate that the efflux of this metabolite from the tissues where it has been found is decreased by paclitaxel, possibly by the same mechanism which causes an increased tissue retention of EpiDx.

For most tissues the increase in EpDx tissue levels following pretreatment with cremophor was much smaller than that seen with paclitaxel, suggesting that paclitaxel itself is mainly responsible for the observed interaction. However, this is not the case in spleen, where cremophor produced the same increase in EpiDx levels as paclitaxel. The reason for this particularly marked effect of cremophor in spleen requires further investigations, as it may be of relevance for the toxicity of the combination.

In conclusion, this study unequivocally demonstrates the existence of a pharmacokinetic interaction between paclitaxel and EpiDx.

This interaction should be considered when clinical trials with the combination of anthracyclins and taxanes are planned. It is possible that EpiDx, being less cardiotoxic than Dx [28, 29] would be more suitable for these combinations. The effect of a longer time interval between the administration of paclitaxel and EpiDx itself is mainly responsible for the observed interaction. However, this is not the case in spleen, where cremophor produced the same increase in EpiDx levels as paclitaxel. The reason for this particularly marked effect of cremophor in spleen requires further investigations, as it may be of relevance for the toxicity of the combination.

In conclusion, this study unequivocally demonstrates the existence of a pharmacokinetic interaction between paclitaxel and EpiDx.

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