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ORIGINAL ARTICLE

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# Bioavailability and pharmacokinetics of the cardioprotecting flavonoid 7-monohydroxyethylrutoside in mice

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**Abstract** *Purpose*: The pharmacokinetics and bioavailability of monoHER, a promising protector against doxorubicin-induced cardiotoxicity, were determined after different routes of administration. *Methods*: Mice were treated with 500 mg.kg$^{-1}$ monoHER intraperitoneally (i.p.), subcutaneously (s.c.) or intravenously (i.v.) or with 1000 mg.kg$^{-1}$ orally. Heart tissue and plasma were collected 24 h after administration. In addition liver and kidney tissues were collected after s.c. administration. The levels of monoHER were measured by HPLC with electrochemical detection. *Results*: After i.v. administration the $AUC_{0-120\ min}$ values of monoHER in plasma and heart tissue were $20.5 \pm 5.3$ μmol.min.ml$^{-1}$ and $4.9 \pm 1.3$ μmol.min.g$^{-1}$ wet tissue, respectively. After i.p. administration, a mean peak plasma concentration of about 130 μ*M* monoHER was maintained from 5 to 15 min after administration. The $AUC_{0-120\ min}$ values of monoHER were $6.1 \pm 1.1$ μmol.min.ml$^{-1}$ and $1.6 \pm 0.4$ μmol.min.g$^{-1}$ wet tissue in plasma and heart tissue, respectively. After s.c. administration, monoHER levels in plasma reached a maximum (about 230 μ*M*) between 10 and 20 min after administration. The $AUC_{0-120\ min}$ values of monoHER in plasma, heart, liver and kidney tissues were $8.0 \pm 0.6$ μmol.min.ml$^{-1}$, $2.0 \pm 0.1$, $22.4 \pm 2.0$ and $20.5 \pm 5.7$ μmol.min.g$^{-1}$, respectively. The i.p. and s.c. bioavailabilities were about 30% and 40%, respectively. After oral administration, monoHER could not be detected in plasma, indicating that monoHER had a very poor oral bioavailability. *Conclusions*: MonoHER was amply taken up by the drug elimination organs liver and kidney and less by the target organ heart. Under cardioprotective conditions (500 mg/kg, i.p.), the $C_{max}$ was 131 μ*M* and the $AUC_{\infty}$ was 6.3 μ*M*.min. These values will be considered endpoints for the clinical phase I study of monoHER.

**Keywords** MonoHER · Cardiotoxicity · Plasma · Heart

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## Introduction

Flavonoids are naturally occurring benzo-γ-pyron derivatives, which are present in the food of humans and animals. 7-Monohydroxyethylrutoside (monoHER) is a semisynthetic member of the flavonoid subclass of flavonols. It is the best antioxidant occurring in the registered hydroxyethylated rutoside mixture Venoruton [1, 2]. In mice, monoHER administered intraperitoneally (i.p.) 60 min before doxorubicin has been successfully used as a protector against doxorubicin-induced cardiotoxicity without interfering with its antitumor effect [3, 4]. It is likely that the cardioprotective effect of monoHER results from its good antioxidant properties, i.e. being a good inhibitor of lipid peroxidation [5] as well as a site-specific scavenger of $Fe^{2+}$ [6].

For the determination of the future phase I study endpoints of monoHER, it is important to characterize the pharmacokinetic profile of monoHER. For this purpose the levels of monoHER have to be determined in plasma and heart tissue of the mice. Different studies have been undertaken to investigate the metabolism and distribution of monoHER in various species [7, 8, 9, 10, 11, 12, 13]. However the methods used are not accurate and specific enough to provide reliable pharmacokinetic

data. Recently, we developed and validated specific and sensitive procedures to measure the levels of monoHER in both plasma and heart [14, 15].

With these analytical tools, we were able to realize the aims of the present study, i.e. the determination of the pharmacokinetics of monoHER in plasma and heart, and liver and kidney tissues after intravenous (i.v.), i.p., subcutaneous (s.c.) and oral administration.

## Materials and methods

### Chemicals and reagents

MonoHER was kindly provided by Novartis Consumer Health (Nyon, Switzerland). Acetic acid, acetone, *o*-phosphoric acid (85%), potassium chloride, sodium bisulfite, silver chloride, disodium hydrogen phosphate and sodium dihydrogen phosphate monohydrate were purchased from Merck (Amsterdam, The Netherlands), EDTA from Sigma-Aldrich Chemie (Zwijndrecht, The Netherlands) and methanol (HPLC grade) from J.T. Baker (Deventer, The Netherlands).

### Animals

The study was approved by the Ethics Committee for Animal Experiments of Vrije Universiteit in Amsterdam, The Netherlands. Male Balb/c mice (20–30 g) obtained from Harlan Nederland (Horst, The Netherlands) were maintained in cages in groups of four to six on regular chow and water ad libitum. The highest standards of animal welfare were applied [16]. Mice were kept in a light- and temperature-controlled room (21–22 °C; humidity 60–65%). The animals were allowed to acclimatize to the animal facility conditions for at least 1 week before starting the experiment. After drug administration the mice were individually housed until they were killed.

### Treatment

Mice were randomized into four treatment groups:

- Group 1 ($n=42$) received 500 mg.kg$^{-1}$ monoHER i.v. via the tail vein as a bolus injection.
- Group 2 ($n=33$) received 500 mg.kg$^{-1}$ monoHER i.p. as a bolus injection.
- Group 3 ($n=33$) received 500 mg.kg$^{-1}$ monoHER s.c. as a bolus injection.
- Group 4 ($n=33$) received 1000 mg.kg$^{-1}$ monoHER orally by means of a stainless steel gastric intubation tube.

The i.p., s.c. and i.v. doses corresponded to the protective dose of monoHER [3, 4]. An oral dose two times higher was chosen because the oral bioavailability of flavonoids is generally low [17, 18].

Solutions were prepared freshly before treatment by dissolving monoHER in alkaline water (pH about 8.4). Slight heating (50 °C) and sonication were required for complete dissolution of monoHER.

Heart tissue was collected immediately after blood withdrawal (by heart puncture) at several time-points within 60 min, and at 1.5, 2, 4, 6, 8, 22 and 24 h after i.v., i.p., s.c. and oral administration. In addition, liver and kidney tissues were also collected after s.c. administration. The experiment was performed three times. In each experiment one mouse was killed at each time-point.

### Sample pretreatment

Blood samples were centrifuged (3000 rpm, 5 min, 4 °C). Plasma, heart, liver and kidney tissues were immediately stored at −80 °C. On the day of analysis, different tissues were further cooled with liquid nitrogen and pulverized with a microdismembranator. Tissue powder was immediately suspended in 25 m*M* phosphate buffer with 0.4% sodium bisulfite for the HPLC analysis.

### HPLC analysis

Concentrations of monoHER were measured in plasma and heart tissue as we have previously described [14, 15]. Likewise, the levels of monoHER in liver and kidney were measured. 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 aliquots 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. Calibration samples of monoHER in heart, liver and kidney tissue homogenates and plasma were freshly prepared on each day of analysis. The detection limits were 0.3 µ*M* and 0.072 nmol.g$^{-1}$ for the assay in plasma and different tissues, 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.

### Data analysis

Concentration-time curves of monoHER were obtained by plotting the mean concentrations at each time-point versus mean time on a semilogarithmic scale. The area under the concentration-time curve ($AUC_\infty$) and mean residence time (MRT) of monoHER were determined after i.p., s.c. and i.v. administration by non-compartmental analysis using the pharmacokinetic computer program WinNonLin, version 1.5 (Pharsight Corporation, Mountain View, Calif.). In addition, the clearance (Cl) and volume of distribution ($V_d^{SS}$) were calculated from the C-t data after i.v. administration. The initial ($t_{1/2\alpha}$) and final half-lives ($t_{1/2final}$) after i.v. administration of monoHER were calculated by CSTRIP from the lnC-t plot using the least-squares method.

Possible differences between the groups were determined by statistical comparison of the $AUC_{0-120\ min}$ values [19]. $AUC_{0-120\ min}$ values were calculated by the trapezoidal rule using the following formula:

$$AUC = \sum_{i=1}^{n} (W_i \times C_i)$$

in which factor $W_i$ was calculated as follows:

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

The calculated $AUC_{0-120\ min}$ values after different routes of administration were statistically compared by calculating the variance [VAR ($C_i$)] of the mean concentration ($C_i$) of the drug in the plasma or heart tissue of the three mice at each time-point. The standard deviations of the AUC values were calculated as:

$$S.D. = \sqrt{\left(\sum_{i=1}^{n} \left(W_i^2 \times (C_i)\right)\right)}$$

With these values Student's *t*-test was used for the statistical comparison.

## Results

### Intravenous administration

The concentration-time plots of monoHER in plasma and heart tissue of mice after i.v. administration of

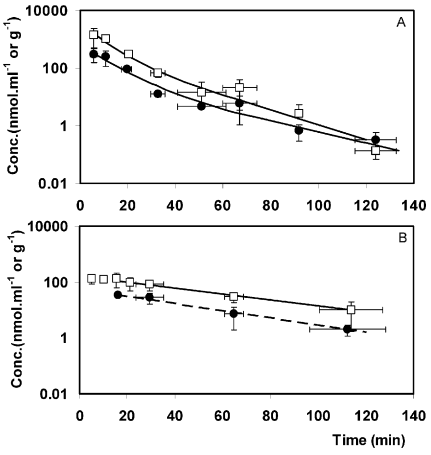


**Fig. 1 A, B** MonoHER levels (means ± SD, $n = 3$) in plasma (□) and heart tissue (●) of mice receiving 500 mg.kg$^{-1}$ i.v. (**A**) or i.p. (**B**)

**Table 1** Summary of the pharmacokinetic parameters of monoHER in plasma and heart tissue of mice treated with 500 mg.kg$^{-1}$ i.v.

| Parameter | Plasma | Heart |
|---|---|---|
| $C_{max}$ (μmol.ml$^{-1}$ or g$^{-1}$ wet tissue) | 2.0 | 0.4 |
| $t_{max}$ (min) | 0 | 0 |
| $T_{1/2\alpha}$(min) | 5.7 | 7.8 |
| $T_{1/2final}$(min) | 11.8 | 16.2 |
| $AUC_{\infty}$(μmol.min.ml$^{-1}$ or g$^{-1}$ wet tissue) | 24.7 | 5.6 |
| $AUC_{0-120\ min}$(μmol.min.ml$^{-1}$ or g$^{-1}$ wet tissue) | 20.5 | 4.9 |
| MRT (min) | 10.9 | 12.1 |
| Cl (ml.min$^{-1}$.g$^{-1}$) | 0.03 | – |
| $V_d^{SS}$ (ml.g$^{-1}$) | 0.34 | – |

500 mg.kg$^{-1}$ monoHER are shown in Fig. 1A. The pharmacokinetic data are given in Table 1. Immediately after monoHER administration, peak values were not only observed in plasma but also in heart tissue. The monoHER concentration in plasma and heart tissue decreased rapidly in a biphasic manner with initial half-lives of 5.7 and 7.8 min and final half-lives of 11.8 and 16.2 min, respectively. MonoHER could not be detected for longer than 2 h after administration. The rapid disappearance of monoHER from the body is also expressed by the total body clearance (0.03 ml.min$^{-1}$.g$^{-1}$) and the short MRT in plasma (10.9 min) and heart tissue (12.1 min). The volume of distribution $\left(V_d^{SS}\right)$ was 0.34 ml.g$^{-1}$. Although monoHER was taken up rapidly by heart tissue, its heart concentrations remained lower than those in plasma. As a consequence, the $AUC_{0-120\ min}$ value in plasma (20.5 ± 5.3 μmol.min.ml$^{-1}$) was higher than that in heart tissue (4.9 ± 1.3 μmol.min.g$^{-1}$; $P < 0.01$). The heart tissue/plasma concentration ratio (t/p ratio) of monoHER was about 0.3 during the first hour and increased to 0.8 when measured up to 2 h after administration.

**Table 2** Summary of the pharmacokinetic parameters of monoHER in plasma and heart tissue of mice treated with 500 mg.kg$^{-1}$ i.p.

| Parameter | Plasma | Heart |
|---|---|---|
| $C_{max}$ (nmol.ml$^{-1}$ or g$^{-1}$ wet tissue) | 131 | 35.3 |
| $t_{max}$ (min) | 5–15 | 5–15 |
| $T_{1/2final}$(min) | 28.5 | 25.7 |
| $AUC_{\infty}$(μmol.min.ml$^{-1}$ or g$^{-1}$ wet tissue) | 6.3 | 1.6 |
| $AUC_{0-120\ min}$(μmol.min.ml$^{-1}$ or g$^{-1}$ wet tissue) | 6.1 | 1.6 |
| MRT (min) | 43.6 | 43.0 |

## Intraperitoneal administration

The concentration-time plots of monoHER in plasma and heart tissue of mice after i.p. administration of monoHER (500 mg.kg$^{-1}$) are shown in Fig. 1B. The pharmacokinetic data are given in Table 2. A peak plasma level of about 130 μ*M* monoHER was found as early as 5 min after monoHER administration and was maintained up to 15 min after administration. During this time the peak concentration in heart tissue was about 35 nmol.g$^{-1}$ wet tissue. The concentration of monoHER in plasma and heart tissue dropped rapidly and monoexponentially with half-lives of 28.5 and 25.7 min, respectively. This rapid disappearance was also expressed by the relatively short final half-life and MRT of monoHER in plasma (28.5 and 43.6 min, respectively) and heart tissue (25.7 and 43.0 min, respectively). MonoHER could not be detected in plasma and heart for longer than 2 h after administration. The heart t/p ratio of monoHER was about 0.25 during these first 2 h after administration. The $AUC_{0-120\ min}$ of monoHER in heart tissue (1.6 ± 0.4 μmol.min.g$^{-1}$) was smaller than that in plasma (6.1 ± 1.1 μmol.min.ml$^{-1}$; $P < 0.005$). The i.p. bioavailability calculated from the ratio of the $AUC_{\infty}$ values in plasma after i.p. and i.v. administration was about 30%.

## Subcutaneous administration

The concentration-time plots of monoHER in plasma, heart, liver and kidney tissues of mice after s.c. administration of monoHER (500 mg.kg$^{-1}$) are shown in Fig. 2. The pharmacokinetic data are given in Table 3. Shortly after monoHER administration (10–30 min), peak values were observed in plasma (230 μ*M*), heart (about 70 nmol.g$^{-1}$), liver (428 nmol.g$^{-1}$) and kidney (345 nmol.g$^{-1}$). The concentration of monoHER in plasma and heart tissue dropped monoexponentially with half-lives of 23.9 and 29.3 min, respectively. MonoHER could not be detected in plasma and heart for longer than 2 h after administration. This rapid disappearance from plasma and heart tissue was also expressed by the short MRT of monoHER (33.0 and 41.5 min, respectively). Longer half-lives and MRTs of monoHER were observed in liver (47.4 and 88 min,

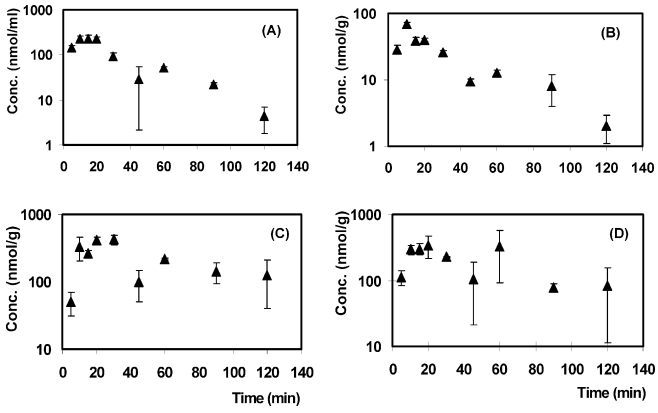


**Fig. 2A–D** MonoHER levels (means ± SD, $n = 3$) in (**A**) plasma, (**B**) heart, (**C**) liver and (**D**) kidney tissues of mice receiving 500 mg.kg$^{-1}$ s.c.

respectively) and kidney (29.9 and 66.6 min, respectively). The heart t/p ratio of monoHER was in the range 0.2–0.4 during the 2 h after administration, while higher t/p ratios were observed for the liver and kidney. The t/p ratios for liver and kidney were about 0.5, 5 and 25 after 5 min, and 1 and 2 h, respectively. The $AUC_{0-120\ min}$ of monoHER in heart tissue ($2.0 \pm 0.1$ μmol.min.g$^{-1}$) was smaller than that in plasma ($8.0 \pm 0.6$ μmol.min.ml$^{-1}$; $P < 0.005$). The $AUC_{0-120\ min}$ values of monoHER in the liver ($22.4 \pm 2.0$ μmol.min.g$^{-1}$) and the kidney ($20.5 \pm 5.7$ μmol.min.g$^{-1}$) were significantly higher ($P < 0.005$) than that in plasma. The s.c. bioavailability was calculated to be about 40%.

### Oral administration

MonoHER could not be detected in plasma of mice treated with 1000 mg.kg$^{-1}$ monoHER orally. To estimate the maximum oral bioavailability under these circumstances, the $AUC_\infty$ of monoHER in plasma was calculated assuming that the lower limit of quantification (0.3 μ*M*) [14] was maintained during the 24 h after monoHER administration. From this calculated $AUC_\infty$ of 430 μ*M*.min, the oral bioavailability was estimated to be less than 1%.

## Discussion

The peak concentration of monoHER in heart tissue was reached immediately after i.v. administration, which indicated a very rapid uptake of monoHER into heart tissue. This observation corresponds with the short distribution half-life ($t_{1/2\alpha}$) of 5.7 min. The rapid elimination of monoHER from the circulation is reflected by the short elimination half-life ($t_{1/2f}$) of 11.8 min, the large total body clearance of 0.03 ml.min$^{-1}$.g$^{-1}$ and the short MRT of 10.9 min. As a consequence, monoHER was only measured in plasma for 2 h after administration. This is in accordance with the rapid excretion of monoHER via the bile and urine as previously observed in different species [13, 20, 21]. On the other hand, it has been reported that radioactivity can be measured in plasma for at least 6 h following the administration of radiolabeled monoHER. The reason for this observation is probably the highly sensitive but unspecific determination of radioactivity originating from monoHER or its metabolites, which may circulate for a longer period. The present data confirm the rapid urinary and biliary excretion routes as shown by the rapid and high uptake of monoHER into the liver and kidney after only 10 min following s.c. administration of monoHER.

The volume of distribution of monoHER was small (0.34 ml.g$^{-1}$). It has been suggested that the constituents of the hydroxyethylated rutosides mixture Venoruton (including monoHER) are taken up by the inner wall of the blood vessels [22, 23]. Considering the very large surface area of the blood vessels over the whole body and the small $V_d^{SS}$, a very specific high uptake of monoHER into the blood vessel walls would not be expected. The low volume of distribution also corresponds with the low concentration of monoHER in heart tissue in comparison to that in plasma as reflected by the $AUC_\infty$ values, the heart to plasma ratio being 0.2.

The oral bioavailability of monoHER was very poor, which can be explained in different ways. First monoHER has a very poor solubility in acidic solutions [24] and therefore a great amount of the administered dose may precipitate in the stomach of the treated mice, which was indeed observed. Secondly, the calculation of the oral bioavailability was based on the concentration of the parent compound only without considering its possible metabolites. Wienert and Gahlen have proposed that a small fraction of the parent compound (the glycoside) is absorbed through the gut and the rest is metabolized to the aglycone by the intestinal microflora [17]. If the aglycones are absorbed then the oral bioavailability will always be too low when considering the parent compound only. Indeed, Ader et al. have shown

**Table 3** Summary of the pharmacokinetic parameters of monoHER in plasma, heart, liver and kidney tissues of mice treated with 500 mg.kg$^{-1}$ s.c.

| Parameter | Plasma | Heart | Liver | Kidney |
|---|---|---|---|---|
| $C_{max}$ (nmol.ml$^{-1}$ or g$^{-1}$ wet tissue) | 229.3 | 68.4 | 427.2 | 345.3 |
| $t_{max}$ (min) | 10–20 | 10 | 10–30 | 10–20 |
| $t_{1/2final}$(min) | 23.9 | 29.3 | 47.4 | 29.9 |
| $AUC_\infty$(μmol.min.ml$^{-1}$ or g$^{-1}$ wet tissue) | 8.0 | 2.0 | 32.2 | 23.9 |
| $AUC_{0-120\ min}$(μmol.min.ml$^{-1}$ or g$^{-1}$ wet tissue) | 8.0 | 2.0 | 22.4 | 20.5 |
| MRT (min) | 34.1 | 41.5 | 88.7 | 66.6 |

that the oral bioavailability of quercetin is only 0.5% for the parent compound, whereas the bioavailability is 35-fold more (17%) when the metabolites are also taken into account [18].

After administration of monoHER under the protective conditions (500 mg/kg, i.p.) [3, 4], the plasma concentration of monoHER reached a mean plateau level of about 130 $\mu M$ between 5 and 15 min after administration. The plasma $AUC_\infty$ under these conditions was 6.3 $\mu mol.min.ml^{-1}$. In contrast to the biphasic decline of monoHER levels after i.v. administration, monoHER disappeared from plasma and heart tissue with a single half-life of about 30 min after i.p. administration. This difference may be explained by the rapidly decreasing release of the drug from the peritoneum during the first part of the distribution and elimination phase. The i.p. bioavailability of monoHER in mice was low (30%), which may be explained by a large first-pass elimination by the liver as previously indicated by a higher biliary excretion of radioactivity after i.p. than after i.v. administration of radiolabeled monoHER in laboratory animals [8]. In addition to the high liver uptake our study also showed a high uptake by the kidney 10 min after s.c. administration of monoHER.

The uptake of monoHER into heart tissue seemed to be independent of the route of administration, because the heart t/p ratios were almost the same during the first hour after i.p. (0.25), s.c (about 0.25) and i.v. (0.3) administration. The i.v. data also indicate that an equilibrium already exists between plasma and heart concentrations at the early time-points. On the other hand the liver and kidney t/p ratios ranged between 0.5 and 5 after 5 min and 1 h, respectively, indicate the much higher uptake of monoHER by the liver and kidney.

## Conclusion

Plateau levels of about 130 $\mu M$ and 35 $nmol.g^{-1}$ monoHER were obtained in plasma and heart, respectively, between 5 and 15 min after i.p. administration of the protective dose of monoHER (500 $mg.kg^{-1}$). By 2 h after administration, monoHER had disappeared from plasma and heart tissue. The $AUC_\infty$ of monoHER under the protective conditions was 6.3 $nmol.min.ml^{-1}$. The i.p. and s.c. bioavailabilities were 30% and 40%, respectively. The oral bioavailability was very poor (estimated to be $< 1\%$). Therefore, monoHER cannot be administered orally. These data will be considered as endpoints for the clinical phase I study of monoHER.

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