RADIATION RESEARCH 129, 281–289 (1992)

Radiation-Induced Heart Disease: Morphology, Changes in Catecholamine Synthesis and Content, β-Adrenoceptor Density, and Hemodynamic Function in an Experimental Model

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SCHULTZ-HECTOR, S., BÖHM, M., BLÖCHEL, A., DOMINIAK, P., ERDMANN, E., MÜLLER-SCHAUENBURG, W., AND WEBER, A. Radiation-Induced Heart Disease: Morphology, Changes in Catecholamine Synthesis and Content, β-Adrenoceptor Density, and Hemodynamic Function in an Experimental Model. *Radiat. Res.* 129, 281–289 (1992).

To study further the pathophysiology of radiation-induced cardiomyopathy, we investigated resting hemodynamics, myocardial catecholamine synthesis and storage, and β-adrenoceptor density after local heart irradiation. In Wistar rats, a radiation dose of 20 Gy eventually leads to compromised myocardial function which is characterized by a reduction in cardiac output to 43 ± 11% and in the left ventricular ejection fraction to 66 ± 7.5%, and an increase in the left ventricular end-diastolic volume to 187 ± 17% of control values. This reduction in function is correlated with focal degeneration of 23 ± 4% of the myocardium. Measurement of tyrosine hydroxylase activity and catecholamine content revealed that catecholamine biosynthesis is unchanged in the adrenals but is significantly reduced in the hearts of irradiated animals, while cardiac β-adrenoceptor density is increased to about 140% of that in age-matched controls. This is in contrast to findings in dilated or ischemic cardiomyopathy. Time-course studies showed that the development of myocardial degeneration starts simultaneously with the decrease in cardiac output and ejection fraction and the increase in β-adrenoceptors at 50–80 days postirradiation. Myocardial degeneration is maximal in extent and severity at 100 days and does not progress thereafter. Cardiac output decreases at 80–100 days postirradiation to 60 ± 7% of control values. A significant further decrease is seen only when congestive heart failure becomes manifest at 249 ± 21 days after 20 Gy. Thus there is a delay between structural myocardial injury and hemodynamic deterioration which could be due to a compensatory increase in β-adrenoceptor density during the initial stages of the cardiomyopathy. A comparison of two strains of rat shows that survival time correlates with the time course as well as the severity of hemodynamic and morphological changes.

INTRODUCTION

During mantle field irradiation of Hodgkin's disease, part of the heart receives a dose close to the tumor dose of 30–45 Gy (*1*). In retrospective studies carried out 2.5–20 years after megavoltage radiotherapy, a reduced left ventricular ejection fraction at rest was found in 4–29% of the patients, with no evidence of other underlying heart diseases such as coronary artery disease (*2*, *3*). The incidence of a reduced ventricular function is a function of radiation dose as well as of the proportion of myocardium included into the treatment beam (*4*). Following partial heart irradiation with 30 Gy, the left ventricular ejection fraction is reduced in 4% of patients (*2*), while following 40–60 Gy the incidence rises to 33% of patients (*5*). On the other hand, with a constant radiation dose of 32–36 Gy and when the portion of the heart included in the treatment field is increased from less than 50% to more than 75%, the incidence of a low left ventricular ejection fraction rises from 11 to 32% (*3*). To clarify further the pathophysiology and time course of the development of radiation-induced cardiomyopathy, we have chosen two different strains of rat in which radiation-induced myocardial damage was inflicted by local heart irradiation. The radiation dose was chosen to be equivalent to maximum organ doses that are delivered during treatment of human malignant disease, e.g., Hodgkin's disease, breast cancer, or lung cancer.

In previous studies it was observed that local heart irradiation in rats causes chronic congestive heart failure after dose-dependent latent periods (*6*). The rat has proven to be a suitable animal model in studies of dose dependence and dose fractionation (*7*). The aim of the present experiments was to characterize the pathophysiology and morphology of radiation-induced heart disease in this model by a sequential study of hemodynamics and histology. In human heart failure, the decline of cardiac output is accompanied by a compensatory activation of the sympathetic nervous system, leading to increased plasma norepinephrine levels (*8*, *9*) and a decreased cardiac norepinephrine content (*10*, *11*). A number of investigators have reported that this permanent activation of cardiac β-adrenoceptors leads to changes in the myocardial cell membrane and a decrease of β-adrenoceptor density (*12–14*). To determine whether the development of radiation-induced myocardial damage in the rats is accompanied by similar changes in catecholamine content

0033-7587/92 $3.00

or synthesis, or in β-adrenoceptor density, these aforementioned biochemical parameters were investigated.

MATERIALS AND METHODS

Animals and Irradiation

All experiments were carried out in parallel in male Wistar/Neuherberg rats and female Sprague–Dawley/Neuherberg rats. At the age of 5 months the animals were given local heart irradiation with a single dose of 20 Gy of 300-kV X rays, administered at a dose rate of 2 Gy/min through a lateral field. The irradiation technique was devised so as to irradiate the entire heart and at the same time to spare the surrounding radiosensitive organs such as esophagus, spinal cord, and lung. The animals were anesthetized with 120 mg/kg ketamine hydrochloride and were fixed in a supine position. Before treatment a lateral chest X-ray picture was taken on Polaroid film using the treatment machine at a reduced voltage. On the basis of this X ray, the treatment field could be shaped individually by movable lead shields for each animal. Esophagus and spinal cord were excluded from the field, and only 15% of the total lung volume received a radiation dose similar to the heart dose (*6*).

Radionuclide Ventriculography

Cardiac output was determined by recording the first passage of a bolus of technetium-99m-pertechnetate through the heart with a gamma camera. Especially for estimation of the left ventricular ejection fraction, it is important to prevent rapid extravasation of the tracer. *In vivo* labeling of erythrocytes with technetium-99m was achieved by injecting 7.2 mg/kg tin(II)-chloride-2hydrate (SOLCO DTPA, SOLCO, Basel, Switzerland) into the right femoral vein under light ether anesthesia, before each study. Ten minutes later the animals were anesthetized with a mixture of 80.6 mg/kg ketamine hydrochloride (Ketanest, Parke-Davis, Berlin, Germany), 4.4 μg dihydroxylidinothiazine (Rompun, Bayer, Leverkusen, Germany), and after another 10 min the animals were placed in a supine position under the pinhole collimator of the gamma camera (NPS, Philips, Hamburg, Germany). After surgical exposure of the left femoral vein, a bolus of 200 MBq technetium-99m in 0.1 ml was injected. The first passage of the tracer through the heart was recorded by counting 10 frames per second over the heart for the first 15 s, and the steady-state count rate in the same region of interest was then determined by counting one frame per 20 s for 5 min. The matrix size was 64 × 64. From cardiac output measurements by Yeung *et al.* (*15*) it was evident that, especially in animals with pleural effusions or subcutaneous edema, estimation of blood volume as a function of body weight can be misleading. Therefore we determined the total blood volume of each individual animal by measuring the dilution of the injected tracer in two blood samples that were taken from the tip of the tail during the steady-state period. Cardiac output is expressed as milliliters per second.

The left ventricular ejection fraction was then estimated in an ECG-gated study that was carried out after each first-pass study. The ECG was recorded by metal electrodes clipped around both forepaws and one hindpaw, the signal was amplified, and R-waves were transformed into a TTL signal that was transmitted to the gamma camera as a trigger signal. Each R-wave initiated registration of a series of 20 frames, the duration of which was 1/20 of an average R–R interval of the rat studied. By adding up the corresponding frames of 600 consecutive cardiac cycles, the contraction and filling of cardiac chambers during the course of one representative cardiac cycle was visualized. Since the interventricular septum in rats is approximately parallel to the sagittal body axis, it is possible to discern the left and right ventricle (Fig. 1). The outline of the left ventricle was defined according to Hutton *et al.* (*16*) in different phases of the cardiac cycle. A time–activity curve over the left ventricle, corresponding to changes in myocardial chamber volume, was then calculated and allowed the estimation of the left ventricular ejection fraction. Each ECG-gated study was analyzed three times. Since arrhythmias, especially AV blocks, occur frequently in both untreated and irradiated rats, a correction was made by excluding from analysis each cardiac cycle that followed a cycle with a duration differing by more than 80 ms from the average R–R period of the individual rat.

The time course of hemodynamic changes was studied in each strain of rat in two parallel cohorts comprising a total of 12 irradiated and 8 age-matched control animals, so that each individual was examined at every second time point. From measurements of heart rate, cardiac output, and left ventricular ejection fraction, stroke volume and end-diastolic left ventricular volume were derived.

Catecholamine Biosynthesis and Content

Tyrosine hydroxylase is the rate-limiting enzyme of catecholamine biosynthesis (*17*). For measurement of tyrosine hydroxylase activity the method of Nagatsu *et al.* (*18*) was modified slightly. The ventricles and adrenal glands of seven irradiated Wistar rats, 250 days postirradiation, and of seven age-matched controls were removed and homogenized. After centrifugation the supernatant was incubated for 20 min at 37°C with 0.2 m*M* L-tyrosine in incubation buffer (0.2 *M* Na-acetate buffer, pH 6.0, containing 100 m*M* mercaptoethanol and 1 m*M* methyltetrahydropterin as cofactor). The reaction was terminated by adding ice-cold dihydroxybenzylamine. Tyrosine hydroxylase activity is represented as synthesized L-DOPA per one gram tissue per one hour. Analysis of dopamine, noradrenaline and adrenaline was performed with Macherey–Nagel columns packed with Nucleosil 5-C18 (Macherey–Nagel, Düren, Germany), using a high-performance liquid chromatography system (HPLC, F. Waters, Eschborn, Germany) and an electrochemical detector (M 460, F. Waters, Eschborn, Germany), as described elsewhere (*19*).

Beta-Adrenoceptor Binding Studies

Adrenoceptor binding studies in Wistar rats have been reported earlier (*20*). In the present study a different radioligand with a much higher receptor affinity was used. The left ventricles were dissected and stored in liquid nitrogen. Only macroscopically intact tissue was used for membrane preparations. For analysis the ventricles were thawed on ice, weighed, and minced with scissors. After disruption of the tissue with an Ultra-Turrax (Jahnke and Kunkel, Staufen, Breisgau, Germany), the suspension was further homogenized by hand with a glass–glass homogenizer. The homogenate was then centrifuged for 10 min at 484*g*. The pellet was discarded while the supernatant was filtered, incubated for 10 min with an equal volume of ice-cold 1 *M* KCl, and finally centrifuged for 45 min at 100,000*g*. The pellet was resuspended in 12 ml buffer (1 *M* Tris with 0.25 *M* EDTA and 0.25 *M* dithiothreitol) and centrifuged again for 30 min at 100,000*g*. This final pellet was resuspended in 12 ml incubation buffer (0.05 *M* Tris with 0.01 M $MgCl_2$) and homogenized for 1 min with a glass–glass homogenizer. Aliquots of 250 μl of the membrane suspension were then incubated at 37°C for 1 h with the radiolabeled ligand [^{125}I]iodocyanopindolol ([^{125}I]Cyp) at concentrations ranging from 0.01 to 0.4 n*M*. The reaction was terminated by vacuum filtration of the incubation medium through Whatman GF/C filters. Filters were rinsed three times with ice-cold incubation buffer and then dried at 90°C. Radioactivity was measured in a scintillation counter. All experiments were carried out in triplicate. Protein content was determined according to Lowry *et al.*

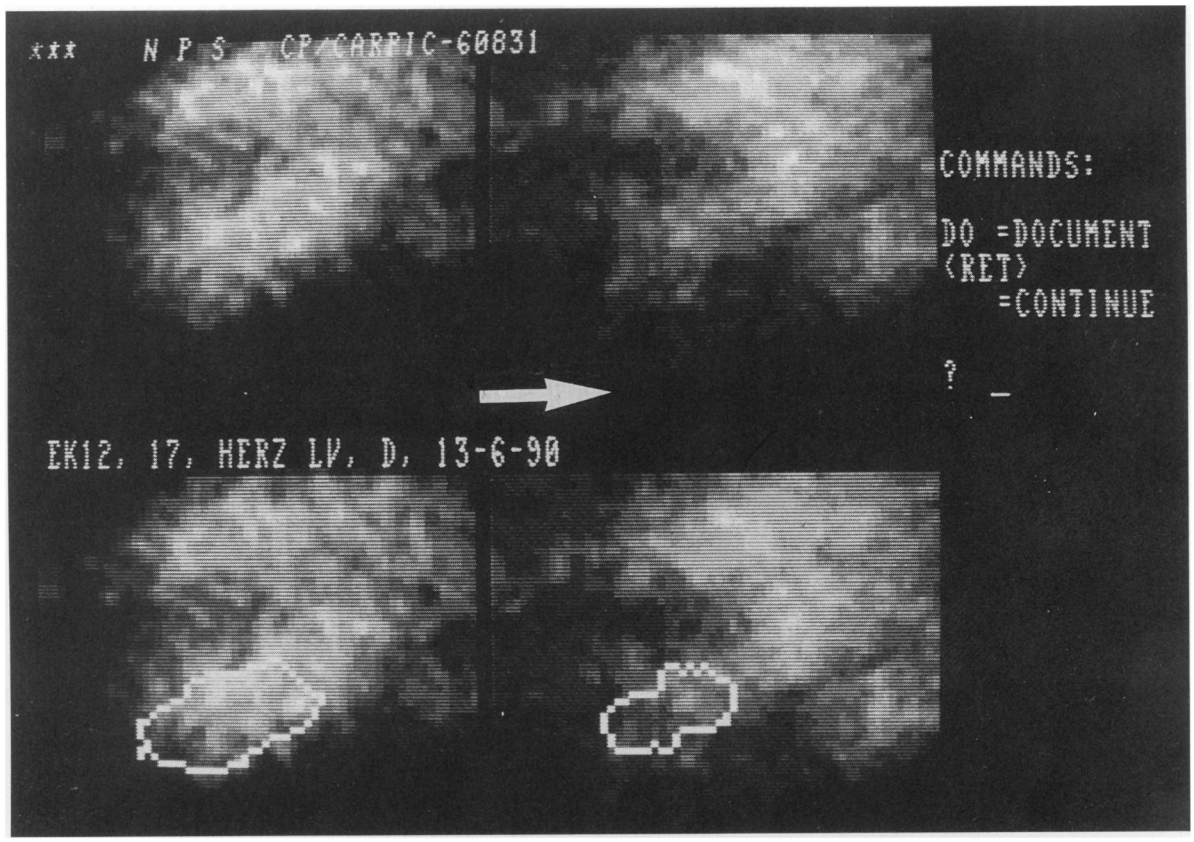

FIG. 1. Cardiac chambers of a control rat in end-diastole (left) and systole (right). The image was generated by superimposing 600 corresponding frames in an ECG-triggered steady state study. In the lower panels the outline of the left ventricle is marked. The arrow points cranially.

(*21*). Specific binding was determined by subtracting binding in the presence and absence of 3 μM (−)-propranolol. The density (B_{max}) and apparent affinity (K_D) of binding sites were obtained in individual experiments from Scatchard plots determined by linear regression analysis. For β-adrenoceptor binding studies each irradiated animal was housed in one cage with an age-matched control and β-adrenoceptor binding experiments were carried out simultaneously. For each time point six to eight pairs of animals were analyzed.

Histology and Morphometry

Animals were sacrificed immediately after the last radionuclide ventriculography for direct comparison of morphological and hemodynamic changes in each individual. The preparation of the tissue and the morphometry technique were identical to earlier studies, in which the time course of morphological changes was described (*22*). Briefly, the hearts were perfusion-fixed with phosphate-buffered Formalin–alcohol. After excision, four transverse slices of 0.5 mm thickness were taken at equal distances through the atria and ventricles and were embedded in glycol methacrylate (Technovit 7100, Kulzer, Friedrichsdorf, Germany) (*22*). Histological sections 3 μm thick were stained with hematoxylin–eosin and projected on paper at a magnification of ×20, and their outlines were traced. Examining the sections under a light microscope, areas of ventricular myocardial degeneration and necrosis were marked in the drawings and their extent relative to the total section area was determined using a semiautomatic image-analysis system (MOP, Kontron, Eching, Germany).

Data Analysis

If not mentioned otherwise, results are expressed throughout the text and figures as arithmetic mean ± standard deviation, derived by analysis of variance according to Wallenstein *et al.* (*23*). Groups of animals were compared by the *t* test.

RESULTS

Radionuclide Ventriculography

Radionuclide ventriculography was found to be a useful noninvasive method for a long-term longitudinal study of hemodynamics in rats. Sequential measurements in control groups showed a consistent time course with increasing age and relatively little interindividual variability of values. Heart rates during measurement were 250 ± 7 min^{-1} in Wistar rats and 206 ± 6 min^{-1} in Sprague–Dawley rats. In comparison to previous measurements in unanesthetized Wistar rats of 395 ± 5 min^{-1} (*24*) these values are clearly reduced due to general anesthesia. This may have influenced absolute values of hemodynamic parameters. However, great care was taken to assure the precise dose

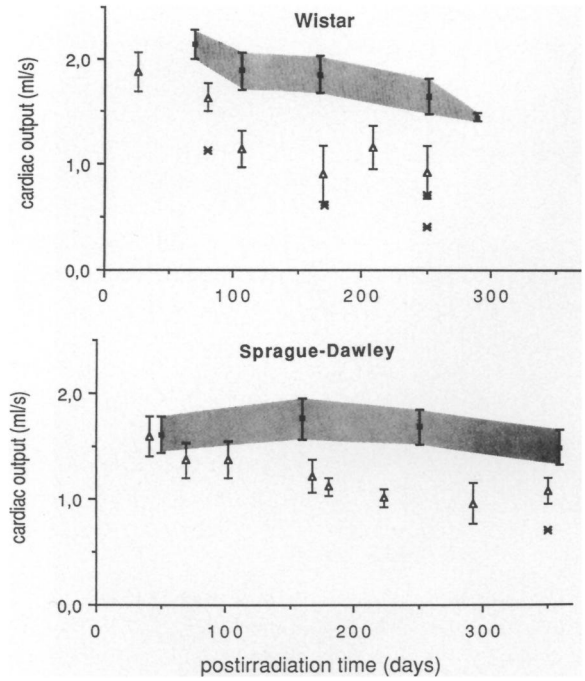

FIG. 2. Sequential changes in cardiac output in untreated control animals (■) and in animals that were irradiated with 20 Gy to the heart (△). Stars represent single measurements in animals with manifest heart failure, while other symbols stand for means ± standard deviations in groups of three to six animals.

and timing of anesthesia to allow comparison of irradiated and unirradiated rats.

Following heart irradiation both strains of rat showed a decrease in cardiac output relative to control values (Fig. 2). In Wistar rats this decrease was obvious at 80 days postirradiation ($P = 0.007$). The difference from control levels, however, did not increase significantly during the course of the study. When symptoms of heart failure, such as dyspnea at rest or subcutaneous edema, were obvious at the time of examination, the cardiac output was close to or below 1 ml/s and the animals had to be sacrificed shortly after radionuclide ventriculography. Since hemodynamic parameters in these animals were significantly lower than for asymptomatic irradiated animals, they are marked out separately in Figs. 2–4. One animal had to be sacrificed with manifest congestive heart failure at 242 days postirradiation, in the interval between two measurements. Therefore, later times do not represent an average of the whole treatment group, but a selection of more resistant animals. In Sprague–Dawley rats, cardiac output in irradiated rats was reduced below control levels at 180 days postirradiation ($P = 0.001$) and did not change significantly thereafter (Fig. 2). In this group, two animals developed heart failure in the interval between measurements, between 300 and 350 days. Only one animal, for which the data are plotted separately in Figs. 3–5, showed signs of a beginning subcutaneous edema when it was examined at 340 days postirradiation. Stroke volume showed a radiation-induced decrease that was equivalent to the decrease in cardiac output in both strains, while heart rates measured in irradiated animals did not differ significantly from those of untreated control animals.

In the ECG-triggered study, gross changes in the motility of the left ventricle were not observed in irradiated or control animals. In Wistar rats, the left ventricular ejection fraction was decreased below control values from 80 days postirradiation onward, and very low values of around 60% were estimated in animals showing clinical symptoms of heart failure. Again, follow-up of individual animals indicated a progressive decrease. In irradiated Sprague–Dawley rats, the left ventricular ejection fraction was not different from control values throughout the observation time, except for the one animal developing congestive heart failure at 340 days.

The end-diastolic left ventricular volume was very different in irradiated animals presenting with and without subcutaneous edema or dyspnea at rest (Fig. 4). In asymptomatic Wistar rats, the values appeared to drop slightly below control levels at around 100 days postirradiation, but then increased and were significantly higher than in controls at 250

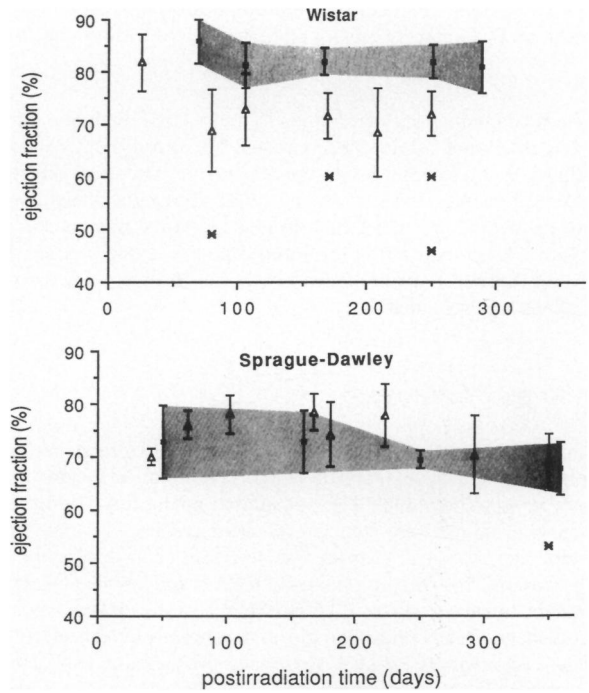

FIG. 3. Sequential changes in left ventricular ejection fraction in untreated control animals (■) and in animals that were irradiated with 20 Gy to the heart (△). Stars represent single measurements in animals with manifest heart failure, while other symbols stand for means ± standard deviations in groups of three to six animals.

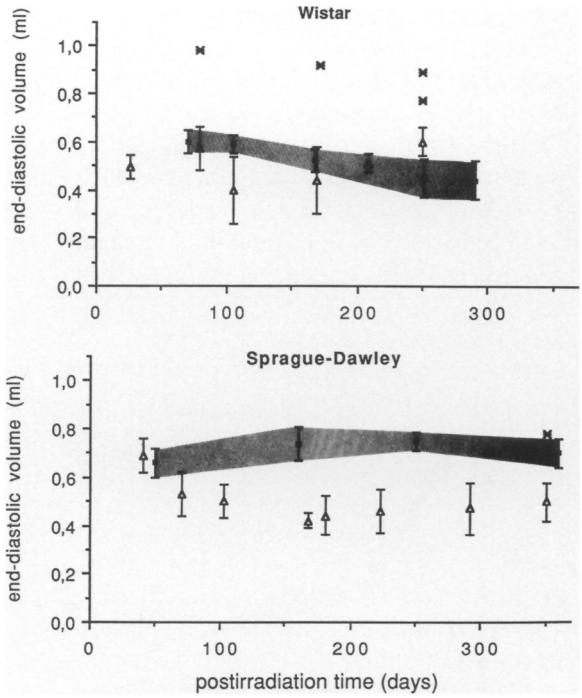

FIG. 4. Sequential changes in left ventricular end-diastolic volume in untreated control animals (■) and in animals that were irradiated with 20 Gy to the heart (△). Stars represent single measurements in animals with manifest heart failure, while other symbols stand for means ± standard deviations in groups of three to six animals.

days. All four animals with manifest congestive heart failure had a markedly increased end-diastolic volume, consistent with a cardiac dilatation. In Sprague–Dawley rats, the left ventricular end-diastolic volume was decreased below control levels from 103 days onward. An exception was one animal that showed dyspnea at rest and incipient subcutaneous edema, and an end-diastolic volume of the left ventricle that was markedly higher than in the rest of the treatment group and slightly higher than in controls.

Survival, Clinical Symptoms and Autopsy Findings

Except for animals with subcutaneous edema, body weights of irradiated animals did not differ from control values at any time. Within the study period of 250 days, 5 of 12 Wistar rats developed heart failure, while only 2 of 12 Sprague–Dawley rats had to be sacrificed within 350 days. Clinical signs of heart failure developed gradually; at first, dyspnea occurred only at exertion, while later the spontaneous activity of the animal appeared to deteriorate. When severe apathy and dyspnea at rest became manifest, 4 of the 6 animals also showed subcutaneous edema of legs and face. At autopsy the liver was congested, weighing 6.2 ± 0.6% of the total body weight, compared to 4.5 ± 0.5% in age-matched controls. When the thorax was opened pleural effusions of 5–15 ml were recovered. The macroscopical appearance of the heart was normal, but the weight of the left ventricle was decreased at 127 and 277 days postirradiation in Wistar rats to 91 ± 7% and 84 ± 7% of control levels, respectively. In Sprague–Dawley rats only animals sacrificed at 245 days postirradiation showed a significant left ventricular atrophy to 91 ± 10%. Accordingly, the thickness of the left ventricular wall of Wistar rats was decreased in comparison to age-matched controls at 250 days postirradiation from 2.7 ± 0.1 mm to 2.1 ± 0.14 mm ($P < 0.0001$). In Sprague–Dawley rats a significant modification of left ventricular geometrical proportions was present only at 350 days postirradiation. At that time the wall thickness was also reduced (2.2 ± 0.18 mm compared to 2.6 ± 0.1 mm, $P = 0.05$). In the ejection phase, the left ventricle builds up pressure which is counterbalanced by wall stress. The exact relationship between intraventricular pressure and circumferential wall stress is determined largely by ventricular geometry. Gülch and Jakob (*25*) demonstrated that left ventricular mechanics can be reasonably approximated by a thick-walled sphere. In this model, the relationship between circumferential mean wall stress (S) and transmural pressure (P) is dependent on the inner ventricular radius (r) and the wall thickness (h), and can be defined as

$$S = P\frac{r^2}{h(2r + h)}.$$

Calculated according to this model, the geometrical factor relating circumferential wall stress to transmural pressure (i.e., $r^2/h(2r + h)$) showed a radiation-induced increase from 0.32 to 0.71 in Wistar rats and from 0.37 to 0.61 in Sprague–Dawley rats. Thus the relatively small decrease in wall thickness represents a mechanically unfavorable situation, imposing a twice-increased wall stress on the already-damaged myocardium.

Catecholamine Biosynthesis and Storage

In the hearts of irradiated Wistar rats, the tyrosine hydroxylase activity and consequently the norepinephrine and epinephrine contents of the heart were reduced significantly at 250 days postirradiation (Fig. 5). However, catecholamine biosynthesis and storage of the adrenal medulla did not change significantly following heart irradiation (Fig. 5).

Beta-Adrenoceptor Binding Studies

In untreated Wistar rats the maximal number of binding sites B_{max} and the antagonist affinity K_D were constant over the time studied. The means from all experiments were 29.3 ± 1.5 fmol/mg protein and 98.7 pmol/liter (95% Cl = 82–105 pmol/liter), respectively. In Sprague–Dawley rats the B_{max} decreased from 43.9 ± 1.8 fmol/mg protein at the

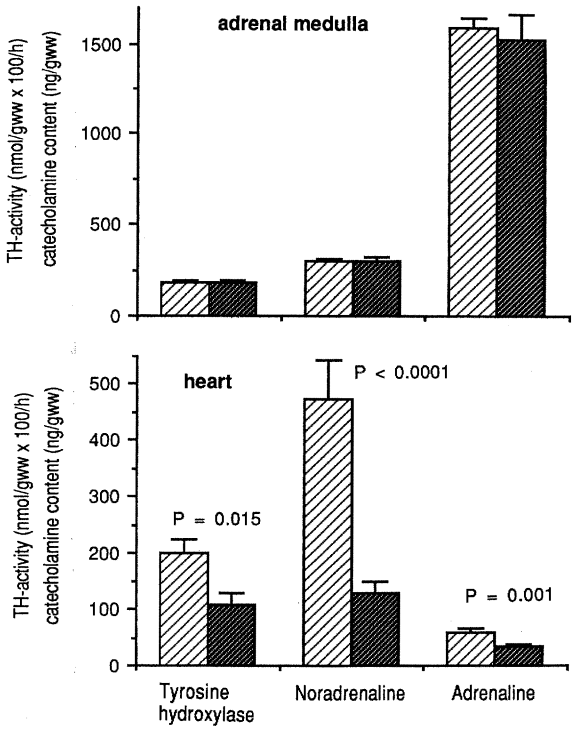

FIG. 5. Tyrosine hydroxylase activity, expressed as nmol/g wet wt × 100/h, as well as norepinephrine and epinephrine content (ng/g wet wt) in the adrenal medulla and the left ventricle in untreated Wistar controls (▨) and at 250 days after local heart irradiation with 20 Gy (■).

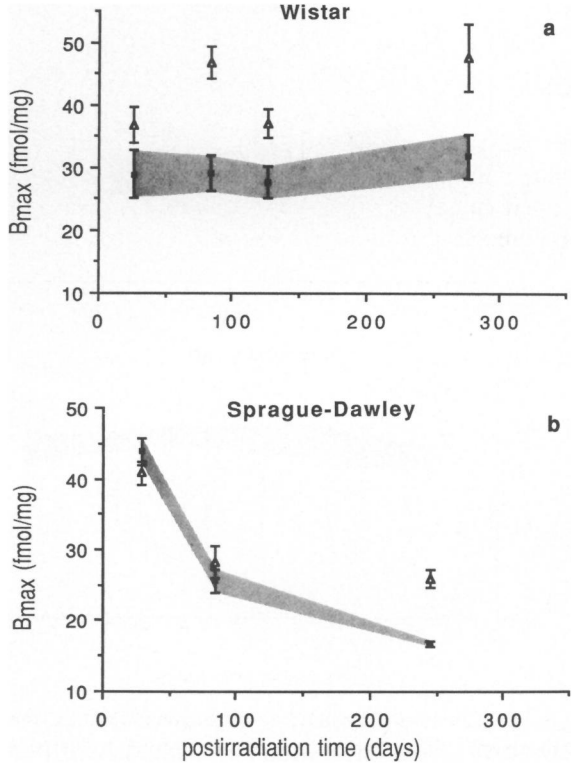

FIG. 6. Sequential changes in β-adrenergic receptor concentration in untreated control animals (■) and in animals that were irradiated with 20 Gy to the heart (△). Symbols stand for means ± SEM in groups of six to eight animals.

age of 180 days to 16.7 ± 0.3 fmol/mg protein at an age of approximately 400 days. K_D did not change systematically with age and the average from all experiments was 95 pmol/liter (95% Cl = 79–103 pmol/liter).

Following irradiation, the β-adrenoceptor density increased above the levels in respective controls in both strains of rat (Fig. 6). This increase was statistically significant by 84 days postirradiation ($P = 0.001$) in Wistar rats and by 127 days ($P = 0.01$) in Sprague–Dawley rats. The antagonist affinities as judged from the K_D values were not significantly different in irradiated and control animals.

Histology

Histological examination of irradiated hearts revealed a subepicardial edema and a swelling of mesothelial cells, but significant epicardial fibrosis or pericardial adhesions were not seen. The myocardium showed a severe focal degeneration and necrosis with severe vacuolization in the periphery of foci and an empty network of sarcolemma in the center. There was no increase in interstitial connective tissue, and the lesions did not show any demarcation reaction or inflammatory infiltration. Foci of degeneration occurred all over the myocardium without a clear pattern of distribution or preferential localization (*6*, *22*). There was no clear correlation between any of the parameters of cardiac function and the extent of myocardial degeneration in individual animals. At the end of the study, myocardial degeneration encompassed about 23 ± 4% of the myocardium in Wistar rats and 13 ± 3% in Sprague–Dawley rats. In Fig. 7

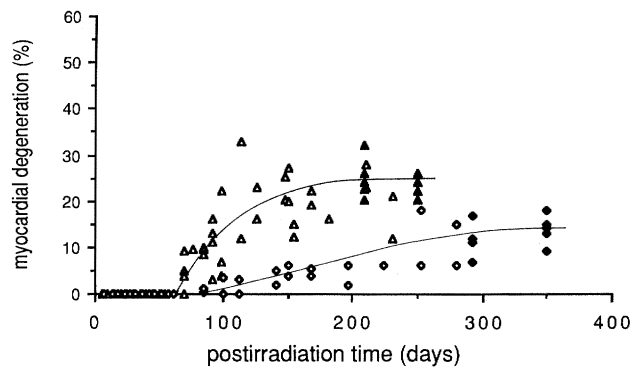

FIG. 7. Changes in the relative extent of myocardial degeneration in Wistar (△ and ▲) and Sprague–Dawley rats (◇ and ◆) after local heart irradiation with 20 Gy. Each symbol stands for an individual animal. Data from earlier experiments (*21*) are represented by open symbols, whereas the present experiment is denoted by closed symbols.

data from earlier studies are included to compare the time course of development of hemodynamic and morphological changes. Myocardial degeneration became evident at about 70 days after 20 Gy in Wistar rats and at about 100 days in Sprague–Dawley rats (Fig. 7). In Wistar rats, the maximal extent of degeneration was reached at about 100 days, while in Sprague–Dawley rats a plateau was not evident before 290 days postirradiation.

DISCUSSION

Partial heart irradiation with 40–60 Gy during radiotherapy of Hodgkin's disease causes a reduction in left ventricular ejection fraction in about 33% of patients (*5*). To characterize myocardial alterations in radiation-induced heart disease experimentally, the entire heart was irradiated with a single dose of 20 Gy. According to fractionation studies in the rat heart (*7*), this can be regarded as equivalent to a clinical treatment schedule delivering a total dose of 70 Gy in 2-Gy fractions. Previous experiments had shown that 20 Gy eventually led to radiation-induced heart failure in all treated animals after 249 ± 21 days in Wistar and 446 ± 15 days in Sprague–Dawley rats (*26*). Thus a higher level of damage than observed clinically was chosen, in order to induce measurable changes in all animals studied.

Sequential studies in the present experiments showed that the latent period of onset of radiation-induced changes was very similar for morphological, hemodynamic, and biochemical parameters. In Wistar rats, myocardial degeneration, reduction in cardiac output, and increase in β-adrenoceptor density started to develop simultaneously, at 70–84 days after 20 Gy. Focal myocardial degeneration progressed rapidly and reached a maximum at 100 days. Cardiac output and left ventricular ejection fraction remained stable at a level of 60 ± 7% of the control values until congestive heart failure developed in the individual, at 249 ± 21 days postirradiation, and then dropped to 43 ± 11% of control values. At this stage the end-diastolic left ventricular volume was increased markedly to 187 ± 16% of control levels. The endogenous cardiac catecholamine biosynthesis in these hearts was reduced, while adrenal catecholamine metabolism was unchanged. The increase in β-adrenoceptor density was maintained throughout the observation period. It is important to note that, in comparison to earlier studies (*20*), the increase in β-adrenoceptors was reproducible in two completely separate experiments, using radioligands of very different affinity.

One interesting finding is that hemodynamic deterioration occurred with a delay of at least 100 days after the full expression of structural myocardial damage. This indicates that myocardial cell damage might be compensated for in the initial state of cardiomyopathy. In animals with radiation-induced myocardial damage, we have observed an increase in myocardial β-adrenoceptors of about 40%. It is not unreasonable to assume that this compensatory increase leads to an enhanced positive inotropic response of the remaining intact cardiomyocytes to β-adrenergic stimulation, and thus contributes to maintaining cardiac function. In light of these findings it is noteworthy that, in patients with compromised myocardial function due to dilated cardiomyopathy, a significant hemodynamic and clinical improvement has been achieved by inducing an increase in β-adrenoceptors by administration of β blockers (*27*, *28*). Hence the clinical observations in patients with cardiomyopathy and the experimental findings reported here offer the interesting hypothesis that the up-regulation of myocardial β-adrenoceptors could be hemodynamically relevant in radiation-induced heart disease. This hypothesis is supported by studies *ex vivo* in isolated working heart preparations of previously irradiated rat hearts. At 2 months after local heart irradiation with 15 or 20 Gy, there was a reduction in stroke volume as well as a modified response of stroke volume on elevated left atrial filling pressures, indicating a loss of myocardial contractility. Experiments at 4 and 6 months postirradiation showed a continuous and steep further decrease in both contractility and stroke volume (*29*). The absence of intermittent adrenergic stimulation by circulating catecholamines could explain why myocardial performance in the denervated working heart preparation *in vitro* deteriorates much more rapidly than in the present *in vivo* study. These experiments illustrate the possible role of increased adrenoceptor density in compensating hemodynamic function *in vivo*.

In Sprague–Dawley rats the onset of functional changes, as well as of myocardial degeneration, is significantly delayed in comparison to Wistar rats. Myocardial degeneration is not evident before 100 days postirradiation. From around 170 days onward, cardiac output is reduced, while the left ventricular ejection fraction remains normal. This is in good agreement with the significantly longer survival time of Sprague–Dawley rats of 446 ± 15 days after 20 Gy, compared to 249 ± 21 days in Wistar rats (*26*), and also with a lesser extent of myocardial degeneration in Sprague–Dawley rats. In contrast to Wistar rats, Sprague–Dawley rats showed a pronounced age-related decrease in β-adrenoceptor concentration in untreated control animals. While it is well known that myocardial response to catecholamine decreases with age (*30*, *31*), the reasons for this phenomenon are unclear. A reduction in β-adrenoceptor density is one possible explanation, although experimental observations are conflicting (*32*, *33*). The earliest receptor binding studies in the present experiments were carried out 26 days postirradiation. However, extrapolating the measurements in control animals strongly indicates that at the time of irradiation β-adrenoceptor density was higher in Sprague–Dawley than in Wistar rats. The present knowledge of the

pathogenesis of radiation-induced heart disease does not allow us to speculate if this could have any influence on the delayed radiation response in Sprague–Dawley rats.

In chronic heart failure unrelated to heart irradiation, reduction of cardiac output induces a sustained activation of the sympathetic nerve system (*8*, *9*) and a consecutive down-regulation of cardiac adrenoceptors (*12–14*). In contrast, the adrenal catecholamine synthesis was unchanged following heart irradiation while cardiac catecholamine synthesis and content were reduced and β-adrenoceptor density was increased. Thus pathophysiology of radiation-induced heart failure appears to be fundamentally different from heart failure due to other causes. Therefore it is not unreasonable to suggest that patients whose hearts have been irradiated in the course of tumor therapy undergo an up-regulation of β-adrenoceptors, leading to a facilitated inotropic stimulation of the heart by the sympathetic nervous system and hence to a compensatory maintenance of hemodynamic function. In that case, clinical assessment of radiation effects in the heart in terms of the incidence and degree of reduction in the left ventricular ejection fraction would lead to a significant underestimation of the actual severity of myocardial damage, i.e., of the susceptibility to cardiac stress or injury in these patients.

ACKNOWLEDGMENTS

The authors thank Mrs. S. Möllenstedt for her excellent assistance and help in performing radionuclide ventriculographies. The study was supported by Philips, Hamburg, Germany and Solco, Basel, Switzerland. Radioligand binding studies were supported by the Deutsche Forschungsgemeinschaft, Grant Bo 896/1-2.

RECEIVED: October 2, 1990; ACCEPTED: August 13, 1991

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