METABOLISM OF THE ONE-RING OPEN METABOLITES OF THE CARDIOPROTECTIVE DRUG DEXRAZOXANE TO ITS ACTIVE METAL-CHELATING FORM IN THE RAT

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ABSTRACT:
Dexrazoxane (ICRF-187) is clinically used as a doxorubicin cardioprotective agent and may act by preventing iron-based oxygen free radical damage through the iron-chelating ability of its fully hydrolyzed metabolite ADR-925 (N,N’-[1S]-1-methyl-1,2-ethanediyl]bis[N-(2-amino-2-oxoethyl)]glycine). Dexrazoxane undergoes initial metabolism to its two one-ring open intermediates and is then further metabolized to its active metal ion-binding form ADR-925. The metabolism of these intermediates to the ring-opened metal-chelating product ADR-925 has been determined in a rat model to identify the mechanism by which dexrazoxane is activated. The plasma concentrations of both intermediates rapidly decreased after their i.v. administration to rats. A maximum concentration of ADR-925 was detected 2 min after i.v. bolus administration, indicating that these intermediates were both rapidly metabolized in vivo to ADR-925. The kinetics of the initial appearance of ADR-925 was consistent with formation rate-limited metabolism of the intermediates. After administration of dexrazoxane or its two intermediates, ADR-925 was detected in significant levels in both heart and liver tissue but was undetectable in brain tissue. The rapid rate of metabolism of the intermediates was consistent with their hydrolysis by tissue dihydroorotase. The rapid appearance of ADR-925 in plasma may make ADR-925 available to be taken up by heart tissue and bind free iron. These studies showed that the two one-ring open metabolites of dexrazoxane were rapidly metabolized in the rat to ADR-925, and thus, these results provide a mechanism by which dexrazoxane is activated to its active metal-binding form.

METABOLISM OF THE ONE-RING OPEN METABOLITES OF THE CARDIOPROTECTIVE DRUG DEXRAZOXANE TO ITS ACTIVE METAL-CHELATING FORM IN THE RAT

Dexrazoxane (ICRF-187, Zinecard; Fig. 1) is clinically used to reduce doxorubicin-induced cardiotoxicity (Hasinoff, 1998; Hasinoff et al., 1998; Cvetkovic and Scott, 2005). There is now considerable evidence to indicate that this toxicity may be due to iron-dependent oxygen free radical formation (Maliszsa and Hasinoff, 1995; Meyers, 1998) on the relatively unprotected cardiac muscle. Thus, dexrazoxane can be considered a prodrug analog of EDTA that is activated upon hydrolysis to its one-ring open intermediates B and C, and then to its fully ring-opened form ADR-925 according to the scheme in Fig. 1 (Hasinoff, 1990, 1994a,b, 1998; Schroeder et al., 2005). Thus, dexrazoxane may act through its ring-opened hydrolysis product ADR-925 (Fig. 1), which can either remove iron from the iron-doxorubicin complex (Buss and Hasinoff, 1993) or bind free iron, thus preventing iron-based oxygen radical formation.

Under physiological conditions (37°C and pH 7.4), dexrazoxane is only slowly hydrolyzed to B and C (t1/2 of 9.3 h), and to the final hydrolysis product ADR-925 (t1/2 of 23 h) according to the kinetic scheme shown in Fig. 1 (Hasinoff, 1994a,b). Given the slow rate at which dexrazoxane hydrolysis-activation occurs in vitro, it is, thus, unclear how sufficient amounts of ADR-925 could be present in heart tissue to chelate iron and prevent oxygen radical damage before dexrazoxane was eliminated (β-phase t1/2 of 4.2 ± 2.9 h in humans) (Hochster et al., 1992). It is, however, observed that in both humans (Schroeder et al., 2003) and rats (Schroeder and Hasinoff, 2002), dexrazoxane is quickly metabolized to B and C, and then to ADR-925. The rapid rate of hydrolysis of dexrazoxane to B and C and the rapid appearance of ADR-925 in plasma in vivo suggested that, first, dexrazoxane and, then, B and C were all enzymatically metabolized.

We previously showed that the dexrazoxane metabolic intermediates B and C are rapidly formed from dexrazoxane in a primary rat hepatocyte suspension (Hasinoff et al., 1994), a result that is consistent with dexrazoxane being metabolized by the zinc hydrolase DH-Pase (EC 3.5.2.2). However, although pure DHPase enzymatically hydrolyzes dexrazoxane to B and C, it is unable to enzymatically hydrolyze B and C to ADR-925 (Hasinoff et al., 1991; Hasinoff, 1993). In other work with purified DHase (EC 3.5.2.3) we showed that this zinc hydrolase is able to enzymatically hydrolyze B and C to ADR-925, but cannot act on dexrazoxane (Schroeder and Hasinoff, 2002). Thus, DH-Pase and DHase may act sequentially, and in concert, to effect the full metabolism of dexrazoxane to its active metal ion-chelating form ADR-925. Whereas DHase is present in a variety of tissues including the heart, liver, and kidney (Kennedy, 1974), DHase is only present in the liver and the kidney, but not in the heart (Dudley et al., 1974; Hasinoff et al., 1991; Hamajima et al., 1996). Because dexrazoxane acts as a cardioprotective agent, we
investigated the metabolism of its one-ring open intermediates B and C, and ultimately to its presumably active strongly metal ion-chelating form ADR-925. Where indicated, only DHPase catalyzes the conversion of dexrazoxane into either B or C, and where indicated, only DHOase catalyzes the conversion of either B or C into ADR-925.

Materials and Methods

Materials. Dexrazoxane hydrochloride and ADR-925 were gifts from Adria Laboratories (Columbus, OH) and were used as supplied. HPLC-grade methanol was obtained from Fisher (Nepean, ON, Canada). Catalase (C-30), Tris, Chelex resin, 1-heptanesulfonic acid, and 1-octanesulfonic acid were obtained from Sigma (St. Louis, MO). Calcein ("high purity") was obtained from Molecular Probes (Eugene, OR). Pharmacokinetic analyses were carried out using WinNonlin 4.0 (Pharsight, Mountain View, CA). Errors where cited or plotted are S.E.s.

Analysis of B, C, and ADR-925. The HPLC analysis of dexrazoxane, B and C using an ion-pair reagent with the reversed-phase C18 column (detection wavelength 205 nm) has been described (Schroeder et al., 2002, 2003, 2005). We have previously described a calcein fluorescence flow-injection analysis determination of ADR-925 on an HPLC apparatus (Schroeder et al., 2002, 2003, 2005). In this study, ADR-925 was determined using the same method except that the fluorescence was determined on a BMG LABTECH (Durham, NC) Fluostar Galaxy 96-well fluorescence plate reader (λex: 485 nm; λem: 520 nm).

DHPase-Mediated Biosynthesis of the B/C Mixture. Milligram quantities of a mixture of B and C were biosynthesized using the dexrazoxane-hydrolyzing activity of DHPase. We previously showed that DHPase rapidly hydrolyzes dexrazoxane to B and C, but not to ADR-925 (Hasinoff et al., 1991; Hasinoff, 1993). DHPase is present as a minor protein contaminant in C-30 Sigma bovine catalase. We and others have previously used it as a source to obtain pure DHPase (Hasinoff et al., 1991; Hasinoff, 1993). Four milliliters of a crystalline suspension of catalase (35 mg of protein/ml) were dialyzed (molecular weight cut-off of 12,000) for 24 h at 4°C with 4 liters of distilled water to remove preservative. Two milliliters of dexrazoxane hydrochloride in water (30 mg/ml) were titrated with 5 M NaOH to pH 7 and then added to the dialyzed catalase/DHPase mixture such that the final dexrazoxane solution concentration was 10 mg/ml. The reaction was carried out at room temperature and was monitored by HPLC, and typically reached completion in 45 to 60 min. The reaction was deemed complete when the dexrazoxane levels were less than 10 μM (or less than 0.03 mol % of the initial dexrazoxane concentration). Acetonitrile (2:1, v/v) was used to precipitate proteins from the reaction mixture. After centrifugation to remove precipitated protein, the B/C mixture was dried under nitrogen and reconstituted in sterile saline (0.9% NaCl w/v) just before use. Under these reaction conditions, dexrazoxane was hydrolyzed to B and C with a B/C ratio of 4:1:1, which was consistent with our previous published enzyme kinetic reports of DHPase-mediated hydrolysis of dexrazoxane (Hasinoff, 1993). The B/C mixture obtained was also analyzed for ADR-925 and was found to contain less then 0.5 mol % ADR-925.

Preparation of ADR-925 from Dexrazoxane. ADR-925 used for the animal experiments was prepared by adding 50 μl of 5 M NaOH per ml to 10 mg/ml dexrazoxane hydrochloride solution for 48 h. We previously showed that the NaOH-catalyzed hydrolysis of dexrazoxane gave ADR-925 (Hasinoff, 1994a). At 48 h, the reaction was quenched with 45 μl of 5 M HCl per ml and was titrated to pH 7. ADR-925 prepared in this fashion contained less then 0.003, 0.006, and 0.006 mol % of dexrazoxane, B, and C, respectively. The ADR-925 was filter-sterilized prior to animal use.

Dosing and Sample Collection. The rats (male Sprague-Dawley, 300–350 g) were allowed food and water ad libitum before the study. The rats were anesthetized i.p. with a combination of ketamine (90 mg/ml) and xylazine (10 mg/kg), and the left jugular vein was cannulated. The B/C mixture and ADR-925 were administered at a dose of 20 mg/kg (5 mg/ml in saline) as an i.v. bolus infusion (2 ml/min) through the tail vein. Blood samples (300 μl) were removed from the left jugular vein, as described (Schroeder and Hasinoff, 2002), from a group of three rats at times of 2, 15, 30, 45, 60, and 90 min. One 300-μl blood sample was drawn from each rat prior to the administration of the B/C mixture or ADR-925 as a control. The blood samples were added to heparin (10 μl of 1000 units/ml) and centrifuged for 5 min at 500g. The plasma was removed, 20 μl of 5 M HCl/ml of plasma was added, and the samples were stored at −80°C to prevent further hydrolysis (Hasinoff, 1994a). To precipitate plasma proteins, acetonitrile ( Fisher ) was added in a 2:1 ratio (v/v). The sample was mixed, allowed to settle for 5 min, and centrifuged at 8000g for 10 min. The supernatant was removed, acidified to pH 3 with HCl, and evaporated to dryness under nitrogen. The samples for the ADR-925 analysis were reconstituted in water to their original volume just before analysis. Samples for dexrazoxane, B, and C analysis were reconstituted in 10 mM HCl/500 μM disodium EDTA to their original volume just prior to analysis. The dexrazoxane hydrochloride (40 mg/kg) for the tissue level studies was administered as described (Schroeder and Hasinoff, 2002). The animal protocol was approved by the University of Manitoba Animal Care Committee.

ADR-925 Levels in Liver, Heart, and Brain Homogenates. After the last blood collection time point (2.8 h after the infusion of dexrazoxane, or 1.5 h after the infusion of the B/C mixture or ADR-925), the heart, brain, and liver were removed and weighed. Using surgical scissors, the organs were cut into small pieces (<5 mm³), washed three times in a 50-ml centrifuge tube by adding 30 ml of 10 mM HCl to stimulate dexrazoxane and B and C (Hasinoff, 1994a), and rapidly swirled for about 1 min, after which the wash solution was discarded. After the third wash, the wash solution was visibly clear of blood. The washed minced organs were homogenized using a Polytron homogenizer (Kinematica, Basel, Switzerland) for 5 min. The homogenate was then centrifuged at 0°C for 2 h at 18,000g. The supernatant was removed and placed in a 1.5-ml microcentrifuge tube and stored at −80°C until analyzed. For the animals treated with dexrazoxane, part of the supernatant sample was treated with 250 mM NaOH for 15 h to convert dexrazoxane, B, and C into ADR-925 (Hasinoff, 1994a) and, thus, have some measure of the total amount (ADR-925tot) of all forms of dexrazoxane in the tissues.

Results

Metabolism of the B/C Mixture in the Rat. As shown in Fig. 2a, when rats were treated with the B/C mixture, both B and C underwent rapid elimination. It is clear that B and C were quickly metabolized to ADR-925 since their hydrolysis product ADR-925 was present in the plasma at its maximum level by the first time point of 2 min.
for ADR-925) were calculated from simultaneously fitting the whole concatenated data set to eqs. 4, 5, and 6. These equations describe a model in which ADR-925 is replotted for times up to 30 min only, for fitting to an ADR-925 formation rate-limited model. The smooth lines (solid lines for B and C and a dashed line for ADR-925) were calculated from simultaneously fitting the whole concatenated data set to eqs. 4, 5, and 6. These equations describe a model in which ADR-925 is formation rate-limited by its rapid in vivo metabolism.

Subsequently, ADR-925 itself was also quickly eliminated from the plasma.

In an initial pharmacokinetic analysis, the plasma concentrations for B, C, and ADR-925 over the first 30 min were each individually fitted to an i.v. bolus one-compartment model with a single exponential term, where \( C_B, C_C, \) and \( C_{adr} \) are the concentrations of B, C, and ADR-925 at time \( t \), respectively. \( C_B, C_C, \) and \( C_{ADR} \) are their respective concentrations at time 0, and \( k_b, k_c, \) and \( k_{adr} \) are their respective first-order elimination rate constants in eqs. 1, 2, and 3:

\[
\begin{align*}
    C_b &= C_B e^{-k_b t} \\
    C_c &= C_c e^{-k_c t} \\
    C_{adr} &= C_{adr} e^{-k_{adr} t}
\end{align*}
\]

The averages of the individually derived pharmacokinetic parameters for B, C, and ADR-925 after the bolus administration of the B/C mixture are given in Table 1. From the elimination rate constants in Table 1, it can be seen that B and C were rapidly eliminated, with half-lives of 4.6 and 3.2 min, respectively. ADR-925 was, likewise, rapidly eliminated, with an apparent half-life of 8.3 min. The \( C_B/C_C \) ratio of 5.5 ± 1.6 (Table 1) compares well with the ratio of the analytically determined starting dose ratio in the B/C mixture of 4.1:1.

Because ADR-925 achieved its maximum plasma concentration at the first time point of 2 min, this result suggested that it was produced from the rapid metabolism of B and C. At 2 min, the plasma concentration of ADR-925 reached a value of 114 ± 15 \( \mu M \), which was already about one-third the value of the concentration of B with a value of 315 ± 42 \( \mu M \) at this time. Inspection of the initial concentration-time data in Fig. 2b, plotted for times up to 30 min, also showed that the initial apparent elimination of ADR-925 paralleled that of B and C. Together, these results suggest that the appearance of ADR-925 was formation rate-limited by the rapid in vivo metabolism of B and C. Given these observations, it was decided to attempt a more complete pharmacokinetic analysis that would contain terms that described the rates at which both B and C were metabolized. Thus, the initial elimination of B and C was modeled assuming that they were eliminated solely through metabolism via a first-order process, and that the ADR-925 metabolite formed was itself eliminated via a first-order process with a rate constant, \( k_m \). Thus, with these assumptions, the elimination of B and C and the first-order formation of ADR-925 for times up to 30 min can be described by eqs. 4, 5, and 6 (Jackson et al., 2004):

\[
\begin{align*}
    C_m &= k_m \cdot C_B(e^{-k_b t} - e^{-k_m t})(k_m - k_b) + k_c \cdot C_C(e^{-k_c t} - e^{-k_c t})(k_c - k_m) \\
    C_B &= C_B e^{-k_b t} \\
    C_C &= C_C e^{-k_c t} \\
    C_{adr} &= C_{adr} e^{-k_{adr} t}
\end{align*}
\]

where \( C_m, C_B, \) and \( C_C \) are the concentrations of the metabolite ADR-925, B, and C, respectively, at time \( t \), and \( k_m, k_b, \) and \( k_c \) are the metabolic formation rate constants for conversion of B and C, respectively, to ADR-925. \( C_B, C_B, \) and \( C_C \) are the concentrations of B and C at time 0, as before. The whole concatenated concentration-time data set for times up to 30 min (Fig. 2b) for all three rats was simultaneously fit, using WinNonlin, to eqs. 4, 5, and 6, weighted with the reciprocal of the calculated concentrations. The best-fit pharmacokinetic parameters obtained are listed in Table 1. The smooth lines in Fig. 2b were calculated from these best-fit pharmacokinetic parameters. As might be expected, \( k_m, k_b, \) and \( k_{adr} \) are close to the values obtained for \( k_m, k_b, \) and \( k_{adr} \) from the analysis of the individual B and C concentration-time data and the fit of those data to eqs. 2 and 3. It can also be seen from Table 1 that \( k_m, k_b, \) and \( k_{adr} \) are similar in value to \( k_m, \) indicating that the rates of

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average ± S.E.</th>
<th>Parameter</th>
<th>Value ± S.E.</th>
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</thead>
<tbody>
<tr>
<td>One-Compartment Individual Pharmacokinetic Analyses</td>
<td></td>
<td>Metabolite Pharmacokinetic Analysis of Concatenated Data</td>
<td></td>
</tr>
<tr>
<td>( k_b (\text{min}^{-1}) )</td>
<td>0.15 ± 0.02</td>
<td>( k_m (\text{min}^{-1}) )</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>( C_B (\mu M) )</td>
<td>420 ± 47</td>
<td>( C_B (\mu M) )</td>
<td>442 ± 40</td>
</tr>
<tr>
<td>( k_c (\text{min}^{-1}) )</td>
<td>0.22 ± 0.04</td>
<td>( k_c (\text{min}^{-1}) )</td>
<td>0.20 ± 0.05</td>
</tr>
<tr>
<td>( C_C (\mu M) )</td>
<td>76 ± 18</td>
<td>( C_C (\mu M) )</td>
<td>76 ± 22</td>
</tr>
<tr>
<td>( k_{adr} (\text{min}^{-1}) )</td>
<td>0.084 ± 0.020</td>
<td>( k_m (\text{min}^{-1}) )</td>
<td>0.32 ± 0.04</td>
</tr>
<tr>
<td>( C_{adr} (\mu M) )</td>
<td>134 ± 10</td>
<td>&amp;</td>
<td></td>
</tr>
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</table>

FIG. 2. a, average rat plasma concentrations of B, C, and ADR-925 after a 20 mg/kg dose (i.v. bolus) of a 4:1:1 mixture of B and C. The straight lines through the B (△), C (○), and ADR-925 (●) data were calculated from a one-compartment fit model described by eqs. 1, 2, and 3 and gave average half-lives of 4.8, 3.3, and 8.3 min, respectively. The data plotted are averages from three rats except for the B datum at 45 min, which was from two rats. b, average rat plasma concentrations of B, C, and ADR-925, but replotted for times up to 30 min only, for fitting to an ADR-925 formation rate-limited model. The smooth lines (solid lines for B and C and a dashed line for ADR-925) were calculated from simultaneously fitting the whole concatenated data set to eqs. 4, 5, and 6. These equations describe a model in which ADR-925 is formation rate-limited by its rapid in vivo metabolism.
formation and elimination of ADR-925 were of the same order of magnitude, which consequently resulted in the initial apparent ADR-925 elimination nearly paralleling the initial elimination of B and C.

**Elimination of ADR-925 in the Rat.** Experiments were also carried out to measure the plasma distribution and elimination of rats treated with 20 mg/kg ADR-925 (the metabolite; Fig. 3). A two-compartment model was used to describe the entire plasma ADR-925 concentration-time data. Thus, the data were fit using WinNonlin to eq. 7.

\[
C_{ADR} = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}
\]  

Equation 7 describes the elimination of ADR-925, where \(\alpha\) and \(\beta\) are the \(\alpha\)-distributive and \(\beta\)-terminal phase elimination rate constants, respectively. \(A\) and \(B\) are their respective pre-exponential factors, the sum of which gives \(C_{\text{max}}\). The average of the best-fit parameters from three rats gave \(\alpha\) and \(\beta\) values of 0.40 ± 0.20 min\(^{-1}\) and 0.024 ± 0.011 min\(^{-1}\), respectively, and a \(C_{\text{max}}\) value of 820 ± 230 \(\mu\)M. The smooth curve in Fig. 3 was calculated from the best-fit parameters for all the data in eq. 7. The average derived overall elimination rate constant, \(k_{10}\), had a value of 0.14 ± 0.06 min\(^{-1}\).

**Tissue Levels of ADR-925 in Rats Treated with Dexrazoxane, the B/C Mixture, or ADR-925.** Because our previous studies showed that dexrazoxane was quickly metabolized in the rat (Schroeder and Hasinoff, 2002) and in humans (Schroeder et al., 2003), it was decided to determine the tissue levels of ADR-925 in heart, liver, and brain tissues to determine whether, in fact, ADR-925 was present in these tissues. Tissues were collected 2.8 h after administration of 40 mg/kg dexrazoxane and 1.5 h after the administration of 20 mg/kg of the B/C mixture or ADR-925. In the dexrazoxane study, part of the tissue to ADR-925. Thus, the ADR-925 tot values in Table 2 are the sum of the dexrazoxane, B, C, and ADR-925 levels in the tissue. The ADR-925 assay is a sensitive fluorescence assay (Schroeder and Hasinoff, 2002) and allowed a determination of low amounts of ADR-925 in these tissues. However, low levels of dexrazoxane, B, and C in these tissues precluded their direct determination by HPLC.

The results in Table 2 show that after administration of 40 mg/kg dexrazoxane, both the heart and liver ADR-925 levels were approximately half the total amount of all forms of dexrazoxane and its three metabolites (ADR-925 tot). The presence of ADR-925 in the heart and liver indicates that dexrazoxane was metabolized in vivo to ADR-925. It is also noteworthy that ADR-925 levels in the brain were undetectable (<2 \(\mu\)mol/kg wet tissue), indicating that the polar dexrazoxane and its three polar metabolites were unable to detectably cross the blood-brain barrier. We previously showed that dexrazoxane, B, C, and ADR-925 were all detectable in the plasma (49, 13, 3.2, and 30 \(\mu\)M, respectively) of similarly dexrazoxane-treated rats at 3 h (Schroeder and Hasinoff, 2002), and thus, it is not surprising that significant levels of ADR-925 were still present in the heart and liver at 2.8 h after dexrazoxane administration.

**Tissue Levels of ADR-925 in Rats Treated with ADR-925.** The ability of the metal-chelating metabolite ADR-925 (Fig. 1) to either remove iron from the iron-doxorubicin complex (Buss and Hasinoff, 1993) or strongly bind free Fe\(^{2+}\) (\(K_f 10^{10.0} \text{M}^{-1}\)) (Huang et al., 1982) or Fe\(^{3+}\) (\(K_f 10^{9.5} \text{M}^{-1}\)) (Diop et al., 2000) is likely the basis of the mechanism by which dexrazoxane prevents iron-based doxorubicin-mediated free radical oxidative stress on the heart muscle. Our previous studies on the metabolism of dexrazoxane in humans (Schroeder et al., 2003) and in the rat (Hasinoff and Aoyama, 1999a,b; Schroeder et al., 2002) showed that B, C, and ADR-925 quickly appeared in the plasma after bolus i.v. administration, a result which suggested that dexrazoxane, and B and C were rapidly metabolized to ADR-925. Thus, these results suggested that rats treated with a B/C mixture might also metabolize B and C to ADR-925.

We also previously showed that DHPase, which is present in the liver and the kidney, can efficiently hydrolyze dexrazoxane (Hasinoff et al., 1991, 1994; Hasinoff, 1993, 1994c; Hasinoff and Aoyama, 1999b) and is likely the enzyme that is primarily responsible for the metabolism of dexrazoxane to B and C. We have also shown that DHPase is unable to convert B and C into ADR-925 (Hasinoff et al., 1991). Likewise, we have shown that DHOase is able to enzymatically hydrolyze B and C to ADR-925 but is unable to hydrolyze dexrazoxane (Schroeder and Hasinoff, 2002).

The results of this study showed that the B/C mixture was very rapidly metabolized to ADR-925, a result that suggested enzyme-mediated metabolism. The peak plasma concentration of dexrazoxane

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**Table 2**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Dexrazoxane</th>
<th>B/C Mixture</th>
<th>ADR-925</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADR-925</td>
<td>ADR-925 tot</td>
<td>ADR-925</td>
</tr>
<tr>
<td></td>
<td>(\mu)mol/kg</td>
<td>(\mu)mol/kg</td>
<td>(\mu)mol/kg</td>
</tr>
<tr>
<td>Heart</td>
<td>51 ± 6</td>
<td>100 ± 7</td>
<td>27 ± 6</td>
</tr>
<tr>
<td>Liver</td>
<td>97 ± 14</td>
<td>232 ± 26</td>
<td>40 ± 16</td>
</tr>
<tr>
<td>Brain</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

The average tissue level of ADR-925 was measured per kilogram of wet tissue harvested from seven rats 2.8 h after dexrazoxane was administered (40 mg/kg), or the levels 1.5 h after the B/C mixture or ADR-925 was administered (20 mg/kg) to three rats. ADR-925 tot is the total amount of ADR-925 measured after treatment of the homogenate with NaOH to convert all forms of dexrazoxane into ADR-925. The limit of detection was 2 \(\mu\)mol/kg.
of 530 µM seen in our previous rat study (Schroeder and Hasinoff, 2002), after administration of 40 mg/kg dexrazoxane, can be compared with peak plasma concentrations of B and C of 442 and 76 µM, respectively, for a 20 mg/kg dose of the B/C mixture (Table 1). In fact, ADR-925 was maximally present in plasma at 2 min when the first blood sample was taken. The rapid appearance of ADR-925 suggested that the initial pharmacokinetics could be analyzed by a model in which the initial concentrations of the ADR-925 metabolite were largely determined by metabolism of B and C. Kinetic analysis of the data (Fig. 2b) in this formation rate-limited model showed that the initial ADR-925 elimination rate constant of 0.32 min⁻¹ was in fact similar in magnitude to the B and C metabolite formation rate constants kfb and kfc of 0.13 and 0.20 min⁻¹, respectively. This simplified model, which only analyzed the data for B and C and ADR-925 up to 30 min, of necessity, involved several simplifying assumptions. It was assumed that B and C, at least initially, were only eliminated through metabolism to ADR-925. It was also assumed that the initial formation rate-limited elimination of B and C occurred through nonsaturating processes. The rapid metabolism of B and C was also a limitation on obtaining a more accurate formation-limited pharmacokinetic analysis. The elimination of ADR-925, when ADR-925 was administered alone, was biphasic and gave an average α-phase distributive phase rate constant of 0.40 min⁻¹ (Fig. 3). This fast distributive phase precluded a valid comparison with peak plasma concentrations of 2002), after administration of 40 mg/kg dexrazoxane, can be compared with peak plasma concentrations of B and C of 442 and 76 µM, respectively (Schroeder et al., 2002). Thus, under these conditions, DHOase would hydrolyze 11 mM, respectively (Schroeder et al., 2002), and these intermediates were the enzyme solely responsible for the metabolism of B and C in vivo. DHOase would be acting on B and C under nonsaturating conditions, given that the kₘ values for B and C are 4.8 and 11 mM, respectively (Schroeder et al., 2002). Thus, under these conditions, DHOase would hydrolyze B and C at a rate ratio equal to the ratio of their Vₜ₅₀/kₘ values of 0.9 (Schroeder et al., 2002). It can be seen from the data of Table 1 that the initial formation rate constant ratio kfb/kfc had a value of 0.68 ± 0.15, a value that compares reasonably well to 0.9 for the pure enzyme. Thus, these results are consistent with DHOase being largely responsible for the in vivo metabolism of the B/C mixture. We also previously reported that plasma, blood, and plasma concentrations of Ca²⁺ and Mg²⁺ were able to promote the hydrolysis of B and C 3- to 5-fold over its background rate of hydrolysis (Buss and Hasinoff, 1997; Schroeder et al., 2005). Thus, these metal ions may partially contribute, along with DHOase, to the metabolism of B and C.

We have also shown in this study that rats treated either with dexrazoxane or the B/C mixture yield tissue levels that are relatively high in ADR-925. The values we obtained can be compared with the total of all metabolite forms of ¹⁴C-labeled dexrazoxane levels determined at 5 min in heart and liver of 320 and 640 µmol/kg, respectively, for mice administered 100 mg/kg dexrazoxane (Mhatre et al., 1982). Because the liver contains both DHPase and DHOase, which may act sequentially to form ADR-925, it was not unexpected that these tissues contained ADR-925. However, the fact that the heart does not contain DHPase (Hamajima et al., 1996) and the supernatant of a heart homogenate does not have dexrazoxane-hydrolyzing activity (Hasinoff et al., 1991) suggests that the ADR-925 found in the heart was from uptake of circulating B and C or ADR-925. In support of this conclusion, we did show that isolated neonatal myocytes were able to slowly take up B, C, and ADR-925 and displace iron from an intracellular fluorescence-quenched iron-calcein complex (Hasinoff et al., 2003). The much lower levels of ADR-925 in heart and liver when the rats were treated with ADR-925 reflect the low cellular permeability of the highly polar dianionic ADR-925.

The lack of detectable ADR-925 in brain tissues is consistent with the polar dexrazoxane and its three metabolites not being able to cross the blood-brain barrier. Dexrazoxane is currently in clinical trials as an extracerebral rescue agent in cancer patients with brain metastases treated with high-dose etoposide (Holm et al., 1998; Schroeder et al., 2003, 2004). Because neither dexrazoxane nor its metabolites were found in brain tissue, these results suggest that dexrazoxane will not antagonize the topoisomerase II poisoning activity of etoposide (Sehested et al., 1993) in cancerous brain tissue.

This study did not directly address how dexrazoxane exerted its cardioprotective effects in the heart. However, in conclusion, these studies showed that the one-ring metabolites B and C of dexrazoxane were rapidly metabolized in the rat to ADR-925. Their rapid in vivo hydrolysis provides a mechanism by which B and C may be activated to its iron-binding form, ADR-925, which has antioxidant activity. The initial plasma levels of ADR-925 that were observed were consistent with the formation rate-limited enzyme-mediated metabolism of B and C. Some of the metabolism of B and C may take place in the heart, which contains the enzyme, DHOase, that can metabolize B and C to ADR-925.

References


Hasinoff BB, Reinders FX, and Clark V (1991) The enzymatic hydrolysis-activation of the
adriamycin cardioprotective agent (+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane. Drug Metab Dispos 19:74–80.


Huang Z-X, May PM, Quinlan KM, Williams DR, and Creighton AM (1982) Metal binding by pharmaceuticals. Part 2. Interactions of Ca(II), Cu(II), Fe(II), Mg(II), Mn(II) and Zn(II) with the intracellular hydrolysis products of the antitumor agent ICRF-159 and its inactive homologue ICRF-192. Agents Actions 12:536–542.


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