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Modification of doxorubicin-induced cardiotoxicity: effect of essential fatty acids and ICRF-187 (dexrazoxane)

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Abstract

The capacity of an oil, containing gamma-linolenic acid (GLA), to reduce the severity of doxorubicin-induced cardiotoxicity has been investigated in a rat model. Groups of 12-week-old, male, Sprague–Dawley rats were injected intravenously (i.v.) with single doses (3 mg/kg body weight) of doxorubicin (DOX). Daily for 1 week prior to DOX administration and for up to 20 weeks afterwards groups of rats received either an oil containing both GLA and linoleic acid (So-1100, Scotia Pharmaceuticals), at two dose levels, or an oil containing linoleic acid, but no GLA (So-1129) by oral gavage. Other groups of rats received water as a control. One of the groups of rats that received water also received i.v. ICRF-187 (60 mg/kg) 15 min prior to DOX. A group of animals acted as age-matched controls. The maximum reduction in body weight in the first 2 weeks after the administration of DOX. was used as a measure of acute toxicity. This was most severe in the group receiving a combination of DOX and ICRF-187 ($5.6 \pm 0.43\%$). Animals receiving 2 ml of either So-1100 or So-1129 were the least affected ($\approx 2.5\%$). Measurements of cardiac volume output made at various intervals after DOX administration indicated a $\approx 35\%$ reduction in cardiac function in the control and So-1129 oil group after 20 weeks. The corresponding reduction in the groups receiving ICRF-187 and 2 ml of So-1100 was $\approx 16\%$. The group receiving daily doses of 1 ml So-1100 showed an intermediate response. The death of an animal with signs of congestive cardiac failure occurred in 40% of the animals in the DOX only control (water) group. There were no deaths in the groups of rats receiving either ICRF-187 or pre- and post-administration of 2 ml of So-1100. It was concluded that an oil containing GLA (So-1100) has similar cardioprotective properties against DOX-induced cardiotoxicity as ICRF-187, but with less general toxicity in this rat model. © 2001 Published by Elsevier Science Ltd.

Keywords: Cardiotoxicity; Cardioprotection; Gamma-linolenic acid; ICRF-87

1. Introduction

The anthracycline antibiotic doxorubicin (DOX) has been in clinical use as an anti-cancer agent for three decades for a wide range of malignant tumours. However, its use has been limited by a dose-related and irreversible cardiotoxicity. The late onset of cardiotoxicity, in terms of both severity and incidence, has only recently been appreciated. Using the relatively crude clinical end-point of overt congestive heart failure it has been estimated that the incidence was 7% after a cumulative dose of 550 mg/m^2 and 18% after 700 mg/m^2 [1, 2].

The late effects of anthracycline-induced cardiotoxicity is of significance when used with curative intent in paediatric cases where a significant deterioration in the quality of life and mortality has been observed. Particular interest has focused on the long-term survivors of paediatric cancer and, using more sophisticated modern methods of detecting ventricular dysfunction, several groups have detected a higher incidence of effects in children than in adults. Sorenson and colleagues [3] noted a 25% incidence of left ventricular dysfunction in children who had received a mean cumulative dose of 300 mg/m^2 of DOX. A higher incidence of 55% was observed in a group of 115 children who had received more than 500 mg/m^2 of DOX and who were in continuous complete remission from acute lymphoblastic leukaemia for a follow-up period of 1–15 years (median 6.4 years) [4].

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The pathology of the anthracycline-induced heart damaged has been described [5,6]. Typical changes include myocytes showing myofibril loss, vacuolar degeneration due to the swelling of the sarcoplasmic endoplasmic reticulum and a loss of myocytes. However, the pathogenesis of anthracycline-induced damage is less well established, although it is commonly regarded as being mediated by free radical damage. Anthracyclines, particularly when complexed with iron, can generate superoxide and hydroxyl radicals either by redox cycling or the intramolecular reduction of chelated iron [7,8]. These free radicals cause marked lipid peroxidation [9] and oxidative damage to cell membranes. The heart is known to lack the normal cellular defence mechanisms against free radical damage, having reduced levels of anti-oxidants (glutathione peroxidase, glutathione reductase and reduced glutathione) in comparison to other tissues. Myocytes are considered to be particularly susceptible to anthracycline-induced damage as they contain numerous mitochondria. Furthermore, anthracyclines are known to lower levels of anti-oxidant enzymes [10].

The use of agents to ameliorate anthracycline-induced cardiac damage has recently excited considerable interest. The drug ICRF-187 (dexrazoxane), originally synthesised as a possible anti-tumour agent [11], acts as an iron chelating agent that removes iron from doxorubicin–iron complexes [12,13]. It has been shown both in animal [14–17] and human [18–20] studies to act as a cardioprotective agent against anthracycline-induced damage. In mammals, the main essential fatty acid in the diet is linoleic acid whose conversion to gamma-linolenic acid (GLA), and hence that of many other biologically important lipids, is rate limited [21,22]. Therapeutic doses of selected essential fatty acids have been demonstrated to modulate radiation-induced injury in the skin and central nervous system [23–26]. The mechanism associated with radiation injury may be direct, by the stabilisation of cell membranes, or via some intermediary steps in the eicosanoid metabolic pathways [27]. There is also a recent report indicating that an oil containing GLA has suppressed bleomycin-induced lung fibrosis in the hamster [28].

Essential fatty acids, including GLA have been shown to have a selective tumoricidal action both *in vitro* and *in vivo* [29,30] or may enhance the effects of cytotoxic drugs including DOX [31]. In the present study, the effects of an oil, So-1100 (Scotia Pharmaceuticals), containing ~9% GLA and ~70% linoleic acid, on the cardiotoxicity of DOX has been examined in the young adult rat. This has been compared with the known effects of ICRF-187. A single dose of 3 mg/kg body weight of DOX was used in this study. In a previous investigation, the effects of free DOX had been compared with either DOX in association with ICRF-187 [17] or as a co-polymer bound to DOX [32], the dose of free DOX used was 4 mg/kg. This had resulted in no animals surviving for longer than 16 weeks. Deaths were due to cardiac failure. On the other hand, a single dose of 3 mg/kg of DOX only produced ~70% mortality, from cardiac failure, over 20 weeks [33].

2. Materials and methods

Sixty-one male, 12-week-old Sprague–Dawley rats, weighing approximately 350–400 g, were used in this study. The animals were caged in groups of two and were maintained on a 41-B cubed diet and water *ad libitum*. They were randomly allocated into one of five groups of 10-11 animals. An additional group of 10 rats acted as age-matched controls:

Group 1: DOX + water (2 ml)
Group 2: DOX + So-1100 (1 ml)
Group 3: DOX + So-1100 (2 ml)
Group 4: DOX + So-1129 (2 ml)
Group 5: DOX + ICRF-187 (60 mg/kg) + water (2 ml)
Group 6: Age-matched controls

Animals in groups 1–4 received either water (2 ml) or the appropriate oil, daily by oral gavage, for 1 week prior to a single intravenous (i.v.) dose of 3 mg/kg body weight of DOX. After the administration of DOX, water or oil was continued daily for a further 20 weeks. Group 5 received water daily for 1 week followed by a single i.v. injection of ICRF-187 (60 mg/kg) delivered 15 min prior to the DOX. Water was given for the remainder of the period of study. For the administration of DOX, animals were anaesthetised using chloral hydrate (300 mg/kg body weight intraperitoneally (i.p.)) and held in the supine position. DOX, and in the relevant cases ICRF-187, was injected via the femoral vein. The oils, So-1100 and So-1129 were similar in their composition except that So-1100 contained ~9% GLA, while So-1129 contained none of this essential fatty acid, but a similar amount of its precursor, linoleic acid (Table 1).

The body weight of all animals was recorded daily for the first 2 weeks of the study, following DOX administration, to assess acute toxicity. The lowest value of the body weight, over that period, for each animal was used

Table 1
Comparison of the fatty acid content of the oils used in the present investigation (%)

	So-1100	So-1129
Linoleic acid	70.2	79.0
Gamma-linolenic acid	8.9	–
Oleic acid	12.7	10.7
Stearic acid	1.8	2.1
Palmitic acid	5.8	6.9
Alpha-linolenic acid	–	0.6
Others	0.8	0.7

to calculate the percentage loss in body weight compared with the body weight in that animal at the time of administration of DOX. Sub-acute toxicity was assessed by recording the body weight of all animals at 4-weekly intervals, for a period up to 20 weeks. Body weight was expressed as the percentage change in weight from the time of injection of DOX.

Prior to DOX administration and at 4-weekly intervals afterwards, for 20 weeks, the cardiac output of all treated and age-matched control animals was assessed repeatedly. An external isotope counting technique was used as previously described in Ref. [34]. Briefly animals were anaesthetised (300 mg/kg body weight chloral hydrate i.p.) and held in the supine position. A bolus of 0.2 ml of $^{99m}TcO_4$ (~74 MBq/ml) was then injected into an exposed femoral vein. The time–activity curve over the heart was recorded for a period of 40 s using a collimated NaI detector, connected to a multi-channel analyser (ND-62, Nuclear Data) which was operated in the multi-scalar mode. The counting interval was 0.1 s. The heart rate was monitored simultaneously using a modified human electrocardiogram (ECG) monitor (Hewlett Packard 7830 A) coupled to a scope memory VIC-12-2 (Seltek Instruments Ltd). Changes in the cardiac volume output index and the heart rate were calculated and expressed as a percentage of the mean value in age-matched controls.

Animals were examined at daily intervals and any showing signs of ill health were killed. The time of death of any animal before the close of the study was recorded as an indicator of overall survival. A post-mortem examination was carried out on all animals that were killed before the end of the study. Animals alive at the end of the study period were anaesthetised by an i.p. injection of chloral hydrate and then killed using an i.v. injection of 0.5 ml of 15% potassium chloride. In the animals that were killed prematurely, the abdominal cavity was examined for signs of ascites and for evidence of an enlargement and/or congestion of the liver. The kidneys, intestines and other organs were also carefully examined. Post-mortem examinations were also carried out on the age-matched control animals at the end of the study period. Animals were considered to have developed terminal heart failure if they were found to have dilated hearts, congested and oedematous lungs with pleural effusion, ascites and/or generalised signs of oedema.

When animals were killed early or were killed after 20 weeks, the hearts were fixed in buffered formalin solution. Following a minimum period of 48 h immersion in fixative, each heart was cut in the coronal plane into three equal parts, identified as the apex, middle and base. The tissue was processed for embedding in paraffin wax. Sections were cut at 4 μm from each block and sequential sections stained with either haematoxylin and eosin or by Masson's trichrome method. The scoring of the severity and/or extent of cardiac lesions was based on a widely used scoring system [6] which has been used in previous studies using this rat strain [32].

Statistical differences between the group means values were analysed using Students *t*-test. Animal survival was expressed as the percentage survival per group and plotted using the Kaplan–Meier method.

3. Results

3.1. Acute toxicity

With the 3 mg/kg dose of DOX, used in this study, the acute toxicity observed in the initial 2 weeks after drug administration, expressed in terms of a loss in body weight, was found to be greatest in the ICRF-187 plus DOX group (Fig. 1). The mean reduction of body weight was 5.6±0.43% of the pre-treatment values. This was significantly greater ($P<0.001$) than the mean loss of body weight in the group receiving DOX only (with water) or with oils (groups 2–4). The smallest reduction in body weight was found in the group receiving 2 ml of So-1100 daily following DOX. The mean reduction in body weight was only 2.38±0.37%.

3.2. Subacute toxicity

3.2.1. Body weight changes

In the 20-week period of this study, the age-matched control animals showed a 41.9±1.56% increase in body weight. This gain in weight was closely replicated by the group receiving DOX and 2 ml of So-1100, where the percentage increase in body weight was 35.2±1.7% (Fig. 2). The increase in body weight in the group receiving So-1100 (2 ml) was significantly greater ($P<0.001$) than that seen in the group receiving only water before and after the administration of DOX. In

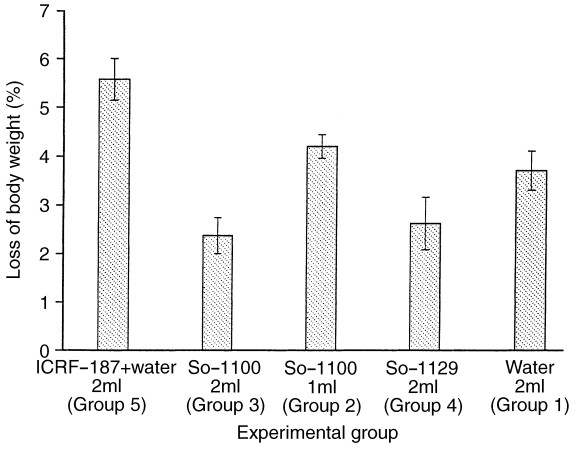

Fig. 1. Mean maximum reduction in body weight in the first 2 weeks after doxorubicin (DOX) administration in the different experimental groups. Error bars indicate ± standard error of the mean (SEM).

this group, the increase in body weight was only ~24% after 20 weeks. This is most likely to represent an overestimate since the more severely affected animals (40%) died over the course of the study. Comparable time-related changes in body weight, to those in the water group, were seen in the group receiving the oil, So-1129 (group 4).

The relative weight-related changes in the groups receiving ICRF-187, and perhaps surprisingly 1 ml of So-1100, were found to be significantly less than in those receiving DOX plus water ($P < 0.001$). There were no deaths in the animals in the ICRF-187 series and only 20% of the animals in the group receiving 1 ml of So-1100 were killed prematurely. However, some animals, especially in the ICRF-187 group, showed signs of debilitation and wasting. However, there was no evidence of subcutaneous oedema or evidence of ascites in either of these two groups in animals killed after 20 weeks.

3.2.2. Animal survival

No premature deaths were observed in the age-matched controls and in the groups receiving ICRF-187 or 2 ml of So-1100 plus DOX. The group receiving 1 ml of So-1100 showed only 20% mortality (2/10) at the end of the study period. There was a 40% loss of animals (4/10) in the group receiving water and a 50% loss of animals (5/10) in the group receiving the oil So-1129 (Fig. 3).

At post-mortem, all the animals that received water by gavage after DOX (group 1) showed signs of cardiac damage, even those that survived for 20 weeks. This was indicated by the gross appearance of an enlarged and congested heart, congested and oedematous lungs, slight serous pleural effusion in most cases, hepatic congestion and ascites. Other abdominal organs, including the kidneys, showed no signs of abnormality. Similar signs of cardiac damage were also found in six out of 10 animals in the group receiving 2 ml of the oil So-1129. However, other abdominal organs including the kidneys again appeared to be normal. In the animals receiving 1 ml of So-1100, signs of cardiac damage were found in three out of 10 animals, i.e. including one surviving for 20 weeks. Oedematous lungs and moderately enlarged and congested livers were found. However, there was no other evidence of cardiac decompensation and the other abdominal organs, including the kidneys, appeared normal. In marked contrast, only one animal receiving 2 ml of So-1100 showed signs of cardiac damage, it had a slightly dilated heart, but no other signs of cardiac failure and the abdominal organs appeared to be normal. In the animals pre-treated with ICRF-187 (group 5), there was no evidence of damage to the heart except in two animals which, at post-mortem, had slightly congested hearts.

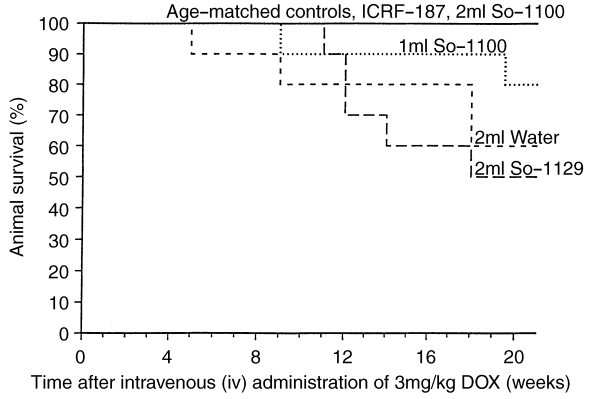

Fig. 3. Time-related changes in survival after doxorubicin (DOX) (3 mg/kg) administration in groups of rats receiving either water (group 1) (- - - -); So-1129 (group 4) (– – –); So-1100/1 ml (group 2) (·····); or So-1100/2 ml (group 3); ICRF-187 (group 5); (—).

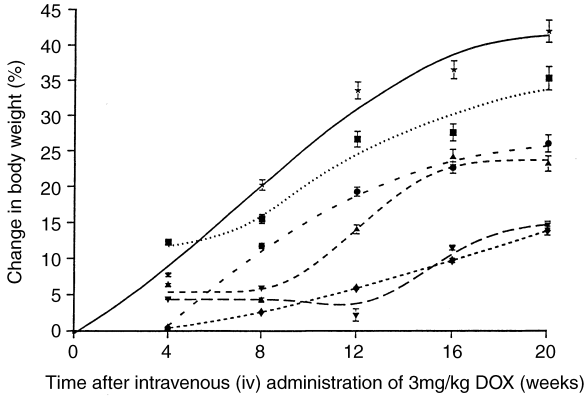

Fig. 2. Percentage increase in body weight (± standard error of the mean (SEM) in the different experimental groups from the time of injection of doxorubicin (DOX) (3 mg/kg). So-1100/2 ml (Group 3) (■·····■); So-1129/2 ml (group 4) (●- - - -●); water (group 1) (▲- - - -▲); So-1100/1 ml (group 2) (◆·····◆); and ICRF-187 (group 5) (▼– – – –▼), compared with age-matched controls (group 6) (⋆ — ⋆).

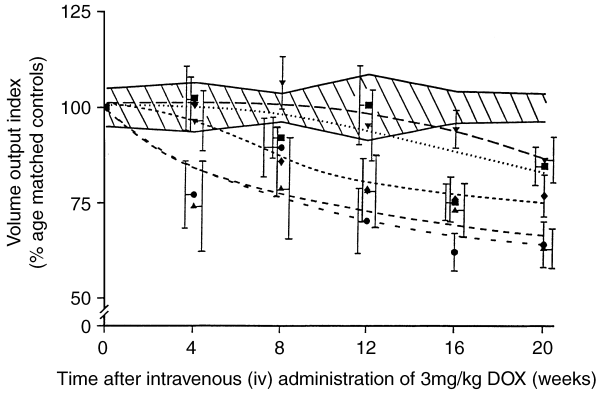

Fig. 4. Time-related changes in the cardiac volume output relative to age-matched controls in the different experimental groups. Age-matched control values ± standard error of the mean (SEM) ((▨)). For key to symbols see Fig. 2.

3.2.3. Changes in cardiac volume output

The time-related changes in the volume output of all groups, expressed as a percentage of the mean value of the age-matched controls, the volume output index (VOI), are shown in Fig. 4. It can be seen that in the animals receiving ICRF-187 (60 mg/kg) 15 min prior to the administration of DOX, the changes in the VOI were not significantly different from those of age-matched controls at 4, 8, 12 and 16 weeks ($P > 0.01$). However, after 20 weeks the VOI was significantly reduced, to 86.0±5.9% ($P < 0.01$) of the aged-matched controls. The group that received 2 ml of So-1100 throughout the period of the study also showed no significant changes in the VOI until 16 weeks after DOX administration. Values at 16 and 20 weeks showed a mean reduction in the VOI of ~20%, significantly different from that of the age-matched controls ($P < 0.01$).

The animals that received DOX alone (with water given daily by gavage) showed a significant reduction in the VOI of ~25% from 4 to 16 weeks ($P < 0.001$) after DOX administration, after which it declined further and at 20 weeks the volume output was ~63% of that of age-matched controls. The pattern of response (for most time points) was similar in the group receiving the oil So-1129 (Fig. 4). The group receiving 1 ml of So-1100, only showed a significant impairment in cardiac function ($P < 0.001$) from 12 to 20 weeks after DOX administration. The average value of the cardiac volume output was ~75% of that of the aged-matched controls over this period. This change could be considered to be representative, since only 2 animals died of cardiac failure over this period.

The interpretation of the cardiac function data, based on the evaluation of the mean VOI, is always complicated by the occurrence of premature deaths from cardiac failure. This will result in an underestimate of the level of cardiac damage. Deaths from cardiac failure are preceded by a ~50% impairment in cardiac function [35]. A subdivision of individual animals into groups on the basis of the degree of impairment in cardiac function, i.e. <20%, 20–40% or >40%, allows animals dying from cardiac failure to be included in the analysis. The distribution of animals, with respect to the degree of impairment in the VOI in the various experimental groups, both at 12 and 20 weeks after the injection of 3 mg/kg of DOX, is shown in Fig. 5. At 12 weeks (Fig. 5a), no animals in the groups receiving ICRF-187 or 2 ml of So-1100 showed individuals with a >40% impairment in the VOI. In the So-1100 group (2 ml), all rats were minimally affected (<20% change in VOI). The remaining experimental groups showed a similar distribution of animals between the various subgroups. At 20 weeks, there was a general trend for animals to be more severely affected. At this time, only the group receiving 2 ml So-1100 showed no individuals with a >40% impairment in the VOI. The water and So-1129 oil groups were the most severely affected with 50% of rats showing a >40% impairment in the VOI (Fig. 5b).

The results at 12 weeks, for the proportion of animals in the various experimental groups showing a ⩾20% impairment in cardiac function, have been compared with the data for the dose-related incidence of this effect (Fig. 6) obtained from animals used in a parallel study. The percentage of animals showing a ⩾20% impairment in cardiac function after 3 mg/kg was 50–60% in rats receiving water, 2 ml of So-1129 and 1 ml of So-1100, comparable to that for animals receiving 3 mg/kg DOX alone [30,31]. In the ICRF-187 group, the incidence was reduced to 27.3±13.4%. In the 2 ml So-1100 group, toxicity was comparable to that of 1 mg/kg of DOX alone. The true shift in the dose–response relationship for a ⩾20% impairment in cardiac function cannot be clearly defined from these data.

3.2.4. Histology

The specimens were identified by a numbering system such that observations were made without knowledge of the treatment received. The histological scores and comments, as assessed by light microscopy, were tabulated and then evaluated with knowledge of the treat-

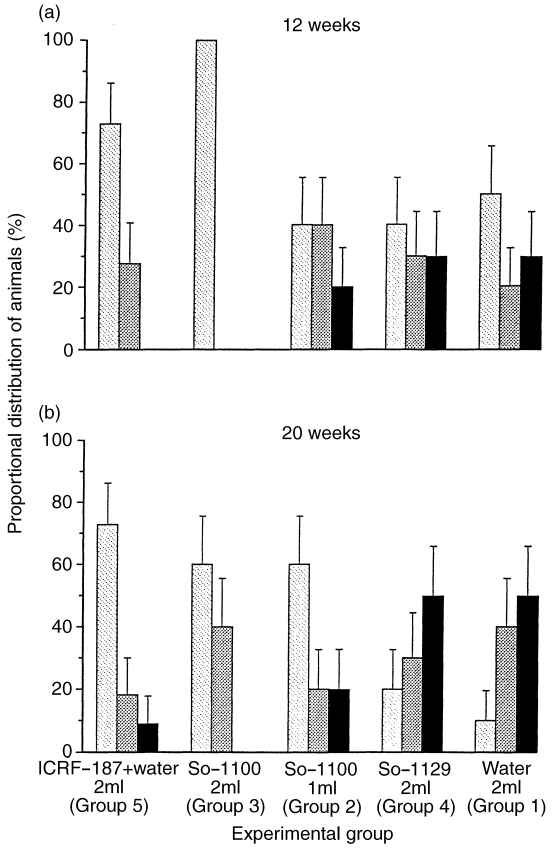

Fig. 5. Proportional distribution of rats (± standard error of the mean (SEM)) showing a specified reduction in cardiac volume output index (VOI) at 12 and 20 weeks after administration of 3 mg/kg doxorubicin (DOX) (<20% ▨; 20–40% ▩; ⩾40% ■).

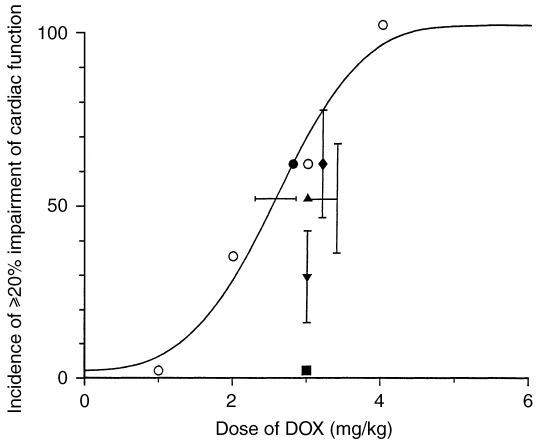

Fig. 6. Incidence of animals (± standard error of the mean) (SEM)) after doxorubicin (DOX) administration (3 mg/kg) in the different experimental groups showing a ⩾20% impairment of cardiac function compared with age-matched controls (○ — ○) dose–incidence data for Hopewell and colleagues . For key to symbols see Fig. 2.

ment received. With the exception of a few rare cases of DOX-induced vacuolated muscle fibres, none of the hearts had lesions that could be considered to be pathognomonic for DOX-induced cardiotoxicity. Some hearts had both acute and chronic foci of myocarditis; these were sometimes present in the same histological section. Acute foci consisted of swollen, hyalinised and fragmented fibres with pyknotic or karyorrhectic nuclei. This was typically accompanied by a mild histiocytic-lymphocytic inflammation. A few acute foci also contained neutrophils and/or haemorrhage. Chronic foci were characterised by irregular areas of fibrous connective tissue with an infiltration of fibroblasts and macrophages, some contained haemosiderin. The presence of collagen in the chronic lesions was highlighted by blue colouration in sections stained by the Mason's trichrome method.

4. Discussion

Considerable clinical interest has been generated by the prospect of selectively modulating anthracycline-induced cardiac damage. This has particular relevance because of the increased appreciation of the scale of the problem, specifically from the late follow-up of paediatric patients [3,4]. The positive results obtained using ICRF-187, as a cardioprotective agent, in human and animal studies [14–20] gives good grounds for continued optimism. The mode of action of ICRF-187 is unknown although one suggested mechanism is that it is converted intracellularly, *in vivo*, to an iron-chelating agent preventing the formation of the doxorubicin-iron complexes that lead to free radical damage [12].

In humans, the co-administration of an agent which itself produces many of the side-effects of cytotoxic chemotherapy [37] together with multi-agent cytotoxic chemotherapy is an important consideration [19,20]. A less toxic and more effective modulator of anthracycline-mediated cardiac damage would be preferable. The results of the present study clearly indicate the enhanced toxicity of DOX when combined with ICRF-187; this combination produced the greatest reduction in body weight in the first 2 weeks after a single i.v. dose of DOX. None of the oils, given daily both before and after administration of DOX, were associated with a similar increase in acute toxicity, indeed animals receiving 2 ml of So-1100, daily, showed the smallest reduction in body weight in the first 2 weeks after i.v. administration of DOX (Fig. 1).

Reports that essential fatty acids, specifically GLA, reduce the severity of late radiation-induced reactions in pig skin [23,25] and in the spinal cord of both rats [26] and pigs [24] are of interest, as is the report of the attenuation of bleomycin-induced lung fibrosis in the hamster [28]. Late radiation damage is likely to be mediated indirectly, possibly though products generated in irradiated tissues, perhaps as a consequence of chronic reperfusion injury [38]. Whether the benefits produced by essential fatty acids are mediated by replacing peroxidised unsaturated fatty acids in lipid-containing structures, such as membranes, or via alterations in eicosanoid metabolic pathways, or indeed some other action, is unknown.

The primary objective of the present study was to examine the capacity of ICRF-187 and an oil containing GLA (So-1100) to ameliorate doxorubicin-induced cardiotoxicity in the rat, using a functional endpoint, impairment of the cardiac volume output. Both ICRF-187 and So-1100, particularly at the higher dose level, produced significant long-term amelioration of functional cardiotoxicity from DOX. Furthermore, data for animal survival also demonstrated significant cardiac protection from the use of ICRF-187 and 2 ml of So-1100. All animals in these groups survived for the entire period of the study (20 weeks).

It is important to point out that in animals receiving ICRF-187 and So-1100 (2 ml daily) there was a small, but significant, decline in the cardiac VOI at the 20-week time-point. It is not possible to predict whether this small fractional reduction in the VOI would be accentuated with a further progression in time. However, earlier studies using this rat model [35,39] have suggested that the degree of reduction in cardiac function at 12 weeks is predictive of the subsequent response. Only animals showing a ⩾40% reduction in cardiac output at 12 weeks showed significant progression of damage, with death from cardiac failure being the usual outcome prior to 20 weeks.

In this study, ICRF-187 was administered using a dose ratio of 20:1 with DOX. It would be reasonable to anticipate that a further increase in the dose of ICRF-

187, while possibly being associated with a higher level of cardioprotection, would have been counterbalanced by an exacerbation of acute toxicity as a result of increased myelosuppression (data not shown). Even at the dose of ICRF-187 used, the long-term general toxicity of the drug combination resulted in a very small rise in body weight (~14%) over the study period (Fig. 2). This effect was clearly not associated with significant cardiotoxicity. On the other hand, animals receiving So-1100, 2 ml daily, gained weight at a similar rate to that of aged-matched controls. The explanation for the poor performance of rats that received only 1 ml of So-1100, daily, in terms of their small gain in body weight, is uncertain. Clearly the oil So-1129, which contained no GLA, did not modulate general long-term body weight-related toxicity when compared with the group receiving a combination of water plus DOX. A similar degree of impairment in cardiac function was also observed in these two groups of rats (Fig. 4).

The degree of cardioprotection obtained using ICRF-187, and indeed more dramatically so with So-1100 (2 ml), is best demonstrated by examining the proportional distribution of animals with respect to the degree of impairment in the VOI at both 12 and 20 weeks after administration of DOX (Fig. 5). All animals receiving 2 ml of So-1100 showed a minimal impairment in the VOI at 12 weeks and only modest impairment in cardiac function (<40% change in VOI) after 20 weeks. All other groups had individual animals showing a ⩾40% impairment in the VOI after 20 weeks. In the groups receiving So-1129 or water, 50% of the animals showed a ⩾40% impairment in the VOI after 20 weeks. An examination of the proportion of animals showing a <20% impairment in VOI at 12 weeks suggested that So-1100 was slightly more effective than ICRF-187, but clearly with greatly reduced acute and general long-term toxicity. Animals receiving So-1129 or water, before and after the administration of DOX, showed similar cardiotoxicity to that seen in a concurrent study where the dose-related incidence of cardiotoxicity was evaluated.

Although the results of the functional studies and the associated clinical observations indicate the cardioprotective effects of So-1100 (in particular 2 ml, daily) and ICRF-187, the histological observations were inconclusive. In previous studies using this rat model, histological investigations were also undertaken [17,32]. Samples of hearts were examined and graded using established scales [5,6,40] by the pathologists responsible for the development of the grading scales. Classical DOX-induced lesions were seen in animals that died within 12 weeks of the administration of DOX (4 mg/kg). Animals that survived for the full 20-week period of these studies, either as a consequence of the co-administration of ICRF-187 [17] or because of the use of polymer-bound DOX [33] had low histological scores and some showed non-specific interstitial myocarditis. The majority of the animals in the present study survived for the full 20 weeks and hence the absence of classical DOX-induced lesions is perhaps not a surprise in view of the fact that earlier experience was with a 33.3% higher dose of DOX than that used in the present study.

Alternative causes of cardiac decompensation also need to be considered. Although anthracycline-induced cardiotoxicity secondary to nephrotoxicity has been reported [41] and has been specifically noted in the Wistar strain of rats [42], it has never been an obvious feature in the Sprague–Dawley strain used in the present study. A gross examination of the kidneys of animals used in the present study, again showed no obvious changes. However, even if a degree of nephrotoxicity was produced, there would appear to be no causal relationship between DOX-induced nephrotoxicity and cardiotoxicity, particularly in this rat strain [43].

In conclusion, the present data appear to suggest a significant role for So-1100 as a protector against DOX-induced cardiac damage. The clinical implications of these observations could be considerable. If the cardioprotection afforded by So-1100 is indeed comparable to that of ICRF-187 and the acute toxicity is less, then this would indeed be of clinical importance.

Given that there is also evidence that GLA may be selectively toxic to a range of tumour cell types [29,30] and may also enhance the efficacy of cytotoxic agents [31] implies that an improvement in the therapeutic ratio may be well be achieved using this approach. It is worthy of further evaluation.

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