7-Monohydroxyethylrutoside Protects against Chronic Doxorubicin-induced Cardiotoxicity When Administered Only Once Per Week

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INTRODUCTION

Doxorubicin is a very effective antitumor agent used in the treatment of a variety of hematological malignancies and solid tumors. The major acute toxicity is bone marrow suppression, and long-term clinical usefulness is limited by a cumulative dose-dependent irreversible chronic cardiotoxicity, which manifests itself as congestive heart failure. The observed cardiotoxicity is generally believed to be caused by the formation of oxygen free radicals (1). The heart is particularly vulnerable to damage induced by free radicals because protective enzymes such as superoxide dismutase and catalase are present at a lower level there than in other tissues of the body (2–4).

The generation of oxygen free radicals is facilitated by the formation of doxorubicin-iron complexes (5, 6). The iron in these complexes can participate in redox cycling and catalyze lipid peroxidation and oxygen radical generation. Iron chelators and free radical scavengers might provide protection by preventing the formation of the extremely reactive hydroxyl radical in the Fenton reaction and by scavenging radicals that have already been formed. The iron chelator ICRF-187 (Fig. 1) has been shown to protect against doxorubicin-induced cardiotoxicity in in vivo experiments. However, its clinical success is limited because it increases the hematological toxicity (7, 8) and reduces the antitumor activity of doxorubicin (9), although other studies have reported no modulation of the antitumor effect (10–12).

Flavonoids are a class of naturally occurring compounds with excellent iron chelating and radical scavenging properties (13–17) and are therefore of interest as possible modulators of doxorubicin-induced cardiotoxicity. Recently, we have shown in mice that the semisynthetic flavonoid monoHER2 (Fig. 1) protects against doxorubicin-induced cardiotoxicity when administered as an i.p. dose of 500 mg/kg five times/week in combination with a weekly i.v. dose of doxorubicin. Additionally, in contrast to ICRF-187, hydroxyethylrutosides are known to have very low toxicity at high concentrations (18). This was shown by the fact that the dose of 500 mg/kg monoHER did not result in adverse effects (19).

However, a dose schedule of monoHER (five times/week) in combination with a single dose of doxorubicin will be very inconvenient in clinical practice. Therefore, the aim of the present investigation was to determine whether a single dose of monoHER just before doxorubicin would also provide complete protection against doxorubicin-induced cardiotoxicity.

MATERIALS AND METHODS

Chemicals. MonoHER (M, 654.6) was kindly provided by Novartis Consumer Health (Nyon, Switzerland). MonoHER

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2 The abbreviations used are: monoHER, 7-monohydroxyethylrutoside; ECG, electrocardiogram.
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were sutured s.c. in lead II position [with the negative (•) lead toward the lower chest] at the right shoulder and the positive (•) lead toward the lower left chest].

Animals. Twenty-six male BALB/c mice (20–25 g) obtained from Harlan Olac Nederland (Horst, the Netherlands) were kept in a light- and temperature-controlled room (21°C–22°C; humidity, 60–65%). The animals were fed a standard diet (Hope Farms, Woerden, the Netherlands) and allowed to eat and drink tap water ad libitum. The animals were allowed to adapt to the laboratory housing conditions for at least 1 week before surgery.

Telemetry System. The telemetry system, which consisted of implantable transmitters (TA10ETA-F20) and telemetry receivers (RPC-1), was obtained from DATA Sciences International (DSI; St. Paul, MN). Output (Hz) from the transmitter was monitored by the telemetry receivers placed underneath the animal’s cage. The signals from the receiver were consolidated by the multiplexer (Data Exchange Matrix; DSI) and stored and analyzed using a personal computer (Compaq Presario 1510) provided with analyzing software from Data Sciences International (Dataquest A.R.T. 1.01).

Surgery. All protocols were approved by the Ethics Committee for Animal Experiments of the Vrije Universiteit (Amsterdam, the Netherlands). The mice were anesthetized i.p. with a 0.07 ml/10 g mixture of Hypnorm (0.315 mg/ml fentanyl and 10 mg/ml fluanisone), Dormicum (5 mg/ml midazolam), and sterilized water in a 1:1:2 ratio. Surgery was performed as described in detail by Kramer et al. (20). In short, the transmitter was implanted in the peritoneal cavity of each mouse 2 weeks before the start of the treatment. The leads of the transmitter were sutured s.c. in lead II position [with the negative (−) lead at the right shoulder and the positive (+) lead toward the lower left chest].

After surgery, the mice were allowed to recover for 2 weeks, and then they were submitted to one of the following weekly dose schedules for 6 weeks: (a) group 1 (n = 7), 0.9% NaCl solution i.v.; (b) group 2 (n = 7), 0.9% NaCl solution i.p., followed by 4 mg/kg doxorubicin i.v. after 1 h; (c) group 3 (n = 5), 500 mg/kg monoHER i.p., followed by 4 mg/kg doxorubicin i.v. after 1 h and 500 mg/kg monoHER i.p. every 24 h for 4 days (MonoHER5; Ref. 19); (d) group 4 (n = 5), 500 mg/kg monoHER i.p., followed by 4 mg/kg doxorubicin i.v. after 1 h (MonoHER1).

The i.v. injections were administered in the tail vein. After these treatments, the animals were observed for 2 additional weeks. In the case of group 4 (monoHER1), animals were observed for 10 additional weeks. During treatment and the observation period, body weight was determined once per week as a measure of general toxicity. ECG and heart rate were registered in the freely moving animal once per week until the end of the study. For the monoHER5 group (group 3), the original data were used, which have been presented previously (19).

Parameters. For interpretation of the ECG, five complexes were analyzed in detail. The ST interval was determined as the mean ± SD of these five complexes. Changes in weight were taken as an indication of general toxicity of the treatments, as were the behavior of the animal and a general impression of the condition.

Statistical Analysis. All parameters were expressed as mean ± SE unless stated otherwise. All parameters were evaluated using ANOVA with Fisher’s LSD test for multiple comparisons when ANOVA indicated significant differences between groups. The program used for this analysis was NCSS (by Dr. J. L. Hintze; Kaysville, UT). The level of significance chosen was 99% (P < 0.01) to correct for weekly comparisons between groups (multiple comparisons).

RESULTS

General Toxicity. After surgery, recovery of the animals was indicated by an increase in weight after an initial decrease (data not shown) and by changes in their behavior such as building a nest of the available paper towel.

Behavior appeared normal in all treatment groups. Animals appeared lively throughout the study, and no behavioral changes were observed compared to mice without transmitters. There were no signs of decreased activity, which would indicate low general toxicity.

Weight Gain. A trend observed was that weight gain in the doxorubicin-treated group was somewhat less than that in the doxorubicin/monoHER and saline groups [percentage weight gain at the end of the study (mean ± SE) in groups 1–4 was 10.3 ± 4.1% (saline), 3.5 ± 1.9% (doxorubicin), 14.1 ± 3.5% (monoHER5; Ref. 19), and 6.7 ± 3.8% (monoHER1), respectively].

Pathology. One non-treatment-related death occurred in the monoHER5 group (group 4) in week 6: an i.p. injection error caused fatal internal bleeding. Two other deaths occurred: (a) one in the control group (group 1) in week 8; and (b) one in the doxorubicin group (group 2) in week 4. The abdominal organs, such as the kidney, liver, and intestine, appeared normal in all

![Chemical structures of monoHER (A) and ICRF-187 (B).](Image)
mice. This indicates that both the transmitter and monoHER did not cause any visible abnormalities.

**ECG Measurements.** The ECG signal in lead II deflection of a mouse is somewhat different from that of man (21, 22) but corresponds with the ECG measured in mice under anesthesia or restraint (23, 24). The ECGs of the control animals did not change during the course of the study. Doxorubicin had a profound influence on the shape of the ECG (Fig. 2). The ST interval increased with time by 16.9 ± 2.7 ms in week 8 (Fig. 3). MonoHER was found to protect against these ECG changes with both dosing schedules used. The monoHER5 schedule reduced the increase of the ST interval to 1.7 ± 0.8 ms (19), and the monoHER1 schedule reduced the increase of the ST interval to 2.3 ± 0.7 ms (in both cases, \( P < 0.001 \) relative to doxorubicin; not significantly different from control). Even after 16 weeks, the increase in the ST interval for the monoHER1 schedule was only 2.6 ± 1.2 ms (\( n = 3 \)).

**DISCUSSION**

In addition to previous studies (19, 25) this study again demonstrates that the flavonoid monoHER is a very effective protector against doxorubicin-induced cardiotoxicity. The involvement of radicals in the mechanism of doxorubicin-induced cardiotoxicity has been the subject of extensive review (3, 26–29). In recent years, several mechanisms have been suggested for doxorubicin-induced cardiotoxicity, but the free radical theory is still the most favored theory. Doxorubicin can generate oxygen radicals in several ways, either by itself or by forming a complex with iron ions. Production of radicals is generally due to redox cycling of doxorubicin. However, studies with a number of antioxidants and iron chelators have had only limited success (30–35).

To date, ICRF-187 is the only cardioprotective agent in clinical use. ICRF-187 is hydrolyzed intracellularly into its ring-opened product ICRF-198, an EDTA-like iron chelator (36, 37). A drawback of ICRF-187 is its own toxicity, which has been shown to include transient leucopenia and moderate thrombocytopenia (36, 38), thus amplifying the acute toxicity of doxorubicin, which is also myelotoxicity. As a result of this, the clinical use of ICRF-187 is limited.

In contrast to ICRF-187, monoHER can protect against doxorubicin-induced cardiotoxicity without causing side effects of its own (19). MonoHER belongs to the hydroxyethylrutoside family, which is known to have very low toxicity at high concentrations (18). Furthermore, we have shown previously that monoHER did not interfere with the antitumor activity of doxorubicin in a 5-day dosing schedule (25). These observations strongly indicate that monoHER is an attractive compound for clinical practice.

A disadvantage for the clinical application of monoHER would have been the dosing-schedule. Until now, a 5-day dosing schedule was advised over a dosing schedule of two times/week because monoHER seemed somewhat more active against doxorubicin-induced cardiotoxicity when given five times/week in combination with a weekly administration of doxorubicin. Eight weeks after starting treatment, the increase in the ST interval for the 5-day schedule and 2-day schedule was 1.7 ± 0.7 and 5.1 ± 1.7 ms, respectively (19). The 5-day dosing schedule of monoHER was based on the prolonged presence of doxorubicin. After administration of doxorubicin to mice, most of the doxorubicin and its major metabolite, doxorubicinol, is cleared from the heart within 5 days (39). Both doxorubicin and doxorubicinol are thought to play a pivotal role in the formation of free radicals. According to the radical scavenging hypothesis, monoHER should be present during these 5 days.

However, Hackett and Griffiths (40) found that plasma levels of monoHER dropped rapidly after i.v. administration of 25 mg/kg \([^{14}C]\)monoHER. After 60 min, monoHER was almost completely eliminated from the plasma. Because we administered a 20× higher dose, we speculated that accumulation would
Cardioprotection by a Weekly Dosing Schedule of MonoHER against doxorubicin-induced cardiotoxicity was demonstrated in mice when given once, i.e., 1 h before doxorubicin (Fig. 3), allowing the most convenient schedule to administer monoHER in combination with doxorubicin. Even after 8 more weeks of observation, the ST interval had not increased (2.6 ± 1.2 ms in week 16 versus 2.3 ± 0.7 ms in week 8). These data show that the heart is protected not only during the administration of doxorubicin, but also for a longer period of time thereafter.

In summary, it was shown that monoHER protects in vivo against doxorubicin-induced cardiotoxicity when administered only 1 h before doxorubicin. The presence of monoHER during the peak plasma levels of doxorubicin and doxorubicinol appeared to be sufficient. It was also demonstrated that monoHER protects the heart not only during the administration of doxorubicin, but also for a longer period of time thereafter. It can therefore be concluded that monoHER is a very attractive candidate for the prevention of doxorubicin-induced cardiotoxicity in clinical practice.

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REFERENCES


