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Accumulation and Metabolism of New Anthracycline Derivatives in the Heart After IV Injection into Mice

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Summary. *In an attempt to establish a relationship between the pharmacokinetics in mouse heart of new anthracycline derivatives and their potential chronic cardiotoxicity and in this way to provide a useful and economical test for screening of new analogs, we followed the accumulation and metabolism of six anthracyclines in the mouse heart after single IV administrations of these drugs at equimolar doses. We found that the six drugs, i.e., daunorubicin (DNR), doxorubicin (DOX), rubidazone (RBZ), detorubicin (DET), N-L-leucyl-DNR (LEU-DNR) and N-L-leucyl-DOX (LEU-DOX), accumulate at various levels in the cardiac tissue and are metabolized to different extents, leading to the appearance in the heart of variable amounts of DNR or DOX. The total exposure of the mouse heart, as evaluated by calculation of the areas under the C×t curves, can be correlated qualitatively with the chronic cardiotoxicity of the six anthracyclines, as recently determined in the rabbit model. We therefore think that our study provides a simple, rapid, and inexpensive predictive test for the screening of new analogs for potential cardiotoxicity. Moreover, it offers the advantage of using the same species for determining the most favorable ratio between therapeutic activity and toxic side-effects.*

Introduction

We have previously studied the distribution and metabolism in mice of new derivatives of daunorubicin (DNR) and doxorubicin (DOX), i.e., 13-benzoylhydrazone-DNR [1] or rubidazone (RBZ), 14-diethoxyacetoxy-DNR [5] or detorubicin (DET), *N*-L-leucyl-DNR (LEU-DNR) [4, 6], and *N*-L-leucyl-DOX (LEU-DOX) [6]. The drug levels reached in the heart 30 min after IV injection were found to be significantly lower after administration of RBZ [1] and the two leucyl derivatives [6] than after their parent anthracyclines DNR or DOX. Moreover, we have observed recently that in the rabbit there is a correlation between the total exposure of the heart to drug and metabolites and the chronic cardiotoxicity of several anthracycline derivatives [8]. In the development of new anthracyclines a great deal of effort is devoted to decreasing the cardiotoxicity of the new compounds while maintaining or improving their therapeutic effectiveness. The possibility of evaluating the cardiotoxicity of anthracyclines by a simple, rapid, and economical test requiring small amounts of drug would therefore speed up and facilitate the screening of new analogs. For this purpose, we followed the accumulation and metabolism of six anthracycline analogs at equimolar doses in mice after a single IV injection, with a view to determining a possible relationship between the pharmacokinetics of the drugs in the heart and their potential chronic cardiotoxicity.

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Materials and Methods

Daunorubicin (DNR; 13 057 R.P.), rubidazone (RBZ; 22 050 R.P.), detorubicin (DET; 33 921 R.P.), *N*-L-leucyl-daunorubicin (LEU-DNR; 20 132 R.P.), *N*-L-leucyl-doxorubicin (LEU-DOX; 39 937 R.P.), and doxorubicin were kindly provided as hydrochlorides by Rhône-Poulenc, SA, Paris, France.

Female NMRI mice (Proefdierencentrum, Heverlee, Belgium) weighing about 24 g received injections into the tail vein of equimolar doses of the different drugs equivalent to 7 mg DNR/kg.

Drugs were dissolved in physiological saline with the exception of RBZ, which was diluted in 0.7 *M* glycine buffer, pH 7.5 [1]. Special care was also taken with DET, which was prepared immediately before use in ice-cold saline because of its instability in physiologic conditions [5].

After various times, the mice were sacrificed by decapitation, blood samples were collected, and the thorax was immediately

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opened. The whole heart was taken, incised in the middle, rapidly rinsed, and put in 1 ml ice-cold saline. The hearts were directly homogenized in 7-ml glass Potter-Elvehjem homogenizers. After rinsing of the homogenizer with 1 ml saline, the pooled suspensions were sonicated for 30 s at 50 W (Sonicator B-12, Branson Sonic Power Co., Danbury, USA). Blood was sonicated in the same manner.

In the case of DET administration, the hearts were collected directly in 1 ml ice-cold DNA solution (type VII, Sigma Chemicals, St Louis, USA) at 2.34 mg/ml. The use of cold DNA avoids an in vitro tranformation of DET to DOX [5]. The heart and blood proteins were measured by the method of Lowry et al. [11]. The amount of blood contaminating each heart sample was carefully measured according to the immunologic method of Mancini et al. [12], by estimating the concentration of serum albumin present in the heart. The rabbit serum directed against mouse serum albumin was kindly supplied by Dr. J. P. Vaerman, Laboratoire de Médecine Expérimentale, ICP, Brussels, Belgium. The drug and protein values of each heart sample were corrected for the respective quantities arising from the blood contamination.

Drugs were analysed on 0.1 ml samples by high-pressure liquid chromatography (HPLC) and fluorometry according to the method described previously [2].

Results

We followed the drug accumulation in the heart in the hours immediately following IV injection into NMRI mice of six anthracycline derivatives: DNR, DOX, DET, RBZ, LEU-DNR, and LEU-DOX, at equimolar doses. The results obtained are reported in Fig. 1 for DNR and its derivatives, while Fig. 2 shows the results obtained with DOX and its derivatives. We have indicated the total amount of drug (intact drug plus fluorescent metabolites) accumulated in the heart in part A of each of Figs. 1 and 2. Figure 1B gives the amount of DNR found in the heart after injection of DNR, RBZ, and LEU-DNR; and Fig. 2B, the amount of DOX found in the heart after injection of DOX, DET, and LEU-DOX.

The heart content in drugs and proteins has been corrected for blood contamination (see *Materials and Methods*); the correction is particularly important with regard to the amount of drug at very short times, but also with regard to the protein. Indeed, we have evaluated the amount of blood present in about 400 heart samples, and have calculated that of a mean total protein content of 6.0 mg/ml in the heart homogenates 1.6 mg protein/ml arose from the contaminating blood.

The highest drug level was reached in the heart within 15 min after IV injection of DNR, RBZ, and LEU-DNR. The peak of maximal accumulation occurred around 30 min for DET and LEU-DOX and at 1 h for DOX.

The two parent anthracyclines, DNR and DOX, and DET accumulated at higher concentrations in mouse heart than did the other derivatives. The leucyl derivatives are characterized by the lowest drug levels.

Using the HPLC technique, we were also able to follow the metabolism of each anthracycline in the heart. We had previously studied the metabolism of DET [5] and RBZ [1] in several tissues, including the heart. Both drugs are rapidly hydrolysed in their parent anthracyclines [1, 5], and RBZ was transformed to DOL to a lesser extent than DNR. Only trace amounts of doxorubicinol (DOX-OL) were found in mouse heart after DOX administration, whereas this metabolite was not detected in the cardiac tissue after injection of DET. LEU-DNR was

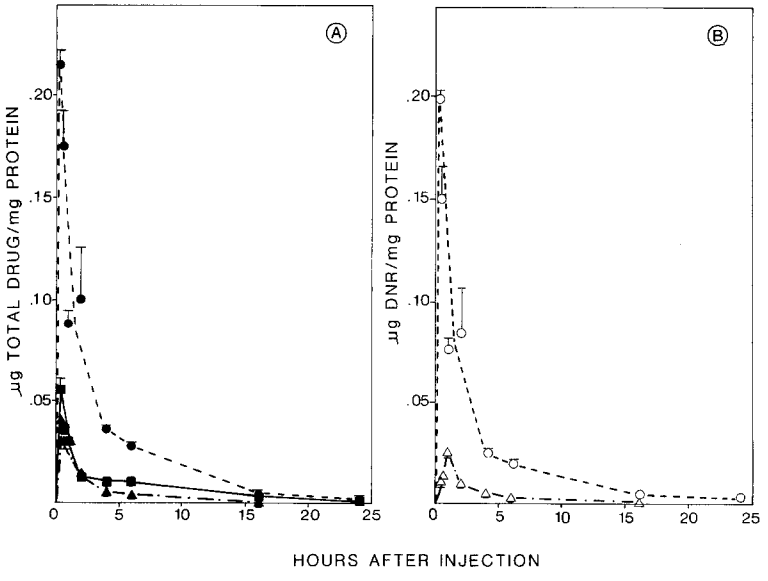

Fig. 1A and B. Drug accumulation in the heart after single IV administrations of DNR, RBZ, and LEU-DNR. The drugs were injected IV into NMRI mice at equimolar doses corresponding to 7 mg/kg for DNR. Means (*bars*; SE) from at least three mice are given for each observation point. **A** Total amount of drug fluorescence after DNR(●), RBZ(■) or LEU-DNR(▲); **B** amount of DNR present in the heart after injection of DNR(○) or LEU-DNR(△)

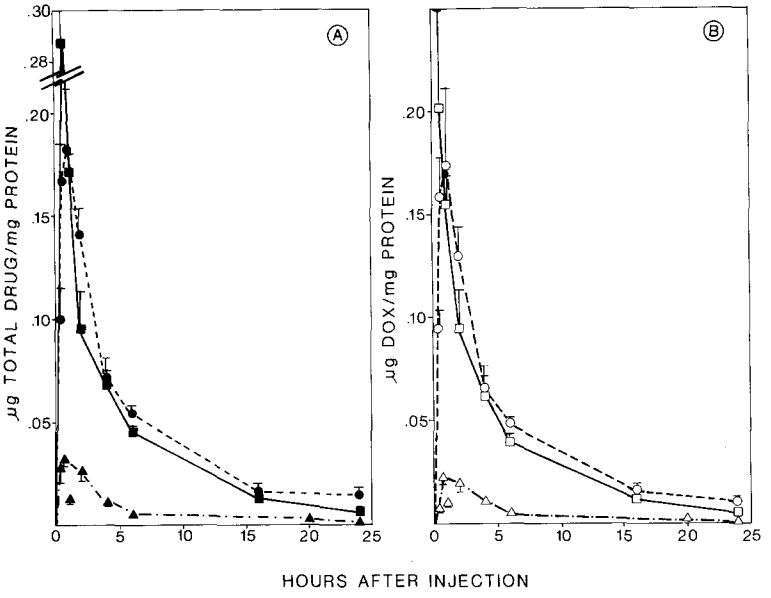

Fig. 2A and B. Drug accumulation in the heart after single IV administration of DOX, DET and LEU-DOX. The drugs were injected IV into NMRI mice at equimolar doses corresponding to 7 mg/kg for DNR. Means (*bars*; SE) from at least three mice are given for each observation point. **A** Total amount of drug fluorescence after DOX (●), DET (■) or LEU-DOX (▲); **B** amount of DOX present in the heart after injection of DOX (○), DET (□), or LEU-DOX (△)

Table 1. Evaluation of the total exposure[a] of the heart to the anthracyclines after single IV administration into NMRI mice

Drug	S (C × t) µg drug/mg protein × hour	
	Total drug	DNR or DOX
DOX	1.28	1.19
DET	1.04	0.92
DNR	0.66	0.54
RBZ	0.26	< 0.21
LEU-DOX	0.15	0.12
LEU-DNR	0.10	0.07

[a] The total exposure of the heart was estimated by calculation of the areas (S) under the curves (concentration × time; C × t) up to 44 h after IV injection of the drugs. The total amount of drug and the amount of DNR or DOX present in the heart after IV injection of the anthracyclines at equimolar doses were taken into account

found mainly in its intact form up to 1 h after IV injection, while DNR, produced by hydrolysis, became the main metabolite after 1 h; DOL was also found in the heart in increasing quantities, but to a lesser extent than DNR. LEU-DOX was more than 50% hydrolysed into DOX even after 30 min, and no other metabolites were detectable in the myocardium. Finally, very small quantities of aglycones were found in some samples.

For each anthracycline we calculated the area under the C×t curve to estimate the exposure of the heart up to 44 h after a single IV injection of the different drugs. We recorded both the total drug and the amount of DNR or DOX only present in the heart tissue after anthracycline administration. These data are reported in Table 1. The total exposure of the heart to the drug was highest after injection of DOX and lowest after administration of the leucyl derivatives.

Discussion

We demonstrated in a previous paper [8] that in the rabbit the chronic cardiotoxicity of several anthracyclines could be related to pharmacokinetic data obtained in the cardiac tissue of this animal species after a single IV injection. The cardiotoxicity of anthracyclines is still poorly understood, and several hypotheses have been proposed. The anthracycline-induced cardiomyopathy is most probably related to the accumulation of the active, i.e., toxic drug or metabolite in the myocardium, however.

We have therefore followed the accumulation and metabolism in the mouse heart of six anthracycline derivatives, closely related chemically and recently characterized as more or less potent cardiotoxic agents in a complete chronic cardiotoxicity study [8]. We have found in mice that, after a single IV injection at equimolar doses, the different anthracyclines accumulate at various levels in the cardiac tissue. They undergo different degrees of metabolic transformation, leading to the production in the heart of various amounts of DNR or DOX. The highest exposure of the heart to anthracyclines, as evaluated by the areas under the C×t curve, is reached after DOX administration, while both leucyl derivatives are characterized by the lowest accumulation. The amount of total drug and of DNR present in the heart after injection of RBZ is at least two times smaller

than that reached after DNR injection. Borderline separation of RBZ from its metabolite DNR in some samples restricted us in the quantitative determination of the exposure of the heart to DNR after RBZ treatment. LEU-DNR accumulates to a lesser degree in the heart than LEU-DOX; moreover, the amounts of DNR found after LEU-DNR injection were smaller than the amounts of DOX present after LEU-DOX administration.

If the total exposure of mouse heart to anthracyclines is considered, especially in terms of the relative amount of DNR or DOX present after administration of the different derivatives, it is possible to establish a classification of the various anthracycline drugs that is qualitatively related to their chronic cardiotoxicity in the rabbit. This estimation is based on the assumption that DNR and DOX, or perhaps DOL and DOX-OL, are the true cardiotoxic compounds. We think it is possible to make a first screening of new analogs with regard to their potential cardiac toxicity by determining simply, rapidly, and at minimal cost, the accumulation and metabolism of each of these new derivatives or of their active metabolites in the mouse heart after a single IV administration. An in vivo determination of pharmacokinetic parameters is of crucial importance for predicting toxic cumulative phenomena like anthracycline cardiotoxicity, and many factors indeed seem to contribute to this chronic toxicity, which can only be analysed in vivo [7].

The evaluation of the exposure of the heart to drug and metabolites is less relevant quantitatively in the mouse than in the rabbit, if relative chronic cardio toxicity in the rabbit is taken as a reference. Indeed, if the total exposure of the heart is compared in terms of the relative amount of DNR or DOX in the rabbit [8], decreasing exposure goes in parallel with decreased chronic cardiotoxicity, and the six anthracyclines studied can be classified in exactly that way. In the mouse, however, the results shown in Table 1 seem to indicate that DOX and DET could be quantitatively more cardiotoxic than DNR, whilst LEU-DNR and LEU-DOX seem to be equally less toxic for the mouse heart. These differences between the two animal models most probably reflect species-specific variations, especially in the extent of drug metabolism.

The mouse model is becoming increasingly appreciated in the evaluation of antitumor drugs. Besides the advantage that a large number of inbred strains and tumors are available, the requirement of smaller amounts of drug in preliminary studies, when compounds may only be available in small quantities and at a high cost, is another great advantage of this model. Recent efforts have been devoted to the study of the cardiotoxicity of anthracyclines in mice to establish for new analogs the ratio between antitumor activity and cardiotoxicity [3, 7, 9, 10, 13]. A chronic cumulative cardiomyopathy has only been described in mice for DOX, however [3, 10]. Alterations in the myocardium after a single IV drug dose have been described for both DNR [14] and DOX [9]; for DNR, in spite of a higher dose (20 mg/kg IV vs. 10 mg/kg for DOX), degenerative lesions could only be observed in the mitochondria.

Our observation that DOX accumulates at the highest levels and consequently induces the greatest exposure of mouse heart to an anthracycline is in agreement with the observation that DOX is a very potent cardiotoxic agent in mice. It would be interesting to compare the six anthracyclines we studied here in a long-term murine cardiotoxicity experiment similar to that reported by Bertazzoli and co-workers [3], allowing the calculation of a median cumulative cardiotoxic dose for each derivative.

In conclusion, we believe that the study of drug accumulation and metabolism in mouse heart at various times after a single IV injection of anthracycline derivatives, allowing a rapid evaluation of the heart exposure to drug and metabolites, can give valuable information on the potential chronic cardiotoxicity of new analogs.

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