RELATIVE PLASMA LEVELS OF THE CARDIOPROTECTIVE DRUG DEXRAZOXANE AND ITS TWO ACTIVE RING-OPENED METABOLITES IN THE RAT

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ABSTRACT:
A postcolumn derivatization reversed-phase high-pressure liquid chromatography method has been developed to detect and separate the one-ring open intermediates of dexrazoxane (ICRF-187) in blood plasma. Dexrazoxane 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 one-ring open intermediates and its fully rings opened hydrolysis product ADR-925. Little is known of the in vivo metabolism of dexrazoxane to its one-ring open intermediates, which may be two of the active forms of dexrazoxane. The one-ring open intermediates were detected within 5 min of i.v. administration of dexrazoxane to rats, suggesting that dexrazoxane is rapidly metabolized in vivo. The plasma concentrations of the one-ring open intermediates varied from 4 to 9% and 6 to 24% of the dexrazoxane concentrations at 5 and 120 min, respectively. The relatively small changes in the levels of the one-ring open intermediates with time suggest that a dynamic steady state is occurring. The ratio of the concentrations of the two one-ring open intermediates was similar to that previously seen for the in vitro dihydropyrimidine amidohydrolase-catalyzed hydrolysis of dexrazoxane. These results are consistent with the hypothesis that dihydropyrimidine amidohydrolase in the liver and kidney is responsible for the metabolism of dexrazoxane in the rat.

Dexrazoxane (ICRF-187, ZINECARD, Fig. 1) A, is clinically used to reduce doxorubicin-induced cardiotoxicity (Swain et al., 1997a, b; Hasinoff et al., 1998; Hasinoff, 1998). Dexrazoxane is the (++)-β)-enantiomer of racemic razoxane (ICRF-159), which was originally developed as an antitumor agent (Creighton et al., 1969; Witjak and Wei, 1991). Dexrazoxane likely acts as a cardioprotective agent by diffusing into the cell and hydrolyzing to its one-ring open intermediates B and C, and then to its fully rings opened metal ion-binding form ADR-925 (Fig. 1D), which has a structure similar to EDTA. ADR-925 may chelate free iron or displace iron bound to the iron-doxorubicin complex (Hasinoff, 1990, 1994a,b), thus reducing iron-based oxygen radical formation. Our previous spectrophotometric and high-pressure liquid chromatography (HPLC) studies (Hasinoff, 1990, 1994a,b) showed that under physiological conditions dexrazoxane hydrolyzed to B and C with a T1/2 of 9.3 h at 37°C and pH 7.4, whereas the final hydrolysis product ADR-925 was produced with a T1/2 of 23 h according to the kinetic scheme shown in Fig. 1. We have also shown that B and C were also good chelating agents and were also able to displace quickly and completely Fe3+ from its anthracycline complexes, suggesting that these intermediates might even be the pharmacologically active species in preventing oxygen radical derived iron-based anthracycline-induced toxicities. We have also shown that a variety of metal ions are able to promote the hydrolysis of B and C to ADR-925 (Buss and Hasinoff, 1995, 1997). Ferric ion and ferrous ion effected a 8- and 6000-fold enhancement in the hydrolysis of B and C (Buss and Hasinoff, 1995), Mg2+ and Ca2+ promoted hydrolysis up to 18-fold, and Zn2+ promoted hydrolysis more than 50,000-fold (Buss and Hasinoff, 1997). It was also shown (Hasinoff et al., 1991) that dexrazoxane underwent an enzymatic ring-opening hydrolysis by the 105,000g soluble supernatant fraction of homogenates of porcine liver and kidney, but not of heart. Dihydropyrimidine amidohydrolase, EC 3.5.2.2 (DHPase), present in the supernatant, was shown to be responsible for this enzymatic hydrolysis (Hasinoff et al., 1991, 1994; Hasinoff, 1993, 1994c). This study also showed that although dexrazoxane was a substrate for DHPase, its one-ring open hydrolysis products B and C were not. We also previously showed that a suspension of primary rat hepatocytes hydrolyzed dexrazoxane (Hasinoff et al., 1994). Sadee et al. (1975), using 14Crazoxane in a rat pharmacokinetic study, found significantly higher total 14C plasma levels compared with intact razoxane plasma levels, indicating that a rapid biotransformation to unknown metabolites occurred. Because of the possible importance of the intermediates B and C as pharmacologically active species, and because these species have not been determined in an in vivo model, it was decided to determine these species in an animal model to see if they were present, and if so, at what levels so that a greater understanding of the metabolism of dexrazoxane to its presumably active metal ion-chelating species could be obtained. A rapid production of B and C in vivo could also be used as an indicator of whether or not DHPase was in part responsible for the metabolism of dexrazoxane to B and C.
Materials and Methods

Dexrazoxane was a gift from Adria Laboratories (Columbus, OH). Ion pair reversed phase HPLC was used to separate B and C and dexrazoxane on the same run. The elution profile at a flow rate of 1 ml/min was: starting with 500 μM Na2EDTA (pH 3.5)/20 mM heptanesulfonic acid (90:10, v/v) the methanol concentration was linearly increased over 10 min from 0 to 8% (v/v). This mobile phase composition (Na2EDTA/heptanesulfonic acid/methanol, 82:10:8, v/v/v) was maintained for another 14 min to the end of the run, whereupon the column was re-equilibrated with the initial mobile phase for 8 min. As before (Hasinoff, 1994a,b), EDTA was used to chelate trace amounts of iron in the flow system and the column that interfered with the analysis. HPLC grade methanol was obtained from Mallinckrodt (Mississauga, Canada) and the heptanesulfonic acid sodium salt was obtained from Sigma (St. Louis, MO). The column was a 10-μm Bondapak 3.9 × 300-mm reversed-phase C18 column (Waters, Mississauga). A Brownlee Labs NewGuard (Applied Biosystems, Santa Clara, CA) C18 guard column (1.5 × 3.2 mm) was placed in front of the separation column. The HPLC apparatus consisted of a programmable Varian (Walnut Creek, CA) 9010 pump, a Varian 9050 variable wavelength absorbance detector (detection wavelength 235 nm), a Varian Star integrator (Varian, Walnut Creek, CA) 9010 pump, a Varian 9050 variable wavelength detection system, and a Rheodyne (Cotati, CA) injector with a 50-μl sample loop. Because of the low absorption peak wavelength and low molar absorptivity of B and C (Hasinoff, 1990, 1994a,b), and the presence of strongly absorbing interfering plasma components, a postcolumn derivatization method was developed. An Eldex A-30-S-Peek pump (Eldex Laboratories, San Carlos, CA) was used to deliver 30 mM NaOH at a flow rate of 0.25 ml/min through a pulsation damper (Varian) to a 3-way mixing tee (Upchurch Scientific, Oak Harbor, WA) that was joined to the outlet tubing from the HPLC column. The outflow from the tee was directed to a reaction/mixing bed consisting of 0.5 m of 0.51-mm i.d. HPLC Teflon tubing (Upchurch) tightly knitted into a rectangular angle 3 × 4 cm, and then to the UV detector. This derivatization method is based on the rapid deprotonation of the imide hydrogen of dexrazoxane, B, or C, to form the corresponding imide anion that has a strong absorption maximum at 227 nm (Hasinoff, 1990, 1994a). The base-catalyzed ring-opening hydrolysis under these conditions is slow enough, and the contact time with NaOH is short enough, that hydrolysis does not affect the analysis (Hasinoff, 1990, 1994a). The HPLC calibration plots using integrated peak areas (3.7–186 μM) and C [preparation of the hydrolysis intermediates A, B, and C and the combined washings were evaporated to dryness under a stream of Ar and stored at −80°C. Just before analysis the samples were reconstituted in 125 μl of 10 mM HCl. Two determinations were carried out on each sample. The HPLC calibration plots using integrated peak areas (3.7–186 μM dexrazoxane, 18–180 μM B and 4–180 μM C, all in plasma) were prepared by adding standards containing known amounts of dexrazoxane, B, and C [preparation as previously described (Hasinoff, 1994b)] to blank rat plasma and treating the samples exactly as described above. The calibration plots (n = 6 for dexrazoxane and C, and n = 4 for B, data not shown) for both dexrazoxane, B, and C were highly linear (r2 = 0.9986, 0.9999, and 0.9998, respectively). The slopes for the B and C calibration plots were very close (within 5%) as might be expected for two such structurally similar compounds (Fig. 1). A second high concentration calibration plot for dexrazoxane was prepared for the 75 to 1900 μM range (n = 5) and gave an r2 of 0.996. Absolute recoveries from spiked plasma ranged from 74 to 97% from 18.6 to 186 μM dexrazoxane, respectively; from 92% to 87% from 18 to 175 μM C, respectively; and from 40 to 82% from 18 to 175 μM B, respectively. Attempts to clean up the plasma samples before HPLC analysis using either Sep-Pak Plus C-8, C-18, or QMA anion solid-phase extraction cartridges were unsuccessful due to poor recoveries of B and C.

Results

The HPLC chromatograms shown in Fig. 2 show that dexrazoxane, B, and C are all detectable in plasma after administration of 40 mg/kg of dexrazoxane. B and C appear in the plasma very quickly after dexrazoxane administration, suggesting that dexrazoxane is rapidly metabolized. These results and those of Fig. 3a show that dexrazoxane over all the times studied is present at much higher concentrations than either B or C. The concentrations of B and C were only 8.6% and 3.6% (mol %), respectively, of the dexrazoxane concentration at 5 min, although this increased to 24% and 5.7% (mol %), respectively, at 120 min. The relative lack of change in levels of B and C with time suggest that the one-ring open intermediates are produced in a dynamic steady-state fashion. This result suggests that B and C were themselves also rapidly excreted or metabolized. It can also be seen from the data in Fig. 3 that the concentration of B was always larger than that of C. This result suggests that dexrazoxane is being metabolized to B and C as well as being eliminated. The peak plasma concentrations of dexrazoxane of 340 ± 20 μM shown in Fig. 3a are comparable to those seen in humans of 340 ± 80 μM at a dose of 600 mg/m2. The dexrazoxane, B, and C plasma concentrations are plotted separately in Fig. 3a in a semilog plot and in Fig. 3b as the B/C ratio to more clearly show the increase in the B/C ratio with time. The S.E. values shown were calculated from data averaged from separate experiments (six rats). Another feature of the appearance of B and C is that the plasma concentrations of these intermediates after about 20 min are nearly constant or increase slightly. As can be seen from the reaction scheme in Fig. 1, this effect is probably due to the fact that B and C are intermediates that are forming and decomposing at the same time. B and C were previously seen to reach a maximum in solution at about 12 h (at pH 7.4 and 37°C) (Hasinoff, 1990, 1994b) and in a hepatocyte suspension (Hasinoff, 1990, 1994b; Hasinoff et al., 1994). Given the slowness of the in vitro hydrolysis of dexrazoxane under physiological conditions, little of the B and C seen in vivo could have been formed from base-catalyzed hydrolysis (Hasinoff, 1990, 1994b) and therefore must have resulted from rapid metabo-
At longer times B and C may still be being formed from dexrazoxane remaining in the plasma or the tissue. Because the plasma concentrations of B and C are only small fractions of the dexrazoxane concentration (4 and 9% of the dexrazoxane concentration at 5 min, for example), we were concerned that some of the B and C that was being measured in the plasma was from B and C present as contaminants in the dexrazoxane used. This was tested for by measuring the plasma concentration of B and C in four rats dosed with a sample of dexrazoxane (a lyophilized preparation) that contained 0.54% and 0.35% (mol %) B and C, respectively, and a second set of two rats dosed with dexrazoxane (a recrystallized preparation) that contained 0.019 and 0.052% (mol %) B and C, respectively. No difference was seen in the plasma concentrations of B and C in the two groups of rats (data not shown) over the whole time course, indicating that the B and C observed in the plasma did not arise from contaminants in the dexrazoxane used.

**Discussion**

The results of this study clearly show that in rats dosed with dexrazoxane the one-ring open hydrolysis intermediates are rapidly formed in vivo. The relatively small changes in either B or C indicate that the one-ring open intermediates were produced in a manner close to that expected for a dynamic steady state. We 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). We also showed that dexrazoxane was enzymatically hydrolyzed by DHPase to B some 6.1-fold faster than to C (Hasinoff, 1993, 1994c), although this factor was reduced to 3.6-fold in a suspension of primary hepatocytes (pH 7.6, 37°C) (Hasinoff et al., 1994). Thus, the B/C ratio of between 2.7 at 5 min and 6.5 at 180 min shown in Fig. 3b is consistent with a contribution from DHPase-catalyzed metabolism of razoxane. The results of this study do not prove that dexrazoxane is metabolized by DHPase in vivo, but are only consistent with this hypothesis. These results could also possibly be explained by metabolism by some other unknown enzyme, or even a preferential tissue uptake or elimination of C compared to B. The rapid appearance of significant quantities of B and C in the plasma so soon after dexrazoxane administration also suggests that dexrazoxane is being metabolized. Distribution and excretion are probably occurring simultaneously with metabolism. The enzymatic hydrolysis of dexrazoxane in the liver and kidney might result in a preferential accumulation of charged and presumably membrane impermeable, metal ion-binding hydrolysis products in these organs. This could have the effect of reducing the concentration of dexrazoxane in the heart where it is needed to protect the tissue from oxygen radical damage.

It was not the purpose of this study to determine the pharmacokinetics of dexrazoxane in the rat because the pharmacokinetics of razoxane have already been measured (Field et al., 1971; Sadée et al., 1975; Collins et al., 1983), but rather to see if the one-ring open intermediates B and C are formed in vivo. Using a bioassay, the half-life of razoxane in the rat was measured to be about 30 min after...
an i.v. dose of 120 mg/kg (Field et al., 1971) and 40 to 45 min by gas chromatography (Sadée et al., 1975). This same study, using \(^{14}\)Crazoxane, found significantly higher total \(^{14}\)C plasma levels compared with intact razoxane plasma levels, which indicated that a rapid biotransformation to unknown metabolites occurred. The results of this study showed that the one-ring open intermediates B and C appear only as minor circulating metabolites compared with the parent razoxane. This result combined with that of Sadée et al. (1975) using \(^{14}\)Crazoxane indicates that the major metabolite or metabolites in plasma have yet to be identified. A likely candidate would of course be the two-ring open intermediate D in Fig. 1, although this remains to be determined. A terminal phase half-life of 40 min by HPLC after a dose of 22 mg/kg has also been measured (Collins et al., 1983). For comparison, the terminal half-life in humans has been measured to be 2.9 ± 1.0 h (Jakobsen et al., 1994) and 4.2 ± 2.9 h (Hochster et al., 1992). Typically, drugs are cleared from the rat at a faster rate than they are in humans.

We previously showed that Fe\(^{+++}\) and Fe\(^{++}\) effected a 8- and 6000-fold enhancement in the hydrolysis of B and C (Buss and Hasinoff, 1995), Mg\(^{++}\) and Ca\(^{++}\) promoted hydrolysis between 2.5- and 18-fold at physiological concentrations of these metal ions, and Zn\(^{++}\) promoted hydrolysis more than 50,000-fold (Buss and Hasinoff, 1997). The free iron concentration is very low (micromolar or lower) in serum (Halliwell and Gutteridge, 1989) as is that of Zn\(^{++}\) (Magneson et al., 1987) and, thus, it seems unlikely that these metal ions could contribute to the elimination of B and C from plasma at least. However, the plasma concentrations of Ca\(^{++}\) and Mg\(^{++}\) are high enough that metal ion-promoted hydrolysis by these metal ions could contribute to the elimination of B and C.

References


