Using telemetry to study the effect of protectors on doxorubicin-induced cardiotoxicity in freely-moving mice

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Summary

Oxygen-free radicals play a role in the cardiotoxicity of doxorubicin (Dox). The clinical use of Dox is limited by a cumulative dose-related cardiotoxicity. In laboratory animals, histology is most commonly used for the study of Dox-induced cardiotoxicity, in combination with biological and functional parameters. However, for monitoring during treatment, a large number of animals is needed. In the literature, there are several reports of electrocardiogram (ECG) changes after chronic Dox-administration in anaesthetized laboratory animals. We developed a new method for measuring ECG in freely-moving mice by telemetry. With this model, we investigated the effect of chronic Dox administration on the ECG of freely-moving telemetered BALB/c mice, and the efficacy of ICRF-187 and 7-monohydroxyethyl rutoside (7-monoHER) as protecting agents.

Anthracyclines are among the most widely used anti-tumour agents, and one of them, doxorubicin (Dox), ranks among the best single agents in the treatment of a variety of haematological malignancies and solid tumours in experimental models and patients. Apart from the common side-effects of anti-cancer therapy, such as bone marrow suppression, alopecia, nausea and vomiting, its clinical use is largely limited by the occurrence of a cumulative dose-related cardiotoxicity, which manifests itself in congestive heart failure.

For monitoring during treatment, a large number of animals is needed. In the literature, there are several reports on ECG changes after chronic Dox administration in anaesthetized laboratory animals (Olson & Mushlin 1990, Calderone *et al*. 1991). Both *in vitro* and *in vivo* models are used to study the cardiotoxicity of Dox. For the *in vivo* treatment models, single or repeated injections of Dox in mice, rats, guineapigs, rabbits or miniature swine resulted in varying degrees of cardiotoxicity. The cardiotoxicity that developed can be evaluated in the following ways. Histology (in combination with biological and functional parameters) is most commonly used to determine the extent of the myocardial damage in laboratory animals because Dox, as with all other anthracyclines, causes very specific changes in heart tissue (Bertazzoli *et al*. 1979). Histological scoring can be performed, among others, according to Billingham's semi-quantitative grading scale (Billingham *et al*. 1978). The grading is based on the number of muscle cells showing myofibrillar loss and cytoplasmic vacuolization. A very detailed histological evaluation is possible with morphometry (van der Vijgh *et al*. 1988).

Less frequently, functional parameters have been used in chronic cardiotoxicity studies in both animals and man. Function-

ality is tested either *in vivo* (Bocherens-Gadient *et al*. 1992) or *ex vivo* (Calderone *et al*. 1991, van Acker *et al*. 1995). In addition, biochemical parameters (ATP/GTP levels, enzymes, antioxidant status) are used to record cardiotoxicity (Hacker *et al*. 1983, Singal *et al*. 1987, Doroshow 1991, Bhanumathi *et al*. 1992, Cini Neri *et al*. 1993). These methods all determine the endpoint parameters since the animals have to be sacrificed to enable measurement. Monitoring of the development of cardiotoxicity is performed by sacrificing small groups of animals at distinct time intervals. Inter-individual variability complicates the interpretation and sensitivity of the results considerably. An alternative is measurement in live animals which allows the monitoring of Dox-induced cardiotoxicity within the same animal by measuring ECG changes. The most common measurement techniques include connecting sensors, lead wires, and cannulae to restrained or tethered awake animals, or taking the desired measurements while the animals are under anaesthesia. One of the problems is that these invasive methods may directly affect the animals' physiological functions, which results in a high variability of the experimental results and even erroneous measurements (Brockway & Hassler 1993). These types of measurements are usually performed in rats, which develop a nephrotic syndrome upon the administration of Dox. Since mice, as well as humans, do not readily develop a Dox-induced nephrotic syndrome like rats, the mouse is the species of choice in such cardiotoxicity studies (van der Vijgh *et al*. 1988).

We have developed a telemetry system for mice so that it is now possible to measure the effect of chronic Dox administration on the heart rate (HR) and the ECG of freely-moving BALB/c mice (Kramer *et al*. 1993) as well as the efficacy of a known (ICRF-187) and a potentially new (7-monoHER) protector against Dox-induced cardiotoxicity.

Materials and methods

The animals used were 20 male mice (BALB/c, 2–3 months old, body weight 22–24 g) obtained from Harlan Nederland (Postbus 6174, 5960 AD Horst, The Netherlands), bred under constant temperature ($24 \pm 2^{\circ}$C), humidity ($60\% \pm 5\%$) and a 12 : 12 h light–dark cycle (lights on 07:00–19:00 h). Throughout these experiments, pelleted food (RMH-TM 1110; Hope Farms B.V., Postbus 85, 3440 AB Woerden, The Netherlands) and water were available *ad libitum*. The animals were allowed to adapt to the laboratory housing conditions for at least one week before starting the operation.

The surgery and the telemetry system have been described (Kramer *et al*. 1993) but, in brief, a small telemetric transmitter (TA10ETA-F20; Data Sciences International, Minnesota 55126–6164, USA) was implanted in the peritoneal cavity under anaesthesia: one part fentanyl-fluanisone (Hypnorm, Janssen Pharmaceutica, 2340 Beerse, Belgium), one part midazolam (Dormicum, Roche, Postbus 42, 3640 AA Mijdrecht, The Netherlands), and two parts sterilized water (0.07 ml/10 g, intraperitoneally). The leads of the transmitter were sutured subcutaneously in the lead II position, the negative lead at the right shoulder, and the positive lead at the lower left chest. After surgery the mice were allowed to recover for 2 weeks, after which they were subject to the administration of the following dose schedules.

(1) Control ($n = 5$): 0.1 ml saline was given intraperitoneally one hour before the intravenous administration of 0.05 ml saline once per week for 6 weeks.
(2) Doxorubicin ($n = 5$): 0.1 ml saline was given intraperitoneally one hour before the intravenous administration of 4 mg/kg Dox (Adriblastina, 2 mg/kg, obtained from Pharmachemie B.V., Haarlem, The Netherlands) once per week for 6 weeks.
(3) ICRF-187 ($n = 5$): 50 mg/kg ICRF-187 (Cardioxane, a gift from EuroCetus Amsterdam, The Netherlands) was injected intraperitoneally one hour before the intravenous administration of 4 mg/kg Dox once per week for 6 weeks.
(4) 7-monoHER ($n = 5$): 500 mg/kg (a gift from Novartis, Nyon, Switzerland) was injected intraperitoneally one hour before the intravenous administration of

4 mg/kg Dox and every 24 h for 5 days for 6 weeks.

After treatment the animals were observed for another 2 weeks and then sacrificed by decapitation. The hearts were removed for histological evaluation.

Results

As described before (Kramer *et al.* 1993), animals lost weight during the first few days after the operation but this was followed by a weight increase. No significant difference in weight was observed between the Dox, ICRF-187, 7-monoHER and control groups. The ECGs of the control animals (see Fig 1) did not alter during the course of the study. Dox had a profound influence on the shape of the ECG. The QT and ST interval increased significantly with time ($P < 0.001$) by 17.7 ± 2.9 ms and 16.7 ± 2.7 ms, respectively, in week 8 (see Fig 2 for the ST interval), whereas the PR segment and the QRS complex remained constant. ICRF-187 and the new compound 7-monoHER completely blocked the increase in ST interval and no difference from the control values was seen (Fig 2). In all animals the maximal heart rate was 700–750 bpm during the entire study.

The histological scores recorded for the myocardial lesions resulting from ICRF-187 and 7-monoHER co-administration protected mice significantly against Dox-induced cardiotoxicity ($P < 0.005$). The histological scores in individual mice were positively correlated with an increase in ST interval ($r = 0.902$, Fig 3).

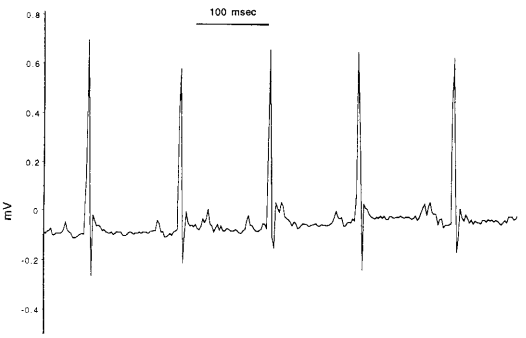

Fig 1 Normal mouse ECG

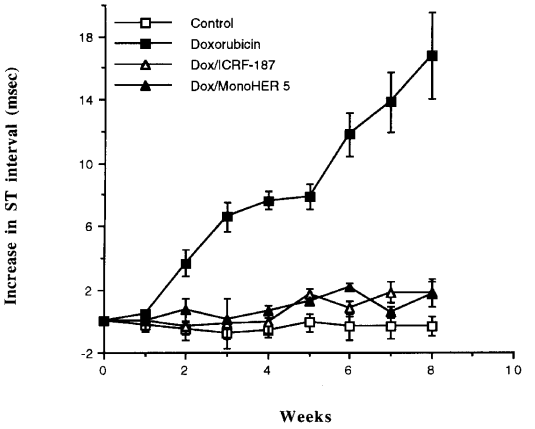

Fig 2 Increase in the ST interval with time. Dox-treated mice ($n = 5$) compared with controls ($n = 5$), ICRF-187 treated mice ($n = 5$) and 7-MonoHER treated mice (n = 5). Data represent mean values ± SEM. All treatment groups relative to Dox $P < 0.001$ from week 2

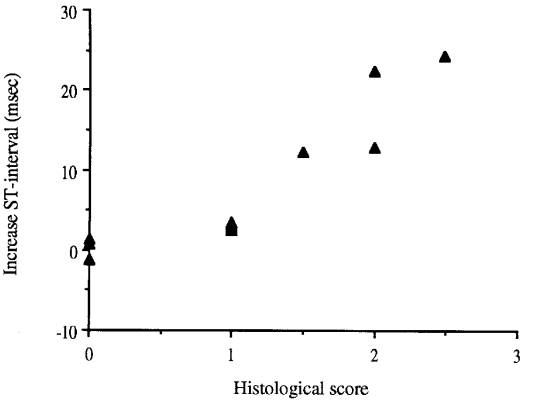

Fig 3 Comparison of the ST interval and the histological score per individual mouse treated with Dox

The transmitter and leads did not appear to cause any abnormality as all the abdominal organs such as the kidney, liver, and intestine appeared normal in the control, Dox, Dox/ICRF-187 and Dox/7-monoHER treated mice, and there were no adhesions.

Discussion

Dox is known to cause a decrease in heart functionality in both humans and laboratory animals (Goebel & Kaplan 1992). Although the ECG is not a functional parameter, sev-

eral authors have described changes in the ECG of laboratory animals upon administration of anthracyclines (Zbinden & Brändle 1975, Danesi *et al*. 1992). Therefore, we followed the ECG changes by monitoring 2 days per week during Dox treatment for 6 weeks. Widening of the ST interval became significant during the second week ($P < 0.001$) and continued to increase during the treatment period (Fig 2). Even after Dox treatment had stopped, ST interval widening continued, which indicates that the development of Dox-induced cardiotoxicity does not stop after therapy. It is not known whether this is caused by the remaining presence of small amounts of Dox or its metabolite, doxorubicinol, in the heart tissue or by a vicious circle of compensating mechanisms in an already damaged heart. Both observations, i.e. late cardiotoxicity and a large variance in toxicity, are also found in patients (Goebel & Kaplan 1992).

The widening of the ST interval, which reflects the prolongation of the repolarization phase, may be explained by prolongation of the action potential. The action potential has been prolonged in Purkinje fibres after incubation with Dox (Le Marec *et al*. 1986), and in isolated myocytes it has been found that oxygen-derived free radicals, which are generated by Dox-iron complexes, can increase the duration of the action potential (Jabr & Cole 1993). Until now, only ICRF-187 has been used clinically as a protecting agent (Koning *et al*. 1991) and we used our model to see if it could predict such protecting drugs. The cardiotoxicity observed in animals in this study appeared acceptable for the animals and sufficient to establish protection as demonstrated by the significant increase in ST interval seen in the Dox-treated mice and the protection provided by ICRF-187 (not significantly different from controls during the entire study: Fig 2). This protection is confirmed by histological data (data not shown) and is in accordance with animal studies and clinical data (Herman & Ferrans 1983, Koning *et al*. 1991). The use of ICRF-187, however, has been limited as it shows bone marrow suppression. A new compound, 7-monoHER, has been found to show no negative influence on Dox cytotoxicity *in vitro* or *in vivo* (Van Acker *et al*. 1997) and is able to fully prevent the Dox-induced changes in the ECG (Fig 2). The heart rate of all groups did not change during the whole study.

In conclusion, the ECG measured by telemetry can be considered a valuable and sensitive tool for measuring the cardiotoxic effects of anti-cancer agents and protectors by monitoring the animals as often as necessary during treatment. In addition, telemetry makes it possible to monitor without introducing interfering factors and is less time-consuming, less labour-intensive and fewer animals are needed compared with histological studies.

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