Cancer Chemother Pharmacol (2003) 51: 306–310
DOI 10.1007/s00280-003-0582-3

ORIGINAL ARTICLE

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The cardioprotector monoHER does not interfere with the pharmacokinetics or the metabolism of the cardiotoxic agent doxorubicin in mice

Received: 21 October 2002 / Accepted: 9 January 2003 / Published online: 22 March 2003

Abstract *Purpose*: Monohydroxyethylrutoside (monoHER) has proved to be a good protector against doxorubicin-induced cardiotoxicity without interfering with the antitumor effect of doxorubicin. The aim of the present study was to determine whether there is a pharmacokinetic interaction between monoHER and doxorubicin which may be involved in monoHER cardioprotection. *Methods*: Mice were treated with monoHER (500 mg.kg^{-1} i.v.) alone, monoHER 5 min after doxorubicin (10 mg.kg^{-1} i.v.), doxorubicin alone and doxorubicin 5 min after monoHER. The levels of monoHER and doxorubicin(ol) in plasma and heart tissue were measured by HPLC 24 h and 48 h after monoHER and doxorubicin administration, respectively. *Results*: The areas under the concentration-time curves (AUCs) of monoHER and doxorubicin(ol) were not affected by the coadministered drug. No changes were observed in pharmacokinetic parameters such as initial and final half-lives, mean residence time, clearance and volume of distribution of monoHER and doxorubicin(ol) after single or combined administration. *Conclusion*: The cardioprotection of monoHER in mice is not caused by a pharmacokinetic interaction between monoHER and doxorubicin.

Keywords MonoHER · Doxorubicin · Doxorubicinol · Plasma · Heart

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Introduction

The cardiotoxic effect of doxorubicin is a major limiting factor in its optimal use in the clinic. This cardiotoxicity is generally thought to be caused by free radicals which are produced during redox cycling of doxorubicin [1, 2, 3, 4]. Doxorubicinol, the main metabolite of doxorubicin, is even more toxic in this respect [5, 6, 7].

We have recently shown that 7-monohydroxyethylrutoside (monoHER), a semisynthetic flavonoid with good antioxidant properties [8, 9], protects against doxorubicin-induced cardiotoxicity in mice [10, 11]. This cardioprotection has been attributed to the scavenging effect of monoHER on the free radicals produced by doxorubicin(ol) [12, 13, 14]. Alternative explanations might be: a possible reduction of the aldoketoreductase activity by monoHER [15, 16] which would result in a reduction of the more cardiotoxic metabolite doxorubicinol and/or an interference of monoHER with the accumulation of doxorubicin(ol) in heart tissue.

In order to investigate these alternative possibilities the aim of our study was to determine whether there is a pharmacokinetic interaction between the two drugs in plasma and heart tissue of mice.

Materials and methods

Chemicals

MonoHER was kindly provided by Novartis Consumer Health (Nyon, Switzerland). Doxorubicin and doxorubicinol were purchased from Pharmitalia (Carlo Erba, Italy). Acetic acid, acetone, *o*-phosphoric acid (85%), potassium chloride, sodium bisulfite, silver chloride, silver nitrate, sodium acetate, sodium hydroxide and sodium dihydrogenphosphate monohydrate were purchased from Merck (Amsterdam, The Netherlands), EDTA was from Sigma-Aldrich Chemie (Zwijndrecht, The Netherlands), triethylamine was from Fluka Biochemika (Buchs, Switzerland), sodium 1-hexanesulfonic acid (HPLC grade) was from Acros (Geel, Belgium), methanol (HPLC grade), chloroform, dimethyl sulfoxide and 2-propanol were from J.T. Baker (Deventer, The Netherlands), and acetonitrile was from BDH (Poole, UK).

Animals

The study was conducted in accordance with the Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1996). The pharmacokinetic study was approved by the Ethics Committee for Animal Experiments of the Free University Medical Center in Amsterdam. Male Balb/c mice (20–30 g) obtained from Harlan Nederland (Horst, The Netherlands) were maintained in cages in groups of four to six mice each, on regular chow and water ad libitum. Mice were kept in a light- and temperature-controlled room (21–22°C; humidity 60–65%). The animals were allowed to acclimatize to the housing conditions of the animal facility for at least 1 week before starting the experiment.

Mice were randomized into four groups each receiving one of the following treatments as a bolus i.v. injection via the tail vein:

- Group 1 ($n = 24$): 10 mg.kg^{-1} doxorubicin,
- Group 2 ($n = 24$): 500 mg.kg^{-1} monoHER 5 min before 10 mg.kg^{-1}doxorubicin,
- Group 3 ($n = 24$): 500 mg.kg^{-1} monoHER,
- Group 4 ($n = 24$): 10 mg.kg^{-1} doxorubicin 5 min before 500 mg.kg^{-1} monoHER.

The coadministered drug was injected 5 min before administration of the drug under investigation (groups 2 and 4) aiming at a maximal pharmacokinetic interaction. After drug administration the mice were individually housed until they were killed.

Groups 1 and 2 were used to study the effect of monoHER on the pharmacokinetics of doxorubicin(ol) by measuring the levels of doxorubicin(ol) in heart tissue and in plasma 0, 2, 7 and 15 min, and 1, 4, 24 and 48 h after doxorubicin administration. In groups 3 and 4 monoHER levels were measured in heart tissue and plasma before and 5, 10, 20, 30, 60, 90 and 120 min after monoHER administration to study the effect of doxorubicin on the pharmacokinetics of monoHER. The experiment was performed in triplicate.

Sample handling

Blood was obtained by heart puncture after ether anesthesia. Hearts were excised and blood samples were centrifuged at 3000 rpm for 5 min at 4°C. Plasma and hearts were stored immediately at −80°C. On the day of analysis, hearts were further cooled with liquid nitrogen and pulverized with a micro-dismembrator (B. Braun Melsungen, Melsungen, Germany).

Homogenates of 125 mg.ml^{-1} were immediately prepared by suspending the appropriate amounts of tissue powder in either water or 25 m*M* phosphate buffer (containing 0.4% sodium bisulfite) for the analysis of doxorubicin or monoHER, respectively.

Drug analysis

The concentrations of monoHER in plasma and heart tissue were measured as previously described [17, 18]. In brief, monoHER was extracted from plasma or tissue homogenate with two volumes of methanol. After centrifugation (9000 rpm, 3 min, 1°C), two 130-µl portions of extract were diluted with 130 µl 25 m*M* phosphate buffer (pH 3.33) and the level of monoHER was measured in the duplicates by HPLC with electrochemical detection. The retention time of the monoHER peak was about 5.2 min. The detection limits were 0.3 µ*M* and 0.072 nmol.g^{-1} in plasma and heart tissue, respectively. The mean within- and between-day accuracy and precision of the quality control samples were less than 15% for all concentrations and less than 20% for the lowest concentration in both matrices.

The levels of doxorubicin(ol) in plasma and heart tissue were measured by HPLC with fluorescence detection using solid-phase and liquid-liquid extraction procedures, respectively, according to a previously described method [5] with slight modifications. Plasma samples (100 µl) were loaded on pre-equilibrated Sep-Pak cartridges followed by washing with 20 m*M* phosphate buffer (pH 4.0)/acetonitrile (9:1 v/v). Doxorubicin was eluted with methanol/chloroform (3:1 v/v). The effluent was evaporated overnight under a stream of nitrogen at 50°C. The remaining residue was carefully reconstituted in the mobile phase, which consisted of acetonitrile and an aqueous solution containing 10 m*M* TEA, 10 m*M* sodium dihydrogenphosphate and 10 m*M* sodium acetate (pH 3.5, 2:1 v/v). The samples were placed in a cooled (4°C) sample tray before injection onto the HPLC.

For heart tissue, doxorubicin was released from DNA and proteins by incubating 200 µl tissue homogenate with 80 µl 1 *M* silver nitrate for 10 min at 37°C. Then 25 µl of an aqueous solution containing 100 m*M* sodium dihydrogenphosphate and 30 m*M* 1-hexanesulfonic acid (pH 8.5) was added. Doxorubicin was extracted from this solution by adding a solution of 1 ml chloroform/2-propanol (2:1 v/v) containing DMSO (9:0.1 v/v) followed by careful sonication and incubation at 37°C for 15 min. Samples were centrifuged at 14,000 rpm for 1 min. The aqueous layer was re-extracted and the resulting organic layers from both extractions were pooled and evaporated under a stream of nitrogen at 50°C. The residue was reconstituted in the mobile phase before HPLC analysis.

The within- and between-day precision and accuracy of the quality control samples of doxorubicin in plasma and heart tissue homogenate were less than 17%. Calibration samples of monoHER, doxorubicin and doxorubicinol in plasma and heart tissue were freshly prepared on each day of analysis.

Pharmacokinetic calculations and statistical analysis

The concentration-time curves of monoHER, doxorubicin and doxorubicinol were obtained by plotting the mean concentrations (C_i) of each drug at each time point (t_i) versus time on a semilogarithmic scale. The area under the concentration-time curve (AUC) of monoHER and doxorubicin(ol) were calculated from 5 to 120 min and from 2 min to 24 h after administration, respectively. The AUC values were calculated by the trapezoidal rule:

$$\mathrm{AUC} = \sum_{i=1}^{n} \left(W_i \cdot \bar{C}_i\right)$$

with

$$W_i = 0.5(t_{i+1} - t_i)$$

The statistical analysis to compare the calculated AUC values of each drug when administered alone or in combination with the other drug was performed by calculating the variance [VAR(C_i)] of the mean concentration (C_i) of the drug in plasma or heart tissue of three mice at each time-point *i*. The standard deviations of the AUC values were calculated by:

$$\mathrm{S.D.} = \sqrt{\left(\sum_{i=1}^{n} \left(W_i^2 \cdot \mathrm{VAR}(\bar{C}_i)\right)\right)}$$

The mean residence time (MRT), clearance (Cl) and volume of distribution $\left(V_d^{SS}\right)$ of monoHER and doxorubicin were determined by noncompartmental analysis using the pharmacokinetic program WinNonlin, version 1.5 (Pharsight Corporation, Mountain View, Calif.). Standard deviations of the MRT, Cl and V_{ss} of monoHER and doxorubicin(ol) were derived from the standard deviations of the respective AUCs by applying the delta method. The values of the aforementioned pharmacokinetic parameters were expressed as 95% confidence intervals.

The confidence intervals of the initial ($t_{1/2\alpha}$) and final half-lives ($t_{1/2\mathrm{final}}$) of monoHER were calculated by CSTRIP. For doxorubicin(ol), the confidence intervals of the initial ($t_{1/2\alpha}$) and final half-lives ($t_{1/2\mathrm{final}}$) were calculated by two-compartmental model analysis. Wald's *t*-test was used for statistical comparison.

Results

MonoHER

Concentration-time plots of monoHER in plasma and heart tissue of mice after the i.v. administration of 500 mg.kg^{-1} monoHER with or without 10 mg.kg^{-1} doxorubicin are shown in Fig. 1. Immediately after monoHER administration, peak values were not only observed in plasma but also in heart tissue. The monoHER concentrations in plasma and heart tissue decreased rapidly in a biphasic manner with initial half-lives of 6 and 7.1 min and final half-lives of 11.4 and 17.1 min, respectively. MonoHER could not be detected in plasma or heart tissue for longer than 2 h after administration. The rapid disappearance of monoHER from the body was also indicated by the fast total body clearance of 3.6 ml.h^{-1}.g^{-1} and short MRT in plasma (9.8 min) and heart tissue (10.6 min). The volume of distribution (V_d^{SS}) was 0.5 ml.g^{-1}. The $AUC_{5\text{-}120\ min}$ values in plasma and heart tissue were 14.1 ± 3.1 μmol.min.ml^{-1} and 2.9 ± 0.7 μmol.min.g^{-1}, respectively. Coadministration of (10 mg.kg^{-1}, i.v.) doxorubicin did not significantly influence ($P > 0.05$) the $AUC_{5\text{-}120\ min}$, initial and final $t_{1/2}$, MRT, clearance or volume of distribution of monoHER in plasma or heart tissue (Table 1, Fig. 1). The heart/plasma ratio of $AUC_{5\text{-}120\ min}$ of monoHER was 0.2. This ratio was also not affected by coadministration of doxorubicin (0.17).

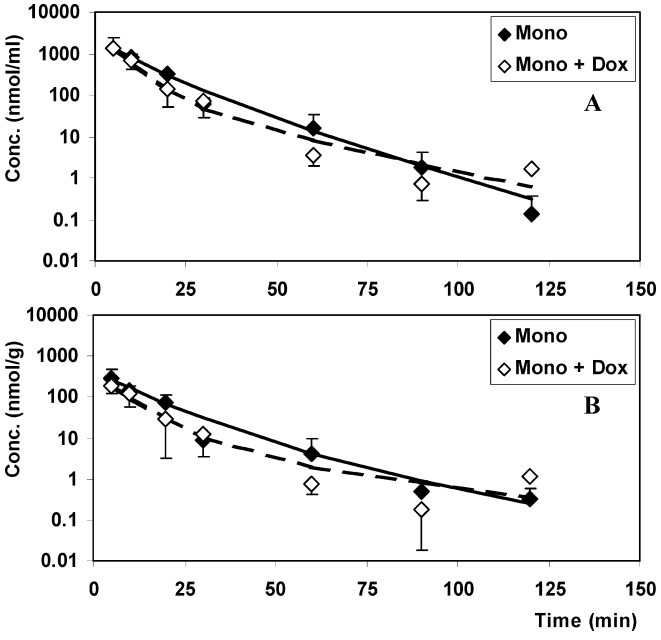

Fig. 1A, B Concentration-time plots of monoHER in (**A**) plasma and (**B**) heart tissue of mice receiving monoHER (500 mg.kg^{-1}, i.v.) with or without doxorubicin (10 mg.kg^{-1}, i.v.)

Doxorubicin

The concentration-time plots of doxorubicin in plasma and heart tissue of mice after the i.v. administration of 10 mg.kg^{-1} doxorubicin with or without 500 mg.kg^{-1} monoHER are shown in Fig. 2A. Doxorubicin was present in plasma and heart tissue for at least 48 h after administration. The concentration of doxorubicin in plasma and heart tissue decreased with initial half-lives of 0.04 and 0.44 h and final half-lives of 12.6 and 11.5 h, respectively.

The slow disappearance of doxorubicin from the body was also indicated by the low total body clearance (2.5 ml.h^{-1}.g^{-1}) and the long MRT in plasma (14.7 h) and heart tissue (13.9 h). The volume of distribution (V_d^{SS}) was 36.8 ml.g^{-1}. The $AUC_{0.03\text{-}48\ h}$ values in plasma and heart tissue were 6.4 ± 1.0 nmol.h.ml^{-1} and 140 ± 32.9 nmol.h.g^{-1}, respectively. Coadministration of (500 mg.kg^{-1}) monoHER did not significantly influence the $AUC_{0.03\text{-}48\ h}$ or the values of the other pharmacokinetic parameters of doxorubicin in plasma and heart tissue ($P > 0.05$, Table 2, Fig. 2A). This also resulted in comparable $AUC_{0.03\text{-}48\ h}$ heart/plasma ratios of 17 and 22 for doxorubicin alone and with monoHER, respectively.

Doxorubicinol

Concentration-time plots of doxorubicinol, the primary and cardiotoxic metabolite of doxorubicin, in plasma and heart tissue of mice are shown in Fig. 2B. In plasma doxorubicinol reached its maximum concentration

Table 1 Pharmacokinetic parameters (95%confidence intervals) of monoHER in plasma and heart tissue of mice receiving monoHER (500 mg.kg^{-1}, i.v.) with or without doxorubicin(10 mg.kg^{-1}, i.v.)

Parameter	Plasma		Heart tissue	
	MonoHER	MonoHER + Dox	MonoHER	MonoHER + Dox
$AUC_{5\text{-}120\ min}$[a]	11.0–17.2	9.1–13.7	2.2–3.6	1.4–2.6
$t_{1/2\alpha}$ (min)	5.8–6.2	3.7–4.3	4.8–10.6	4.0–6.3
$t_{1/2final}$ (min)	10.1–12.8	9.6–21.8	12.3–23.9	9.6–33.5
MRT (min)	7.1–12.5	6.9–10.1	7.5–13.7	10.1–15.3
Cl (ml.min^{-1}.g^{-1})	0.04–0.09	0.05–0.10	–	–
V_d^{SS} (ml.g^{-1})	0.4–0.6	0.5–0.7	–	–

[a]$AUC_{5\text{-}120\ min}$ is expressed as micromoles×minute per milliliter of plasma or per gram of wet heart tissue

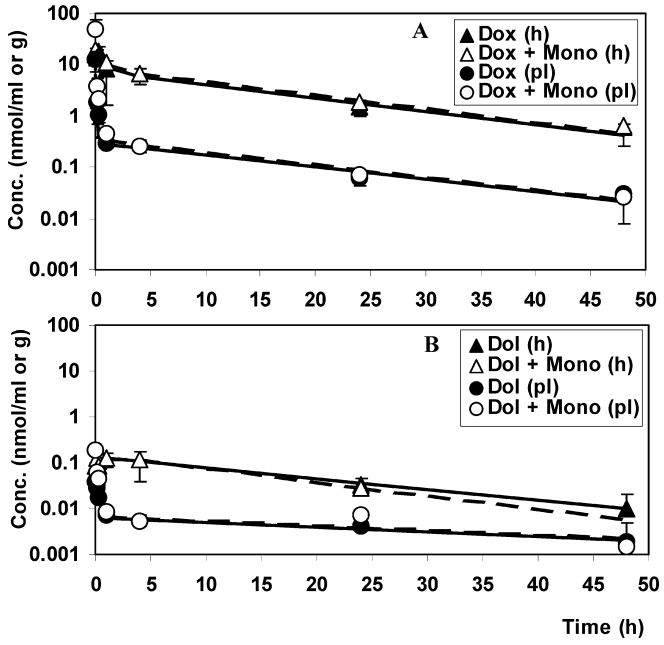

Fig. 2A, B Concentration-time plots of doxorubicin (*Dox*, **A**) and doxorubicinol (*Dol*, **B**) in plasma and heart tissue of mice receiving doxorubicin (10 mg.kg^{-1}, i.v.) with or without monoHER (500 mg.kg^{-1}, i.v.)

immediately after doxorubicin administration. In heart tissue the peak concentration of doxorubicinol was attained at about 1 h after administration. The concentration of doxorubicinol in plasma and heart tissue decreased with final half-lives of 29.2 and 12.3 h, respectively. The MRTs of doxorubicinol in plasma and heart tissue were 39.8 and 14.8 h, respectively. $AUC_{0.03\text{-}48\ h}$ values of doxorubicinol were 0.2 ± 0.1 nmol.h.ml^{-1} and 2.5 ± 0.7 nmol.h.g^{-1} in plasma and heart tissue, respectively. Coadministration of monoHER did not significantly affect the $AUC_{0.03\text{-}48\ h}$, $t_{1/2final}$ or MRT of doxorubicinol in plasma or heart tissue ($P > 0.05$, Table 3, Fig. 2B).

Discussion

MonoHER has been found to selectively protect against doxorubicin-induced cardiotoxicity without interfering with its antitumor activity [10, 11]. Because monoHER is an iron chelator and an antioxidant [12, 13, 14], its cardioprotective effect has been attributed to its ability to reduce the formation of free radicals produced by doxorubicin [3, 19, 20] and to scavenge them. Other mechanism(s) might also play a role. One such possible mechanism is a pharmacokinetic interaction between monoHER and doxorubicin. We therefore explored this alternative mechanism in the present study.

The concentration-time curves of monoHER in plasma and heart tissue and the values of its main pharmacokinetic parameters are in accordance with those of our previous study (Abou El Hassan et al., submitted for publication). MonoHER was rapidly taken up into heart tissue, which corresponds with a short distribution half-life in plasma. The rapid elimination was reflected by short final half-lives in plasma and heart tissue. Coadministration of doxorubicin had no significant effect ($P > 0.05$) on the $AUC_{5\text{-}120\ min}$ of monoHER in plasma or heart tissue or on the other values of the main pharmacokinetic parameters of monoHER in plasma and heart tissue.

The pharmacokinetics of doxorubicin in our study were in good agreement with the results obtained by Van der Vijgh et al. in a previous study [5]. Coadministration of monoHER did not cause a significant change in the $AUC_{0.03\text{-}48\ h}$ of doxorubicin ($P > 0.05$) in either plasma or heart tissue. Comparable values were obtained for the main pharmacokinetic parameters of doxorubicin in plasma and heart tissue in the presence or absence of monoHER. Also, the heart/plasma ratios of $AUC_{0.03\text{-}48\ h}$ of doxorubicin with and without monoHER were comparable.

In contrast to the results of previous studies [15, 16], the results of the present study indicate that monoHER does not influence the activity of aldoketoreductase, which reduces doxorubicin to doxorubicinol. Coadministration of monoHER did not significantly affect the $AUC_{0.03\text{-}48\ h}$, final half-life or MRT of doxorubicinol in plasma or heart tissue (Table 3).

Although monoHER is present in plasma and heart tissue for a shorter time than doxorubicin, it is still present in appreciable levels at the time of peak levels of doxorubicin in plasma and heart tissue when doxorubicin is given after monoHER. Thus, from a pharmacokinetic point of view, monoHER protects at least when

Table 2 Pharmacokinetic parameters (95% confidence intervals) of doxorubicin in plasma and heart tissue of mice receiving doxorubicin (10 mg.kg^{-1}, i.v.) with or without monoHER (500 mg.kg^{-1}, i.v.)

Parameter	Plasma		Heart tissue	
	Dox	Dox + MonoHER	Dox	Dox + MonoHER
$AUCAUC_{0.03\text{-}48\ h}$[a]	5.4–7.4	7.6–10.8	108.0–173.8	139.7–172.5
$t_{1/2\alpha}$ (h)	0.03–0.05	0.02–0.04	0.14–0.74	0.013–0.93
$t_{1/2final}$ (h)	9.4–15.8	8.5–15.7	7.6–15.4	8.5–14.5
MRT (h)	12.6–16.8	9.3–11.7	11.7–16.0	13.5–15.1
Cl (ml.h^{-1}.g^{-1})	1.9–3.4	1.3–2.5	–	–
V_d^{SS} (ml.g^{-1})	29.7–43.9	15.9–21.7	–	–

[a] $AUC_{0.03\text{-}48\ h}$ is expressed as nanomoles×hour per milliliter of plasma or per gram of wet heart tissue

Table 3 Pharmacokinetic parameters (95% confidence intervals) of doxorubicinol in plasma and heart tissue of mice receiving doxorubicin (10 mg.kg^{-1}, i.v.) with or without monoHER (500 mg.kg^{-1}, i.v.)

Parameter	Plasma		Heart tissue	
	Dol	Dol + MonoHER	Dol	Dol + MonoHER
$AUC_{0.03\text{-}48\ h}$[a]	0.1–0.3	0.1–0.5	1.8–3.2	1.4–3.2
$T_{1/2final}$ (h)	7.8–26.6	4.8–22.6	10.3–15.1	8.9–12.3
MRT (h)	33.4–46.2	29.0–40.0	11.9–17.7	10.4–15.6

[a]$AUC_{0.03\text{-}48\ h}$ is expressed as nanomoles×hour per milliliter of plasma or per gram of wet heart tissue

high doxorubicin levels are present during its distribution phase. This corresponds with previous observations that reducing peak levels of doxorubicin by, for example, long-term infusion of doxorubicin decreases its cardiotoxicity [21].

In conclusion, monoHER did not affect the pharmacokinetics of doxorubicin in plasma or heart tissue. MonoHER also had no influence on the conversion of doxorubicin into doxorubicinol in plasma or heart tissue. Thus an influence of monoHER on the pharmacokinetics and metabolism of doxorubicin can be excluded as a possible explanation for the cardioprotection of monoHER.

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