CURRENT TOPIC

Anthracyclines: cardiotoxicity and its prevention

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The anthracycline antibiotics have now been in use in curative chemotherapy protocols for over two decades. They are highly active against a wide range of haemopoietic and solid tumours in children and adults. Unfortunately, their antineoplastic use is limited by significant and irreversible cardiotoxicity, the full extent of which continues to emerge. As more children become long term survivors of cancer, the relative impact of anthracycline induced cardiac damage has increased but, despite an understanding of the pathogenesis, consensus strategies for detection and prevention have not been established.

Historical perspective

Daunorubicin, the first anthracycline antibiotic to be used, was isolated in Italy in 1963 from cultures of *Streptomyces peucetius*.¹ In phase II trials it showed activity against acute lymphoblastic leukaemia and acute myeloid leukaemia. Doxorubicin, isolated shortly afterwards,² showed demonstrable activity against a wider range of tumours in children, including soft tissue and bone sarcomas, Wilms' tumour and lymphomas, as well as lymphoblastic and myeloid leukaemia. Early phase I trials involved patients with widespread disease and reports of cardiotoxicity³ could only uncertainly attribute toxicity to the drug rather than the disease. With increasing use of anthracyclines, however, it became apparent that daunorubicin and doxorubicin were directly cardiotoxic in children⁴ and adults.⁵

Originally cardiac toxicity was described as being acute or subacute and was seen during or immediately after one dose or course of anthracycline. Additionally, late or chronic toxicity occurred after prolonged administration of anthracycline.⁶ Acute toxicity was seen as transient changes on electrocardiograms, typically ST and T wave flattening, and arrhythmias, typically sinus tachycardia.^{5,7} These changes usually reverted to normal, were independent of dose, and were mainly seen in adults. The subacute toxicity was manifest as a pericarditis-myocarditis syndrome or as acute left ventricular failure⁶ and was particularly seen in early trials of anthracyclines in adults when scheduling led to doses as high as 160 mg/m² being delivered over times as short as four days.

Late cardiotoxicity developing after repeated

administration of anthracyclines was first recognised when patients presented clinically with symptoms of congestive heart failure⁵ within six months of their last dose of anthracycline. The outcome was often fatal and necropsy showed the cytoplasmic vacuolisation and myocyte necrosis since recognised as typical of anthracycline damage.⁵ This review will focus on late toxicity.

Risk factors and incidence

The prime factor determining the risk of developing anthracycline induced cardiomyopathy is the cumulative dose of anthracycline received.⁵ Estimates of the incidence of cardiomyopathy vary depending on the end point being used. Von Hoff and coworkers' large series from the 1970s^{7,8} using early clinically overt congestive cardiac failure as the end point estimated the incidence of daunorubicin induced cardiomyopathy to be 2% at 600 mg/m² and 17% at 1050 mg/m². The equivalent figures for doxorubicin were 7% at 550 mg/m² and 18% at 700 mg/m². The rapid increase in toxicity above 550 mg/m² led to this being used as a limiting dose for the two drugs.

The risk of cardiotoxicity is potentiated by mediastinal radiation,⁹ advancing age,^{8,9} and pre-existing cardiac disease or hypertension,⁸ although the effects of advancing age and cardiac disease may not be independent. The effect of young age at the time of treatment is less certain. Von Hoff and coworkers^{7,8} found that children younger than 15 years had a higher risk of developing cardiac failure than adults with daunorubicin and doxorubicin. Within a paediatric group Lipshultz *et al* found an increased risk for children treated under 4 years of age of developing subclinical cardiomyopathy with long follow up,¹⁰ but this was not confirmed by Steinherz *et al*.¹¹ Although early studies found no effect of gender, it has been suggested that girls may be at increased risk of cardiotoxicity.¹²

The increasing numbers of long term survivors and the availability of routine echocardiography in the late 1970s meant that reports began to appear of cardiac decompensation occurring for the first time 10 years after initial treatment¹³ and of subclinical cardiomyopathy.¹⁴ Steinherz *et al* assessed cardiac function by measuring fractional shortening in patients treated in childhood up to 20 years from completing treatment and found an

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overall 23% incidence of abnormal cardiac function.¹¹ This increased to 63% in the subgroup who had received more than 500 mg/m² and had been followed up for more than 10 years. Late decompensation as a result of pregnancy or weight training was well recognised. Using more sensitive load independent echocardiographic measures of cardiac function, Lipshultz *et al* found an overall incidence of 57% abnormalities,¹⁰ with up to 15 years' follow up. The full extent of the problem and the functional significance of asymptomatic abnormalities is as yet unclear.

Pathology

Electron microscopic examination of endomyocardial biopsy samples from patients treated with anthracyclines shows myofibril loss, swelling of the sarcoplasmic reticulum and mitochondria, cytoplasmic vacuolisation, and widespread damage with necrosis of myocytes.⁹ These changes may be focal or diffuse.

The number of myocytes present in the adult heart is established by the age of 6 months. Myocytes lost by necrosis are not replaced, but the remaining myocytes increase in size to compensate. There is also an increase in the amount of interstitial tissue and fibrosis. The changes seen in children and adults are the same.

For children sustaining anthracycline cardiotoxicity, myocyte loss means the left ventricular wall becomes relatively thin and particularly fails to keep pace with pubertal or growth hormone induced growth spurts,^{10 13} occasionally causing subclinical damage to become clinically overt at this time.

Mechanism of damage

At the current level of understanding the mechanisms by which anthracyclines produce antitumour activity and cardiotoxicity appear different. The antitumour activity of anthracyclines seems to be exerted by intercalation in double stranded DNA and stabilisation of the topoisomerase II-DNA complex, which is an essential stage of DNA replication, resulting in DNA strand breaks.¹⁵ In contrast, the cardiotoxicity of anthracyclines is almost certainly mediated by free radical damage which is not involved in the antitumour effect. Anthracyclines, particularly when complexed with iron, can generate superoxide and hydroxyl radicals either by redox cycling or intramolecular reduction of the chelated iron.¹⁶ These free radicals then cause lipid peroxidation¹⁷ and damage a variety of cellular membranes, causing cell death. The heart is thought to be particularly susceptible to this damage because it has a large number of mitochondria, which are a site of free radical generation, and because it has low levels of antioxidant enzymes.¹⁸

Detection of cardiotoxicity

Detection of cardiac damage at the point of cardiac failure is too late. There is a need for a

method of cardiac assessment which detects early cardiac damage, is easily performed, and has predictive power.

The electrocardiogram was the earliest method used for monitoring toxicity, but the transient changes seen with acute toxicity had little predictive value for the development of cardiomyopathy.⁷ Schwartz *et al* suggest that the prolongation of the QT interval (QTc) is a useful screening test for early cardiac damage when echocardiography remains normal,¹⁹ but its relation with clinical outcome is unproved.

In Stanford, Billingham and coworkers pioneered the development of taking right ventricular endomyocardial biopsy samples and a biopsy scoring system which, in the hands of an experienced pathologist, correlates well and linearly with the cumulative anthracycline dose.⁹ Using endomyocardial biopsy samples it is possible to predict the rate of progression of damage for any individual,²⁰ but there is an associated morbidity and therefore there are concerns about the invasiveness of repeated treating in children.²¹

Echocardiographic measurement of fractional shortening and ejection fraction as indicators of left ventricular systolic function is the most widely used method of assessing cardiotoxicity, but is complicated by the ability of the myocardium to compensate for early damage. The sensitivity of systolic echocardiography can be improved by exercise testing²² when failure of the fractional shortening to increase in response to exertion may indicate early cardiomyopathy. Similar, asymptomatic cardiomyopathy can be unmasked by echocardiography during low dose dobutamine infusions at a time when other physiological studies are normal.²³

Fractional shortening is affected by variables such as anaemia, fever, and volume infusions that influence left ventricular loading conditions and interfere with the relation between fractional shortening and contractility. Attention has focused on load independent echocardiographic measures of systolic function such as end systolic wall stress and the stress-velocity index. These are sensitive indicators of cardiac dysfunction,^{10 24} but are complicated to measure and require cooperation in young children. Follow up studies using these measures¹⁰ show increasing end systolic wall stress with increasing anthracycline dose. This is the result of increased left ventricular afterload, which is the result of reduced left ventricular wall thickness and seems a particular problem in children treated at young age. Direct measurement of the left ventricular posterior wall dimension and thickening has also proved a sensitive indicator of asymptomatic damage.²³ The value of these measures in prospective studies remains to be proved.

There has been interest in echocardiographic measurement of left ventricular diastolic function, in particular early diastolic filling, as a more sensitive measure of cardiac damage.^{24 25} Again this is awkward to measure in children and comparison with stressed systolic assessment has shown diastolic measures to be less sensitive.²³

Left ventricular contractility can also be measured by radionuclide angiography. Two studies in adults have used radionuclide angiography to measure ejection fraction prospectively^{26 27} and developed guidelines to tailor anthracycline dose to individual tolerance, thereby preventing early onset cardiac failure. Radionuclide angiography has not been used in any large paediatric series and accuracy might be expected to be more of a problem in children where movement and anatomical considerations make the images less clear.

Overall, the issue of selecting an ideal monitoring investigation is unresolved. Endomyocardial biopsy probably remains the investigation with the best linear correlation with cardiac toxicity and power of predicting the onset of early cardiac failure. It has not been validated against the risk of developing late cardiac decompensation or subclinical cardiomyopathy, however. On the other hand, load independent and stressed measurement of systolic function can detect a large amount of subclinical cardiomyopathy, but the importance of this for future cardiac function has not been determined and the predictive power of this measure has not been validated prospectively. Fractional shortening is easily measured and testing is widely available. Increasing its sensitivity by exercise stressing may well make it the best measure to use prospectively to monitor cardiotoxicity and limit future cardiac dysfunction.

Prevention of cardiotoxicity

DOSE LIMITATION

The earliest strategy of cardioprotection adopted was limitation of the cumulative dose of anthracycline received. Studies showing a small risk of early congestive heart failure below cumulative doses of 550 mg/m²^{7 8} meant that this was used as a safe dose.

Current paediatric protocols rarely use total anthracycline doses greater than 450 mg/m² and this has been one of the most important factors to date in limiting the incidence of heart failure. The fallacy of this approach is that there is no safe dose as the risk of early cardiomyopathy increases progressively and not in an all or none fashion. It also does not take into account the risks for subclinical cardiomyopathy, although the long term functional significance of this remains unknown.

Variation in the individual tolerance of anthracyclines is unacknowledged, to the detriment of patients who develop significant cardiac damage at doses below those deemed safe, and also to the detriment of those who are more tolerant but are denied further doses of an effective antitumour drug above the safety threshold.

SERIAL MONITORING

The difficulty of choosing a single method for monitoring cardiac function which is sensitive and predictive of future dysfunction was addressed earlier. It would, however, allow

tailoring of cumulative anthracycline dose to individual tolerance.

Endomyocardial biopsy samples²⁰ and radionuclide angiography²⁵ scans have been used in prospective monitoring studies and in both instances management protocols were devised which limited anthracycline dose based on changes in the biopsy score or ejection fraction. The incidence of early onset cardiac failure was successfully reduced but, as is now understood, this is only a small part of the anthracycline cardiotoxicity problem.

Echocardiography is widely used for monitoring in paediatric practice. Because of the patchy nature of early cardiac monitoring there are few serial data with long follow up to allow predictions of risk to be developed. Steinherz *et al*¹¹ and Lipshultz,¹⁰ however, showed that fractional shortening at the end of treatment correlated fairly well with fractional shortening at long term follow up. In Steinherz's series only 11% of children with normal end of treatment fractional shortening developed mildly abnormal fractional shortening at follow up. For children with abnormal end of treatment fractional shortening there was some continued deterioration. Unfortunately, fractional shortening does not necessarily change in a linear fashion with increasing dose, but the Children's Cancer Study Group (CCSG) have proposed guidelines for dose limitation based on fractional shortening monitoring.²⁸

ANTHRACYCLINE ANALOGUES

The search for analogues of doxorubicin and daunorubicin with a better therapeutic index has been relatively disappointing. Epirubicin, idarubicin, and aclarubicin A are semisynthetic anthraquinones closely related structurally to doxorubicin and daunorubicin. All three drugs are effective antitumour agents, with epirubicin being similar to doxorubicin in the range of tumours affected and idarubicin and aclarubicin A being active in acute myeloid and lymphoblastic leukaemia.

When epirubicin was first introduced, phase I studies suggested it should be used in a dose relative to doxorubicin 1.2:1. This recommendation was based on the doses at which dose limiting haematological toxicity was produced. Unfortunately, epirubicin produces identical cardiotoxicity to doxorubicin, although at higher doses. The dose ratio at which about 5% congestive heart failure occurs is of the order of 1.8–2.0:1 (epirubicin:doxorubicin) based on data gathered by Farmitalia in 9144 patients²⁹ and compared with the series of Von Hoff *et al*⁸ for doxorubicin. The lower cardiotoxicity of epirubicin has been confirmed by endomyocardial biopsy samples.³⁰ Analysis of several patient series using epirubicin or doxorubicin as a single drug on the same schedule in breast cancer and soft tissue sarcomas in adults suggests that epirubicin is equipotent with doxorubicin for antitumour effect in these situations,³¹ but there are no equivalent data in paediatric tumours. Furthermore, short term response rates do not necessarily correlate with long term outcome.

The relative cardiotoxicity of idarubicin and aclarubicin A remains to be established although damage is produced by the two drugs.

In an attempt to synthesise an anthracycline analogue without cardiotoxicity the structurally less similar anthraquinone, mitoxantrone, was developed. It has antitumour activity in leukaemias, but again causes cardiac damage with an incidence of 3% of heart failure at cumulative doses greater than 160 mg/m².³² Antineoplastic doses are also lower than doxorubicin, but there has been no direct comparison of cardiotoxicity for equivalent antineoplastic doses.

Several synthetic anthrapyrazole drugs are currently undergoing evaluation. Their iminoquinine structure should prevent free radical generation, so assessment of their cardiotoxic potential may be promising.

LIPOSOMAL ANTHRACYCLINES

Investigation of liposomes as potential drug carriers began in the 1970s. Animal studies of liposome encapsulated doxorubicin clearly showed reduced cardiotoxicity³³ and in experimental models antitumour activity was good, particularly for hepatic tumours, as might be expected from the preferential hepatic and splenic uptake of liposomes. Liposomal doxorubicin was well tolerated in phase I clinical trials,³⁴ although at relatively low doses, and has entered phase II trials.³⁵ The clinical role of this mode of delivering anthracyclines remains to be established.

SCHEDULING

Rather than reducing cardiotoxicity by changing the form of anthracyclines, other investigators have explored the effect of altering drug scheduling.³⁶

The original studies of doxorubicin used doses of 60–90 mg/m² given every three weeks. The dose was either given as a single intravenous bolus or fractionated over three to six days. Initially there was no evidence that cardiotoxicity may be schedule dependent until pilot studies³⁷ suggested that dividing the dose every three weeks into weekly boluses was associated with a lower incidence of cardiomyopathy. The retrospective study of Von Hoff confirmed that, independent of other risk factors, there was a significant reduction in the probability of clinically overt cardiomyopathy occurring at a cumulative dose of 550 mg/m² when doxorubicin was given weekly as opposed to every three weeks.⁸ There was little difference between fractionated and single doses every three weeks. Subsequent prospective studies,³⁸ which included examination for subclinical cardiotoxicity, also confirmed the cardioprotective effect of weekly bolus administration for the same dose intensity.

In the belief that the reduced cardiotoxicity of weekly doxorubicin was the result of reduced peak plasma concentrations, other groups investigated enhancing this effect by

administering doxorubicin as a prolonged intravenous infusion. Unequivocal data supporting reduced toxicity with prolonged infusions are limited, with much of the work being carried out in adults in small series with historical controls. Some of the most convincing data came from Legha *et al* at the M D Anderson Center³⁹ where, in a non-randomised study monitoring changes in endomyocardial biopsy score, reduced cardiotoxicity was seen for the group receiving 24–96 hour infusions as opposed to bolus doses. The infusion time for the prolonged infusion group of 21 patients was escalated in steps from 24 to 96 hours. Five patients were maintained on 48 hour infusions, the remainder at 96 hours making it impossible to ascertain the relative toxicity of the different times, but it seems likely that extending the infusion time to 96 hours is cardioprotective.

Casper *et al*, in a prospective randomised study, showed using radionuclide angiography that doxorubicin given over 72 hours was less cardiotoxic than bolus injections for a given cumulative dose, but that protection was by no means absolute.⁴⁰ Two small series with historical controls have suggested that 24 and 48 hour infusions may also be less toxic.^{41 42}

The original study of Speyer *et al* of six hour infusions was uncontrolled, but again showed that prolonging doxorubicin infusion time is not absolutely protective, with 48% of patients showing a decrease in ejection fraction of 10% or more.⁴³ This is comparable with the 42% showing a 10% decrease in Casper's study of 72 hour infusions. Subsequently, Shapira *et al* have confirmed in a controlled trial that six hour infusions are less cardiotoxic than bolus doses.⁴⁴

All studies of prolonged anthracycline infusions have been carried out in adults, although feasibility in children has been established in some small paediatric series. No study has examined late cardiotoxicity, nor the relative cardiotoxicity of different infusion times. Most importantly, no study has established how much it is necessary to prolong bolus doses to reduce cardiotoxicity.

An essential correlate of reducing toxicity by increasing infusion times is that antineoplastic efficacy is preserved. In vitro studies suggested that the efficacy of weekly or infused anthracyclines may be better than conventional schedules. For weekly doses there are clinical data that efficacy in breast carcinoma and soft tissue sarcomas is maintained.⁴⁵ For prolonged infusions there are only three controlled studies, one of which has no data on antitumour effect,⁴⁴ one of which concludes that efficacy is similar,⁴⁶ and the study of Casper *et al*,⁴⁰ which suggests that the prolonged infusion group may have a worse outcome. In all other uncontrolled studies the response rate has been compared with historical controls. It must be concluded that for adults it has not been safely established that antineoplastic efficacy is unaltered by anthracycline scheduling changes, and whether the existing data can be extrapolated to children with their very different tumours is uncertain.

CARDIOPROTECTANTS

A further strategy to ameliorate anthracycline induced cardiotoxicity has been to develop cardioprotective agents. ICRF-159 (Razoxane), a cytotoxic chelating agent based on ethylenediaminetetra-acetic acid (EDTA), was originally developed as a potential antineoplastic agent.⁴⁷ Although early studies were promising, phase II trials showed no significant antitumour activity. Before cardiotoxicity was understood to be the result of free radical generation by anthracycline iron complexes, Herman *et al* discovered that ICRF-159 protected isolated heart preparations from acute anthracycline cardiotoxicity.⁴⁸ Subsequent animal experiments from Herman's laboratory established that ICRF-159 and its D-isomer ICRF-187 protected against acute and chronic anthracycline induced cardiotoxicity.

It was postulated that ICRF-187 worked by reacting with the doxorubicin-iron complex to chelate and remove the iron, thereby reducing free radical formation, and there is evidence to support this.⁴⁹ ICRF-187 did not interfere with antitumour effect in animal studies, but did add some myelotoxicity of its own. There has been a suggestion in clonogenic assay that ICRF-187 might interfere with the topoisomerase II inhibitory effects of anthracyclines,⁵⁰ however, this contradicts earlier reports of ICRF-187 potentiating the effects of doxorubicin.⁵¹ Free radical scavengers such as acetylcysteine and α -tocopherol had an insignificant cardioprotective effect.

Phase I trials had established the dose limiting toxicities of ICRF-187 to be myelosuppression and, in children, transient hepatic toxicity.⁵² Speyer *et al* initiated a phase III study in adults with advanced breast cancer,⁵³ which clearly showed significant cardioprotection by ICRF-187 using radionuclide angiography and biopsy monitoring. There was no significant reduction in antitumour activity and a slight increase in myelosuppression. The degree of protection against changes in ejection fraction and biopsy score was striking, allowing cumulative doxorubicin doses up to 2150 mg/m².⁵⁴ Unfortunately, disease progression in these patients prevented comment about long term cardioprotection. There has been no phase III trial in children to date, although case reports suggest similar cardioprotection.⁵⁵

In response to the urgent need for a paediatric phase III study the Medical Research Council and UK CCSG were hoping to open a trial to examine the cardioprotective effect of ICRF-187 in acute myeloid leukaemia and Ewing's sarcoma; however, this has been delayed by the company because of concerns about possible interference with anthracycline activity, as outlined here. There is an urgent need to resolve this issue.

Our practice

Based on our own analysis of published reports and experience, current practice is as follows:

(1) Doxorubicin and daunorubicin are always administered as infusions with a minimum infusion time of one hour. Some UK/European study treatment protocols

request administration times of greater than one hour with which we comply. We have not been involved in studies of prolonged anthracycline infusion.

(2) Standard monitoring is carried out on all patients receiving anthracyclines using electrocardiography and echocardiography. Wherever feasible, baseline assessment takes place before the initiation of treatment; however, because assessment is at another hospital, it is not always possible to do this in seriously ill children. We broadly follow CCSG guidelines²⁸ in that we obtain further echocardiograms or electrocardiograms, or both, before alternate courses of anthracyclines at doses less than 300 mg/m² and before every course above this dose level.

(3) We use fractional shortening as our main guide to tailoring treatment. A decrease in fractional shortening of more than 10% from baseline or fractional shortening less than 29% will trigger a discussion about further anthracycline treatment. We would usually repeat the test one to two weeks later and discontinue anthracyclines if the result is confirmed.

(4) Our follow up policy after completion of treatment varies depending on the end of treatment cardiac function. In those with a 'normal' or 'acceptable' cardiac function (fractional shortening >29%), echocardiography is carried out at one, three, and five years after the end of treatment and our aim is then to repeat this every five years or sooner if there is any other potential problem – for example, growth hormone treatment or pregnancy. In those with abnormal end of treatment function, echocardiography is carried out between three and six months after the completion of treatment, at one year, and then at least annually for the next five years, although a number of children have required more frequent monitoring.

Conclusions

For paediatric oncologists the use of anthracyclines to treat children with malignancies causes a dilemma. Anthracyclines undoubtedly damage the myocardium irreversibly and an important minority of children have survived their tumour to die of cardiomyopathy. Furthermore, because more than 50% of children with cancer become long term survivors, the fact that 23–57% of those who received anthracyclines have evidence of subclinical myocardial damage has worrying implications for the future. Anthracyclines form an important part of the curative chemotherapy given to children with acute myeloid leukaemia, Wilms' tumour, and sarcomas and may only be discarded at the potential cost of a decrease in survival rates. Cardioprotective strategies require careful controlled clinical trials before they are accepted into routine clinical use.

1 Grein A, Spalla C, Di Marco A, *et al*. Decrizione e classificazione di un attinomicete (*Streptomyces peucetius* sp. nova) produttore di un sostanza ad attivita antitumorale: la Daunomicina. *G Microbiol* 1963; 11: 109–15.

- 2 Bonnadonna G, Monfardini S, DeLena M, *et al.* Clinical evaluation of Adriamycin, a new antitumour antibiotic. *BMJ* 1969; **iii**: 503-6.
- 3 Tan CT, Tanaka H, Yu KP, *et al.* Daunomycin: an anti-tumour antibiotic in the treatment of neoplastic disease. *Cancer* 1967; **20**: 333-53.
- 4 Gilladoga AC, Manuel C, Tan CT, *et al.* The cardiotoxicity of Adriamycin and daunomycin in children. *Cancer* 1976; **37**: 1070-8.
- 5 Lefrak EA, Pitha J, Rosenheim S, *et al.* A clinicopathological analysis of Adriamycin cardiotoxicity. *Cancer* 1973; **32**: 302-14.
- 6 Bristow MR, Billingham ME, Mason JW, *et al.* Clinical spectrum of anthracycline antibiotic cardiotoxicity. *Cancer Treatment Reports* 1978; **62**: 873-9.
- 7 Von Hoff DD, Rozenzweig M, Layard M, *et al.* Daunomycin-induced cardiotoxicity in children and adults. A review of 110 cases. *Am J Med* 1977; **62**: 200-8.
- 8 Von Hoff DD, Layard MW, Basa P, *et al.* Risk factors for doxorubicin-induced congestive heart failure. *Ann Intern Med* 1979; **91**: 710-7.
- 9 Bristow MR, Mason JW, Billingham ME, *et al.* Doxorubicin cardiomyopathy; evaluation by phonocardiography, endomyocardial biopsy and cardiac catheterisation. *Ann Intern Med* 1978; **88**: 168-75.
- 10 Lipshultz SE, Colan SD, Geiber RD, *et al.* Late cardiac effects of doxorubicin therapy for acute lymphoblastic leukaemia in childhood. *N Engl J Med* 1991; **324**: 808-15.
- 11 Steinherz LJ, Steinherz PG, Tan CTC, *et al.* Cardiac toxicity 4 to 20 years after completing anthracycline therapy. *JAMA* 1991; **266**: 1672-7.
- 12 Silber JH, Jakacki RI, Larsen RL, *et al.* Increased risk of cardiac dysfunction after anthracyclines in girls. *Med Pediatr Oncol* 1993; **21**: 477-9.
- 13 Goorin AM, Chauvenet AR, Perez-Atayde AR, *et al.* Initial congestive heart failure six to ten years after doxorubicin chemotherapy for childhood cancer. *J Pediatr* 1990; **116**: 144-7.
- 14 Dresdale A, Bonow R, Wesley R, *et al.* Prospective evaluation of doxorubicin-induced cardiomyopathy resulting from post-surgical adjuvant treatment of patients with soft tissue sarcomas. *Cancer* 1983; **52**: 51-60.
- 15 Bodley A, Liu LF, Israel M, *et al.* DNA topoisomerase II-mediated interaction of doxorubicin and daunorubicin congeners with DNA. *Cancer Res* 1989; **49**: 5969-78.
- 16 Keizer HG, Pinedo HM, Schuurhuis GJ, Joenje H. Doxorubicin (Adriamycin): a critical review of free radical-dependent mechanisms of cytotoxicity. *Pharmacol Ther* 1990; **47**: 219-31.
- 17 Myers CE, McGuire WP, Liss RH, *et al.* Adriamycin: the role of lipid peroxidation in cardiac toxicity and tumour response. *Science* 1977; **197**: 165-7.
- 18 Doroshow JH, Locker GY, Myers CE. Enzymatic defences of the mouse heart against reactive oxygen metabolites; alterations produced by doxorubicin. *J Clin Invest* 1980; **65**: 128-35.
- 19 Schwartz CL, Hobbie WL, Truesdell A, *et al.* Corrected QT interval prolongation in anthracycline-treated survivors of childhood cancer. *J Clin Oncol* 1993; **11**: 1906-10.
- 20 Billingham ME, Bristow MR. Evaluation of anthracycline cardiotoxicity: predictive ability and functional correlation of endomyocardial biopsy. *Cancer Treatment Symposiums* 1984; **3**: 71-6.
- 21 Pegelow CH, Popper RW, de Wit SA, *et al.* Endomyocardial biopsy to monitor anthracycline therapy in children. *J Clin Oncol* 1984; **2**: 443-6.
- 22 Weesner KM, Bledsoe M, Chauvenet A, Wofford M. Exercise echocardiography in the detection of anthracycline cardiotoxicity. *Cancer* 1991; **68**: 435-8.
- 23 Klewer SE, Goldberg SJ, Donnerstein RL, *et al.* Dobutamine stress echocardiography: a sensitive indicator of diminished myocardial function in asymptomatic doxorubicin-treated long-term survivors of childhood cancer. *J Am Coll Cardiol* 1992; **19**: 394-401.
- 24 Hausdorf G, Morf G, Beron G, *et al.* Long term doxorubicin cardiotoxicity in childhood: non-invasive evaluation of the contractile state and diastolic filling. *Br Heart J* 1988; **60**: 309-15.
- 25 Marchandise B, Schroeder E, Bosly A, *et al.* Early detection of doxorubicin cardiotoxicity: interest of Doppler echocardiographic analysis of left ventricular filling dynamics. *Am Heart J* 1989; **118**: 92-8.
- 26 Schwartz RG, McKenzie WB, Alexander J, *et al.* Congestive heart failure and left ventricular dysfunction complicating doxorubicin therapy: a seven year experience using serial radionuclide angiography. *Am J Med* 1987; **82**: 1109-18.
- 27 Palmeri ST, Bonow RO, Myers CE, *et al.* Prospective evaluation of doxorubicin cardiotoxicity by rest and exercise radionuclide angiography. *Am J Cardiol* 1986; **58**: 607-13.
- 28 Steinherz LJ, Graham T, Hurwitz R, *et al.* Guidelines for cardiac monitoring of children during and after anthracycline therapy: report of the Cardiology Committee of the Children's Cancer Study Group. *Pediatrics* 1992; **89**: 942-9.
- 29 Praga C, Trave F, Petroccione A. Anthracycline-induced cardiotoxicity and its relevance in cancer treatment. In: Nimmo WS, Tucker GT, eds. *Clinical measurement in drug evaluation*. London: Wolfe, 1991: 131-42.
- 30 Bonnadonna G, Gianni L, Santoro A, *et al.* Drugs ten years later: epirubicin. *Ann Oncol* 1993; **4**: 359-69.
- 31 Lanchbury AP, Habboubi N. Epirubicin and doxorubicin: a comparison of their characteristics, therapeutic activity and toxicity. *Cancer Treat Rev* 1993; **19**: 197-228.
- 32 Shenkenberg TD, Von Hoff DD. Mitoxantrone: a new anti-cancer drug with significant clinical activity. *Ann Intern Med* 1986; **105**: 67-81.
- 33 Herman EH, Rahman A, Ferrans VJ, *et al.* Prevention of chronic doxorubicin cardiotoxicity in beagles by liposomal encapsulation. *Cancer Res* 1983; **43**: 5427-32.
- 34 Gabizon A, Peretz T, Sulkes A, *et al.* Systemic administration of doxorubicin-containing liposomes in cancer patients: a phase I study. *Eur J Cancer Clin Oncol* 1989; **25**: 1795-803.
- 35 Treat J, Greenspan A, Forst D, *et al.* Antitumour activity of liposome-encapsulated doxorubicin in advanced breast cancer: a phase II study. *J Natl Cancer Inst* 1990; **82**: 1706-10.
- 36 Bielack SS, Erttmann R, Winkler K, Landbeck G. Doxorubicin: effect of different schedules on toxicity and antitumour efficacy. *Eur J Cancer Clin Oncol* 1989; **25**: 873-82.
- 37 Weiss AJ, Manthel RW. Experience with the use of adriamycin in combination with other anticancer agents using a weekly schedule with particular reference to lack of cardiotoxicity. *Cancer* 1977; **40**: 2046-52.
- 38 Torti FM, Bristow MR, Howes AE, *et al.* Reduced cardiotoxicity of doxorubicin delivered on a weekly schedule. *Ann Intern Med* 1983; **99**: 745-9.
- 39 Legha SS, Benjamin RS, Mackay B, *et al.* Reduction of doxorubicin cardiotoxicity by prolonged continuous intravenous infusion. *Ann Intern Med* 1982; **96**: 133-9.
- 40 Casper ES, Gaynor JJ, Hajdu SI, *et al.* A prospective randomized trial of adjuvant chemotherapy with bolus versus continuous infusion of doxorubicin in patients with high-grade extremity soft tissue sarcoma and an analysis of prognostic factors. *Cancer* 1991; **68**: 1221-9.
- 41 Speyer JL, Green MD, Bottino J, *et al.* A phase I/II study of 6 hour and 24 hour intravenous infusion of doxorubicin. In: Muggia F, Young C, Carter S, eds. *Anthracycline antibiotics in chemotherapy*. The Hague: Martinus Nijhoff, 1982: 398-411.
- 42 Tannir N, Yap HY, Hortobagyi GH, *et al.* Sequential continuous infusion with doxorubicin and vinblastine: an effective chemotherapy combination for patients with advanced breast cancer previously treated with cyclophosphamide, methotrexate, 5-FU, vincristine. *Cancer Treatment Reports* 1984; **68**: 1039-41.
- 43 Speyer JL, Green MD, Dubin N, *et al.* Prospective evaluation of cardiotoxicity during a 6-hour doxorubicin infusion regimen in women with adenocarcinoma of the breast. *Am J Med* 1985; **78**: 555-63.
- 44 Shapira J, Gotfried M, Lishner M, Ravid M. Reduced cardiotoxicity of doxorubicin by a 6-hour infusion regimen: a prospective randomized evaluation. *Cancer* 1990; **65**: 870-3.
- 45 Borden EC, Amato DA, Rosenbaum DH, *et al.* Randomized comparison of three Adriamycin regimens for metastatic soft tissue sarcomas. *J Clin Oncol* 1987; **5**: 840-50.
- 46 Zalupski M, Metch B, Balcerzak S. Phase III comparison of doxorubicin and dacarbazine given by bolus versus infusion in patients with soft tissue sarcomas: a Southwest Oncology Group study. *J Natl Cancer Inst* 1991; **83**: 926-32.
- 47 Hellman K, Newton KA, Whitmore DN, *et al.* Preliminary clinical assessment of ICRF-159 in acute leukaemia and lymphosarcoma. *BMJ* 1969; **i**: 822-4.
- 48 Herman EH, Mhatre RM, Lee IP, Waravdekar VS. Prevention of the cardiotoxic effects of Adriamycin and daunomycin in the isolated dog heart. *Proc Soc Exp Biol Med* 1972; **140**: 234-49.
- 49 Rajagopalan S, Politi PM, Sinha BK, Myers CE. Adriamycin-induced free radical formation in the perfused rat heart: implications for cardiotoxicity. *Cancer Res* 1988; **48**: 4766-9.
- 50 Sehested M, Jensen PB, Sorensen BS, *et al.* Antagonistic effect of the cardioprotector (+)-1,2-bis (3,5-dioxopiperazinyl-1-yl) propane (ICRF-187) on DNA breaks and cytotoxicity induced by the topoisomerase II directed drugs daunorubicin and etoposide (VP-16). *Biochem Pharmacol* 1993; **46**: 389-93.
- 51 Monti E, Sinha BK. Potentiation of doxorubicin cytotoxicity by (+)-1,2-bis (3,5-dioxopiperazinyl-1-yl) propane (ICRF-187) in human leukemic HL-60 cells. *Cancer Communications* 1990; **2**: 145-9.
- 52 Holcenberg JS, Tutsch KD, Earhart RH, *et al.* Phase I study of ICRF-187 in pediatric cancer patients and comparison of its pharmacokinetics in children and adults. *Cancer Treatment Reports* 1986; **70**: 703-9.
- 53 Speyer JL, Green MD, Kramer E, *et al.* Protective effect of the bispiperazinedione ICRF-187 against doxorubicin-induced cardiac toxicity in women with advanced breast cancer. *N Engl J Med* 1988; **319**: 745-52.
- 54 Speyer JL, Green MD, Zeleniuch-Jacquotte A, *et al.* ICRF-187 permits longer treatment with doxorubicin in women with breast cancer. *J Clin Oncol* 1992; **10**: 117-27.
- 55 Bu'lock FA, Gabriel HM, Oakhill A, *et al.* Cardioprotection by ICRF-187 against high dose anthracycline toxicity in children with malignant disease. *Br Heart J* 1993; **70**: 185-8.