Monohydroxyethylrutoside, a Dose-dependent Cardioprotective Agent, Does Not Affect the Antitumor Activity of Doxorubicin

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ABSTRACT

The cumulative dose-related cardiotoxicity of doxorubicin is believed to be caused by the production of oxygen-free radicals. 7-Monohydroxyethylrutoside (monoHER), a semisynthetic flavonoid and powerful antioxidant, was investigated with respect to the prevention of doxorubicin-induced cardiotoxicity in mice and to its influence on the antitumor activity of doxorubicin in vitro and in vivo. Non-tumor-bearing mice were equipped with a telemeter in the peritoneal cavity. They were given six weekly doses of 4 mg/kg doxorubicin i.v., alone or in combination with either 100 or 250 mg/kg monoHER i.p., 1 h prior to doxorubicin administration and for the following 4 days. Cardiotoxic effects were measured from electrocardiogram changes up to 2 weeks after treatment. Protection against cardiotoxicity was found to be dose dependent, with 53 and 75% protection, respectively, as calculated from the reduction in the ST interval. MonoHER and several other flavonoids with good antioxidant properties were tested for their antiproliferative effects in the absence or the presence of doxorubicin in A2780 and OVCAR-3 human ovarian cancer cells and MCF-7 human breast cancer cells in vitro. Some flavonoids were directly toxic at 50 and 100 μM, whereas others, including monoHER, did not influence the antiproliferative effects of doxorubicin at these concentrations. The influence of monoHER was further tested on the growth-inhibitory effect of 8 mg/kg doxorubicin i.v., given twice with an interval of 1 week in A2780 and OVCAR-3 cells that were grown as s.c. xenografts in nude mice. MonoHER, administered 1 h before doxorubicin in a dose schedule of 500 mg/kg i.p. 2 or 5 days per week, was not toxic and did not decrease the antitumor activity of doxorubicin. It can be concluded that monoHER showed a dose-dependent protection against chronic cardiotoxicity and did not influence the antitumor activity of doxorubicin in vitro or in vivo.

INTRODUCTION

Doxorubicin, an anthracycline with potent antitumor activity against a wide range of human malignancies, has the major acute toxicity of bone marrow suppression. The long-term clinical usefulness is limited by a cumulative dose-related cardiotoxicity. The chronic toxicity to the heart is believed to be caused by the formation of oxygen-free radicals (1), although other mechanisms of toxicity have been suggested. With the current use of hematopoietic growth factors, which allow for high-dose chemotherapies, including anthracycline therapy, cardiotoxicity will become an even more frequent treatment-limiting factor. This is because of higher peak plasma levels, which are more cardiotoxic than low levels and, in turn, lead to the maximum cumulative dose in an earlier stage of treatment.

Recently, we reported that the semisynthetic flavonoid antioxidant monoHER 1 (Fig. 1) provides excellent protection against chronic doxorubicin-induced cardiotoxicity in a mouse model at a dose of 500 mg/kg i.p., given 1 h before doxorubicin and for the next consecutive 4 days. At that dose level, it showed full protection, and no toxic side effects were observed (2). This is in contrast with the clinically successful compound Cardioxane (ICRF-187), which has the disadvantage of being toxic to bone marrow (3). We found that the protection offered by monoHER against doxorubicin-induced cardiotoxicity was comparable to that of ICRF-187, but the toxicity, expressed by weight loss, was more severe in ICRF-187-treated mice than it was in monoHER-comedicated mice (2).

MonoHER is the best antioxidant in the registered flavonoid mixture Venoruton (4, 5), which possesses protective activity against doxorubicin-induced cardiotoxicity (6). To evaluate its possible clinical application, we investigated the protecting capacity of monoHER in two lower doses, 250 and 100

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1 The abbreviations used are: monoHER, 7-monohydroxyethylrutoside; ECG, electrocardiogram; T/C%, treated versus control, multiplied by 100%.
mg/kg, which were compared with the initial 500 mg/kg dose, to obtain insight in the presence of a dose dependency with respect to cardioprotection. Furthermore, we investigated the influence of monoHER on the in vitro and in vivo antitumor activity of doxorubicin.

MATERIALS AND METHODS
Chemicals
MonoHER (MW 654.6) was kindly donated by Zyma S.A. (Nyon, Switzerland). Doxorubicin (Adriblastina; 2 mg/ml) was obtained from Farmitalia Carlo Erba (Milan, Italy). ICRF-187 (Cardioxane; 20 mg/ml) was kindly provided by Chiron (Amsterdam, the Netherlands). Doxorubicin and ICRF-187 were dissolved in 0.9% NaCl solution and stored at -20°C until use. monoHER was dissolved in 36 mM NaOH in sterile water (final concentration, 33 mg/ml; pH approximately 7.8–8) and stored at 4°C for a maximum of 3 days.

Cardiotoxicity Study
Telemetry System
As described earlier (7), the telemetry system, which consisted of implantable transmitters (model TA10ETA-F20), a telemetry receiver (model RA1010), and an analogue ECG adapter (option R08), was obtained from DATA Sciences (St. Paul, MN). The data acquisition system consisted of a MacLab (ML020 MacLab/8; ADInstruments Ltd, London, England), which was connected to an Apple Macintosh LCII 4/80 computer with the program Chart from MacLab. The transmitter was activated by a magnet, after which the output of the transmitter was received by an antenna that was mounted in a receiver board, which was placed under the animal cage. This board was connected to the data acquisition system. The sampling rate was 400 samples/s.

Surgery
For the chronic cardiotoxicity study, male BALB/c mice (20–25 g) obtained from Harlan CPB (Zeist, The Netherlands) were kept in a light- and temperature-controlled room (21–22°C; humidity 60–65%). The animals were fed a standard diet (Hope Farms, Woerden, the Netherlands) and were allowed tap water ad libitum. Animals were kept in quarantine for at least 1 week before surgery.

The mice were anesthetized i.p. with 0.07 ml per 10 g of a mixture of 0.315 mg/ml fentanyl and 10 mg/ml fluanisone (Hypnorm), midazolam (Dormicum, 5 mg/ml), and sterilized water in the ratio 1:1:2. Surgery was performed as described in detail by Kramer et al. (7). In short, the transmitter was im-
planted in the peritoneal cavity of each mouse at least 2 weeks before the start of the treatment. The leads of the transmitter were sutured s.c. in the lead II position [the (−) lead at the right shoulder and the (+) lead toward the lower left chest].

**Experimental Design**

After surgery, the mice were allowed to recover for 2 weeks, after which they were submitted to one of the following weekly dose schedules for 6 weeks: group 1 (*n* = 6), 0.05 ml of a 0.9% NaCl solution i.v.; group 2 (*n* = 5), 4 mg/kg doxorubicin i.v.; group 3 (*n* = 6), 500 mg/kg monoHER i.p., 4 mg/kg doxorubicin i.v. after 1 h, followed by 500 mg/kg monoHER i.p. every 24 h for 4 days (monoHER 500), group 4 (*n* = 6), 250 mg/kg monoHER i.p., 4 mg/kg doxorubicin i.v. after 1 h, followed by 250 mg/kg monoHER i.p. every 24 h for 4 days (monoHER 250); or group 5 (*n* = 5), 100 mg/kg monoHER i.p., 4 mg/kg doxorubicin i.v. after 1 h, followed by 100 mg/kg monoHER i.p. every 24 h for 4 days (monoHER 100).

i.v. injections were administered in the tail vein. After 6 weeks of treatment, the animals were observed for another 2 weeks. During treatment and the observation period, the body weights were determined once a week as a measure of general toxicity. ECG was registered in the freely moving animal once a week until the end of the study.

**Parameters**

**Telemetry Parameters.** For interpretation of the ECG, four consecutive complexes were analyzed in detail. The PR segment, QRS complex, QT interval, and ST interval were calculated as the mean ± SD of these four complexes. In case of an unacceptable variation in the complexes due to noise caused by movement of the animal (coefficient of variation >10%), four other consecutive complexes were selected for evaluation.

**Other Parameters.** Changes in weight were taken as a measure of general toxicity, as were behavior of the animal and general impression of the condition. At the end of the study, the animals were sacrificed by decapitation. The organs were submitted to pathological examination and investigated for any abnormalities, either due to doxorubicin or to the transmitter.

**Cell Culture**

Three cell lines were used, the human ovarian cancer cell lines A2780 (8) and OVCA3-3 (9) and the human breast cancer cell line MCF-7 (10). The cells were routinely grown at 37°C and 5% CO₂ in DMEM (Flow Laboratories, Irvine, Scotland) supplemented with 7.5% heat-inactivated FCS (Life Technologies, Inc., Gaithersburg, MD).

Stock solutions of the flavonoids tested were freshly prepared in DMSO. Doxorubicin was dissolved in sterile 0.9% NaCl solution. Drugs were diluted in culture medium immediately prior to their addition to the culture plates. The DMSO concentration never exceeded 0.55%, which was found not to influence cell growth.

Exponentially growing cells were harvested and plated as single-cell suspensions in 96-well flat-bottomed microtiter plates (Greiner, Solingen, Germany). Cells were seeded in triplicate in 100 μl of medium at a density of 5000 cells/well for A2780 and MCF-7 and 8000 cells/well for OVCAR-3. After 24 h (day 1), 100 μl of medium containing the test compound were added, together with 25 μl of doxorubicin or medium. The total exposure time to the drugs was 72 h. At the end of the drug exposure period (day 4), growth-inhibitory effects were evaluated with the standard sulforhodamine B test (11). Absorbance was read at 540 nm using an automated spectrophotometric microplate reader (Argus 400; Canberra Packard, Tilburg, The Netherlands). Data were collected and analyzed using KinetiCalc (Bio-Tek EIA Application Software, Bio-Tek Instruments, Winooski, VT). The results were expressed as the IC₅₀, which is the concentration of the drug giving a 50% inhibition of cell growth of treated cells when compared to the growth of control cells. Doxorubicin growth inhibition curves were plotted, and IC₅₀ values were calculated in the presence and absence of the flavonoids.

**Antitumor Activity Study**

**Experimental Design**

Nude female mice (Hsd:Athymic-nu) were obtained from Harlan CPB at the age of 6 weeks. The animals were maintained in isolation under controlled atmospheric conditions (temperature, 23–25°C; humidity, 50–60%). Animal handling was carried out under sterile conditions. For drug tolerance studies, non-tumor-bearing mice (8 weeks old) were used. Each group contained three mice. After treatment, the mice were weighed daily to determine toxicity.

For the antitumor studies, 10⁶ cells from A2780 and OVCA3 were inoculated s.c. into both flanks of 8-week-old mice. The solid tumors arising were used for the transplantation of small fragments in subsequent recipients. As xenografts, A2780 shows an undifferentiated pattern and has a volume doubling time of approximately 3.5 days. OVCAR-3 shows a pattern of a poorly differentiated serous adenocarcinoma and has a volume doubling time of approximately 5 days. Treatment was started when the tumor size was approximately 600 mm³ for A2780 and 150 mm³ for OVCAR-3; the first treatment day was designated as day 0. Treatment and control groups consisted of five or six mice each. Mice were weighed and tumors were measured twice a week for A2780 and once a week for OVCAR-3.

**Treatment Evaluation**

Treatments were as follows: group 1, no treatment; group 2, 8 mg/kg doxorubicin i.v., days 0 and 7; group 3, 8 mg/kg doxorubicin i.v., days 0 and 7, 500 mg/kg monoHER i.p., days 0 and 7 (1 h before doxorubicin), 1–4, and 8–11; group 4: 8 mg/kg doxorubicin i.v., days 0 and 7, 500 mg/kg monoHER i.p., days 0 and 7 (1 h before doxorubicin) and days 1 and 8; group 5, 8 mg/kg doxorubicin i.v., days 0 and 7, 100 mg/kg ICRF-187 i.p., days 0 and 7 (1 h before doxorubicin); and group 6, 500 mg/kg monoHER i.p., days 0–4 and 7–11.

Earlier, we established the maximum tolerated dose of 8 mg/kg i.v. for doxorubicin for two weekly injections. At this schedule, tumor-bearing nude mice will show a reversible weight loss of approximately 10% of the initial weight within 2 weeks after the first injection.

Tumor volumes were calculated according to the formula 0.5 × length × width × height (12, 13). The tumor volumes from the start of treatment (*V₀*) until any given time (*Vₜ*) was calculated for each tumor and expressed as the relative tumor volume.
Fig. 2 Weight gain per treatment group in the cardiotoxicity study. Data points, mean percentage of weight gain; bars, SE. Control, 0.9% NaCl solution i.v. once weekly for 6 weeks; doxorubicin, 4 mg/kg i.v. once weekly for 6 weeks. MonoHER 500, 250, and 100 indicate 500, 250, and 100 mg/kg, respectively, i.p., 1 h prior to doxorubicin and every 24 h for 4 days.

Table 1 Changes in ST interval per treatment group in the cardiotoxicity study

<table>
<thead>
<tr>
<th>Week</th>
<th>0.9% NaCl</th>
<th>Doxorubicin</th>
<th>monoHER 100</th>
<th>monoHER 250</th>
<th>monoHER 500</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>D</td>
<td>I</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>3</td>
<td>D</td>
<td>I</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>I</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>5</td>
<td>D</td>
<td>I</td>
<td>D</td>
<td>D</td>
<td>D</td>
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<tr>
<td>6</td>
<td>D</td>
<td>I</td>
<td>D</td>
<td>D</td>
<td>D</td>
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<tr>
<td>7</td>
<td>D</td>
<td>I</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>8</td>
<td>D</td>
<td>I</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
</tbody>
</table>

ANOVA at $P < 0.01$ with Fisher’s least significant difference test (multiple comparisons).
*ND, no difference indicated by ANOVA; NS, not significant.*
* D, significant decrease in ST interval when compared to mice treated with doxorubicin.*
* I, significant increase in ST interval when compared to mice treated with 0.9% NaCl solution.*

RESULTS
Cardiotoxicity Study

General Toxicity/Weight Loss. After surgery, recovery of the animals was indicated by an increase in weight after an initial decrease. Behavior appeared normal in all treatment groups during the entire study as compared to control mice without transmitters. There were no signs of decreased activity, which indicated low general toxicity. This was confirmed by the weight gain (Fig. 2), which revealed no significant differences between groups. There was, however, a trend ($P = 0.098$) toward somewhat less weight gain in the doxorubicin-treated group than in the other groups (Fig. 2). Pathological examina-
cytotoxic at concentrations of 50 and 100 μM. doxorubicin than were MCF-7 and OVCAR-3 cells, as deter-

any group.

weekly doxorubicin injections, the increase in the ST interval

monoHER was given 5 days a week in combination with the

was able to protect against this ECG change. When 500 mg/kg

segment and the QRS complex remained constant. monoHER

3 and Table I) by 16.7 ± 2.7 ms in week 8, whereas the PR

deflection of a mouse is somewhat different from that of man

malalties caused by either the treatment or the transmitter.

activity at 50 μM.

the investigated cell lines. Taxifolin showed some cytostatic

they did not significantly influence the IC50 of doxorubicin in

X

Table Culture

under anesthesia or restrained (15, 16). The T wave immediately

(14),

As described earlier, the ECG signal in lead II

ECG.

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. The ST interval increased with time (Fig.

3 and Table 1) by 16.7 ± 2.7 ms in week 8, whereas the PR

segment and the QRS complex remained constant. monoHER

was able to protect against this ECG change. When 500 mg/kg

monoHER was given 5 days a week in combination with the

weekly doxorubicin injections, the increase in the ST interval

was 1.7 ± 0.8 ms after 8 weeks (P < 0.001, relative to
doxorubicin, not significantly different from control; Ref. 2). In

a dose of 250 or 100 mg/kg, monoHER protected to a somewhat

lesser extent. The increase in ST interval after 8 weeks for 250

mg/kg monoHER was 4.6 ± 0.7 ms, and for 100 mg/kg mono-
HER, it was 7.8 ± 1.7 ms (both values had P < 0.01, relative
to doxorubicin). No arrhythmias were observed in animals of

any group.

Cell Culture

A2780 cells were approximately 10-fold more sensitive to
doxorubicin than were MCF-7 and OVCAR-3 cells, as
determined from the IC50 of 2.05 × 10⁻⁸, 2.09 × 10⁻⁷, and 3.28 × 10⁻⁷ M, respectively. As can be seen from Table 2, the
flavonoids cyanidanol, monoHER, and rutin were found not to be
cytotoxic at concentrations of 50 and 100 μM. More importantly, they did not significantly influence the IC50 of doxorubicin in the investigated cell lines. Taxifolin showed some cytostatic activity at 50 μM, which was indicated by the fact that the maximum cell growth could not be reached. This effect was the largest in OVCAR-3 cells. The slight cytostatic activity of 50 μM taxifolin had no influence on the IC50 of doxorubicin, which
could still be calculated. At a concentration of 100 μM, taxifolin was toxic (no cell growth) in all cell lines. Kaempferol and quercetin were toxic in all cell lines at 50 μM; no living cells were found after the incubation period.

**Antitumor Activity Study**

First, we investigated the toxicity of monoHER and ICRF-187 in combination with 8 mg/kg doxorubicin, which is the maximum tolerated dose in a schedule of two weekly injections. Three non-tumor-bearing nude mice were given 8 mg/kg doxorubicin i.v. on days 0 and 7. The toxicity of monoHER was determined in three non-tumor-bearing nude mice receiving 8 mg/kg doxorubicin i.v. on days 0 and 7 and 500 mg/kg monoHER i.p. on days 0 and 7 (1 h before doxorubicin) and days 1–4 and 8–11. The toxicity of ICRF-187 was also determined in three non-tumor-bearing nude mice receiving doxorubicin as described above and 100 mg/kg ICRF-187 i.p. on days 0 and 7, 1 h before doxorubicin. ICRF-187 in combination with doxorubicin appeared to cause more pronounced weight loss than doxorubicin alone (Table 3). MonoHER did not affect the weight loss caused by doxorubicin.

In the antitumor activity study, doxorubicin treatment resulted in a significant growth delay of A2780 xenografts, with a T/C% of 30% (P < 0.05; Table 4). Treatment with 500 mg/kg monoHER alone in a 5-day schedule did not influence the tumor growth; the T/C% was 61% (not significantly different from doxorubicin-treated and control tumors) in A2780 xenografts. The addition of monoHER or ICRF-187 to doxorubicin did not affect the growth delay. No differences were observed between the 2-day and the 5-day schedules of monoHER (Table 4 and Fig. 4A). Also, in OVCAR-3 xenografts, doxorubicin showed a significant growth delay, with a T/C% of 45% (P < 0.001). Treatment with 500 mg/kg monoHER alone did not influence the tumor growth, as can be seen from the T/C% of 97% (not significantly different from control tumors). Also, for OVCAR-3 tumors, coadministration of doxorubicin and ICRF-187 or monoHER by either schedule did not affect the growth delay caused by doxorubicin (Table 4 and Fig. 4B).

**DISCUSSION**

The formation of oxygen-free radicals is believed to be the cause of doxorubicin-induced cardiotoxicity, although compounds that reduce free radical formation, such as antioxidants and iron chelators, have had limited success in its prevention (17–20). Until now, only ICRF-187, which is hydrolyzed intracellularly into an iron chelator, found its way to clinical practice

<table>
<thead>
<tr>
<th>Table 2</th>
<th>IC50 of growth inhibition by doxorubicin in the presence of flavonoida</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell line</td>
<td>Doxorubicin + flavonoid (μM)</td>
</tr>
<tr>
<td>Noneb</td>
<td>2.05 ± 0.66</td>
</tr>
<tr>
<td>Cyanidanol (50)</td>
<td>1.52 ± 0.16</td>
</tr>
<tr>
<td>Cyanidanol (100)</td>
<td>0.85 ± 1.25</td>
</tr>
<tr>
<td>monoHER (50)</td>
<td>2.57 ± 1.95</td>
</tr>
<tr>
<td>monoHER (100)</td>
<td>2.00 ± 1.82</td>
</tr>
<tr>
<td>Taxifolin (50)</td>
<td>1.11 ± 2.82</td>
</tr>
<tr>
<td>Taxifolin (100)</td>
<td>Toxic</td>
</tr>
<tr>
<td>Rutin (50)</td>
<td>2.71 ± 1.36</td>
</tr>
<tr>
<td>Rutin (100)</td>
<td>1.83 ± 0.25</td>
</tr>
<tr>
<td>Kaempferol (50)</td>
<td>Toxic</td>
</tr>
<tr>
<td>Quercetin (50)</td>
<td>Toxic</td>
</tr>
</tbody>
</table>

a Mean ± SD of at least three independent experiments performed in triplicate. None of the flavonoids had an influence on the control cell growth, except for those labeled toxic and 50 μM taxifolin, which had a small influence.

b Doxorubicin in the absence of flavonoid.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Weight loss of non-tumor-bearing nude mice</th>
</tr>
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<tbody>
<tr>
<td>Groupa</td>
<td>Days of treatment</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0, 7</td>
</tr>
<tr>
<td>+ monoHER</td>
<td>0–4, 7–11</td>
</tr>
<tr>
<td>+ ICRF-187</td>
<td>0, 7</td>
</tr>
</tbody>
</table>

a Doxorubicin, 8 mg/kg i.v.; monoHER, 500 mg/kg i.p.; ICRF-187, 100 mg/kg i.p. 
b Weight on day 0 is 100%. No significant differences were found between groups.
Table 4  Antitumor activity of doxorubicin with or without monoHER or ICRF-187 in nude mice bearing A2780 or OVCAR-3 xenografts

<table>
<thead>
<tr>
<th>Group</th>
<th>Days of treatment</th>
<th>A2780</th>
<th>OVCAR-3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T/C% (day 17)</td>
<td>Toxic death</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0, 7</td>
<td>30</td>
<td>0/6</td>
</tr>
<tr>
<td>+ monoHER, 5 days</td>
<td>0–4, 7–11</td>
<td>29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/6</td>
</tr>
<tr>
<td>+ monoHER, 2 days</td>
<td>0, 1, 7, 8</td>
<td>34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/6</td>
</tr>
<tr>
<td>+ ICRF-187</td>
<td>0, 7</td>
<td>41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1/6</td>
</tr>
<tr>
<td>monoHER, 5 days</td>
<td>0–4, 7–11</td>
<td>61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0/6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Not significantly different from doxorubicin-treated tumors.
<sup>b</sup> Not significantly different from control tumors.

(3, 21). Pretreatment with ICRF-187 allowed a 4-fold escalation in the cumulative dose of doxorubicin, with attenuation of the doxorubicin-induced cardiomyopathy in animals, and an escalation of at least 2-fold has been reported in patients (21). ICRF-187 did not prevent alopecia and other anthracycline-induced toxicities. A clear disadvantage of ICRF-187 is its toxicity to mitotically active tissues, e.g., bone marrow, lymphoid tissue, testes, and gastrointestinal mucosa (3). Because of this toxicity and because there is concern about the drug’s impact on antitumor efficacy, it has not become a popular addition to doxorubicin treatment.

The success of ICRF-187 in preventing cardiotoxicity, however, indicates that free radicals are likely to be involved because iron-catalyzed reactions play an important role in the formation of radicals in biological systems. In addition, it demonstrates that antitumor activity and cardiotoxicity are probably caused by different mechanisms or, at least, can be separated because ICRF-187 does not influence the antitumor activity of doxorubicin. This has given researchers a new incentive to study nontoxic iron chelators and other antioxidants.

Flavonoids are known to be excellent transition metal chelators and radical scavengers. The combination of these properties enables “site-specific” scavenging. If oxygen radicals can still be formed around the iron, which is chelated by the flavonoid, then these radicals are formed in the vicinity of the flavonoid and can be scavenged immediately (5). MonoHER is a powerful antioxidant, the most active compound of the hydroxyethylrutosides that are present in Venoruton (5, 22). Venoruton itself was found earlier to partly protect against doxorubicin-induced cardiotoxicity (4). The protection may, therefore, be caused by the scavenging of oxygen-free radicals that are generated by doxorubicin or by the chelation of iron, which catalyzes the formation of more reactive radicals.

As a model for cardiotoxicity, we have used the changes in ST interval, which have been found earlier to correlate with the degree of cardiotoxicity in both rats (23) and mice (24, 25). In a previous study, 500 mg/kg monoHER, given as an i.p. injection 5 days a week, was able to completely prevent the cardiotoxicity in the mouse, which was caused by 4 mg/kg doxorubicin given in six weekly i.v. injections. This treatment resulted in an increase in the ST interval of only 1.7 ± 0.8 ms after 8 weeks versus 16.7 ± 2.7 ms for doxorubicin (P < 0.001, relative to doxorubicin, not significantly different from control; Ref. 2). In the same study, monoHER alone was found not to exert any effect on the ECG.

The present results show a clear dose dependency in the

![Fig. 4](image-url) Antitumor activity of doxorubicin, with or without cardioprotector. Data points, mean relative tumor volume; bars, SE. A. A2780 xenografts; B. OVCAR-3 xenografts. Control, no treatment; doxorubicin, 8 mg/kg i.v., days 0 and 7; + monoHER 5-day, + 500 mg/kg i.p., days 0–4 and 7–11; + monoHER 2-day, + 500 mg/kg i.p., days 0 and 1 and 7 and 8; + ICRF-187, + 100 mg/kg ICRF-187 i.p., days 0 and 7; monoHER 5-day, 500 mg/kg i.p., days 0–4 and 7–11 without doxorubicin. MonoHER and ICRF-187 at days 0 and 7 were given 1 h prior to doxorubicin.
protective effect of monoHER. After 8 weeks, doxorubicin combined with a dose of 250 mg/kg monoHER caused a small but steady increase in ST interval (4.6 ± 0.7 ms, 73% protection, versus 16.7 ± 2.7 ms for doxorubicin). The combination of doxorubicin with 100 mg/kg monoHER gave approximately 53% protection from the toxicity of doxorubicin (increase in ST interval, 7.8 ± 1.7 ms). As already mentioned above, doxorubicin combined with 500 mg/kg monoHER resulted in an increase in the ST interval of 1.7 ± 0.8 ms in week 8, which was not significantly different from the ECG in control animals.

It appears that a relatively high dose of flavonoid is needed for optimal protection against doxorubicin-induced cardiotoxicity. This is probably caused by the high clearance of the flavonoid (26), which, as a consequence, requires repeated dosing. This in contrast to ICRF-187, which is given only once, 1 h before doxorubicin. As the hydrolyzed product (ICRF-198) is charged, it cannot easily leave the cell and, therefore, remains at its site of action. MonoHER is neutral at physiological pH and can, therefore, probably cross the cellular membrane either way.

Venoruton, which contains about 10% monoHERs (2% specifically 7-monoHER), was found not to have an influence on the antitumor activity of doxorubicin in L1210 and P388 cells in vitro and in vivo with a dose of 1.5 g/kg (18, 27). In the antitumor activity experiments in human tumor xenografts described here, the higher dose of 500 mg/kg monoHER was also found not to influence the growth inhibition of doxorubicin in A2780 and OVCAR-3 xenografts in either the 2-day or the 5-day dose schedule. In addition, monoHER showed no increase in weight loss caused by doxorubicin, whereas there was a trend that weight loss was longer and more pronounced in mice treated with ICRF-187 and doxorubicin than in mice given doxorubicin alone (Table 3). It should be noted that cardioprotection in the antitumor activity study (8 mg/kg doxorubicin in combination with 500 mg/kg monoHER) is probably comparable to the 250 mg/kg monoHER dose with 4 mg/kg doxorubicin in the cardiotoxicity study, whereas ICRF-187 was dosed in the same concentration ratio versus doxorubicin in both the antitumor study and the cardiotoxicity study (2, 25).

High doses of monoHER (up to 100 μm) did not influence the antiproliferative effects of doxorubicin in three cell lines derived from human tumor types that are known to be sensitive to anthracyclines in the clinic. Some other flavonoids tested in vitro were cytotoxic at concentrations of 50 μm, which was the lowest concentration tested. The cytotoxicity of flavonoids, including quercetin, has been observed earlier, and some authors have studied the effect of quercetin as an antitumor agent in patients (28). Our observations indicate that flavonoids such as monoHER might be used in vivo to modulate cardiotoxicity because high doses do not affect the antitumor effect of doxorubicin.

In our experiments, monoHER was shown to protect against chronic doxorubicin-induced cardiotoxicity in a dose-dependent way. At a dose of 500 mg/kg, it appeared to be at least as effective as ICRF-187 (2), a compound that has shown promising results in clinical practice. MonoHER is of interest, despite the high dose needed for protection, because hydroxyethylrutosides have little toxicity of their own. Importantly, monoHER does not have a negative influence on the antiproliferative effects of doxorubicin in A2780, OVCAR-3, and MCF-7 cells in vitro and in A2780 and OVCAR-3 xenografts grown in nude mice. Therefore, it can be concluded that monoHER merits further investigation as a possible protector against doxorubicin-induced chronic cardiotoxicity in cancer patients.

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