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Effects of *in Vivo* Heart Irradiation on Myocardial Energy Metabolism in Rats

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To investigate the effect of *in vivo* heart irradiation on myocardial energy metabolism, we measured myocardial adenosine nucleotide concentrations and mitochondrial oxygen consumption in left ventricular tissue of rats 0–16 months after local heart irradiation (20 Gy). At 24 h and 2 months no difference in myocardial adenosine nucleotide concentration was apparent between irradiated and control hearts. The total myocardial adenosine nucleotide concentrations in irradiated hearts compared to those of nonirradiated controls tended to be lower from 4 months onward. The rate of oxidative energy production (state 3 respiration) in irradiated hearts was significantly reduced compared with that of age-matched controls from 2 months onward. Moreover, as a result of aging, a time-dependent decrease in the rate of oxidative energy production was observed in both irradiated and control hearts ($P < 0.001$). The respiratory control index (RCI = oxygen consumption in state 3/oxygen consumption in state 4) in irradiated hearts was not different from the RCI measured in age-matched control animals. During the period of study the RCI diminished significantly with age in both groups ($P < 0.005$). The number of oxygen atoms used per molecule of ADP phosphorylated (P/O ratio) was not influenced by the irradiation. The P/O ratio for the NAD^+-linked substrates remained unchanged at a value of about 3 during the period studied. At 6 months after irradiation activities of myocardial enzymes such as lactate dehydrogenase, creatine kinase, citrate synthase, and cytochrome *c* oxidase were reduced. The reduction in myocardial energy production and the changes in energy supplies provide a mechanism to explain impaired contractility after local heart irradiation.

INTRODUCTION

Clinical studies have demonstrated that patients treated for Hodgkin's disease frequently develop cardiac complications long after radiotherapy of the mediastinum has been applied (*1–3*). Radiation-induced cardiac damage may lead to functional impairment and is associated with a variety of morphological changes (*4–6*).

Impairment of cardiac function after local heart irradiation has been demonstrated in experimental studies both *in vivo* (*7–9*) and *in vitro* using the isolated working heart preparation (*10, 11*). Experiments in our laboratory with the isolated working heart preparation revealed depressed left ventricular stroke volumes and deterioration of *in vitro* pump function of the heart after irradiation with doses ranging from 15 to 30 Gy (*11*).

By 2 months after irradiation with a single dose of 15 or 20 Gy, changes in the function of the hearts of rats were observed, but there was no sign of histopathological changes (*11*). Therefore, we hypothesized that biochemical changes in the myocardium are responsible for radiation-induced impairment of contractile function.

The energy for cardiac contraction is supplied by ATP, which is produced in the mitochondria by a process of oxidative phosphorylation. The energy required for the phosphorylation of ADP to ATP is liberated through electron transfer from substrate molecules to oxygen. Oxidation of NAD^+-linked substrates (e.g., β-hydroxybutyrate) yields three molecules of ATP per atom of oxygen (P/O = 3). Radiation might lead to impairment of mitochondrial oxidative phosphorylation resulting in depressed ATP synthesis [see review in Ref. (*12*)], and as a consequence in loss of contractile function of the heart [see review in Ref. (*13*)].

The pathogenesis of several heart diseases has been shown to be associated with alterations in mitochondrial function and diminished enzyme activities leading to impaired contractility (*13–15*). Reductions of myocardial lactate dehydrogenase and creatine kinase activities are often used as an indicator for heart damage, i.e., necrosis and atrophy.

In the present paper we report on the adenosine nucleotide concentrations, mitochondrial ATP production, and several enzyme activities in the rat myocardium after whole-heart irradiation of rats with a single dose of 20 Gy.

MATERIALS AND METHODS

Animals

Female Sprague–Dawley rats (obtained from IFFA/CREDO Broekman, Someren, The Netherlands) 12–14 weeks old and weighing approximately

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250 g were used. Fifteen minutes before irradiation animals were anesthetized using Ketamine (intramuscular, 40 mg/kg body wt; Aesculaap, Boxtel, The Netherlands) and Rompun (subcutaneous, 1.6 μg/kg body wt; Bayer, Leverkusen, Germany). After irradiation the rats were housed three to a cage and received food pellets and drinking water *ad libitum*. At 24 h and 2, 4, 6, 8, 12, and 16 months after treatment irradiated animals and age-matched controls were sacrificed and used for the experiments.

The experiments were conducted with the permission of the animal welfare committee of the University of Leiden (UDEC) as required by Dutch law.

Irradiation

A single dose of 20 Gy was given to the heart. The details of the irradiation technique have been described (*11*). Briefly, irradiation was performed with a Philips RT250 X-ray generator (Eindhoven, The Netherlands) operated at 250 kV and 15 mA. X rays were filtered with a 0.4-mm thoraeus filter resulting in an HVL of 2.5 mm copper. The dose rate was 1.95 Gy/min. The rats were irradiated individually using parallel opposed fields (anterior–posterior 1:1). During irradiation the thorax of the rat was shielded with two 4-mm-thick lead plates with a hole of 19 mm diameter located precisely over the heart.

Surgical Procedure

After weighing, the rats were anesthetized with diethylether. The thorax was opened and a piece of about 50 mg of left ventricle was removed as quickly as possible (within 5 s) using a precooled (in liquid nitrogen) clamp. This piece, always from the same anatomical site, was lyophilized overnight and stored at −70°C. The rest of the heart was removed rapidly from the animal and placed in ice-cold buffer (*16*). Transmural blocks of the left ventricular wall were cut free, weighed, and homogenized.

Determination of the Adenosine Nucleotide Concentration

About 10 mg of freeze-dried heart tissue was homogenized using a Heidolph pestle homogenizer at 2800 rpm in 1 ml PCA/DTE (3 *M* perchloric acid/5 m*M* dithioerythritol) at −12°C. During the complete procedure, samples were kept on ice. After the tissue had been dissolved, 5 ml of ice-cold H_2O was added and mixed. Then 5 ml of the solution was transferred and centrifuged for 3 min at 10,000*g*. Subsequently, 4 ml of the supernatant was transferred to a second tube and $KHCO_3$ was added (±200 mg) until the pH was 7.5. Finally the sample was centrifuged for 3 min at 10,000*g*. Then the supernatant was filtered (0.20 μm) and 20 μl was injected into the HPLC system (Applied Biosystems) for determination of the adenosine nucleotides according to the method of Wynants and Van Belle (*17*).

Evaluation of Oxidative Energy Production

Fresh tissue homogenates were prepared according to the method of Starnes *et al.* (*18*). Briefly, myocardial samples were homogenized using a Heidolph potter homogenizer at a concentration of 100 mg of tissue per 1 ml ice-cold KEA buffer [180 m*M* KCl, 10 m*M* EDTA (ethylenediaminetetraacetic acid) and 0.5% bovine serum albumin, pH 7.4]. Aliquots of 200 μl were used to measure the mitochondrial function parameters. Oxygen consumption was measured polarographically (*19*) in a thermostatted (at 25°C) glass oxygraph with a Clark oxygen electrode (Yellow Springs Instruments, Inc., Yellow Springs, OH). The signal was registered on a chart recorder (Salm & Kipp, Breukelen, The Netherlands). The concentration of dissolved oxygen in air-equilibrated water at 25°C was assumed to be 250 μ*M*. In the oxygraph 200 μl of homogenate was mixed with 1.5 ml of a solution containing 250 m*M* sucrose, 10 m*M* Tris–HCl, 10 m*M* K_2HPO_4, and 10 m*M* β-hydroxybutyrate (pH 7.4). In separate experiments 20 μl of one of the following substrates was added to the reaction mixture instead of

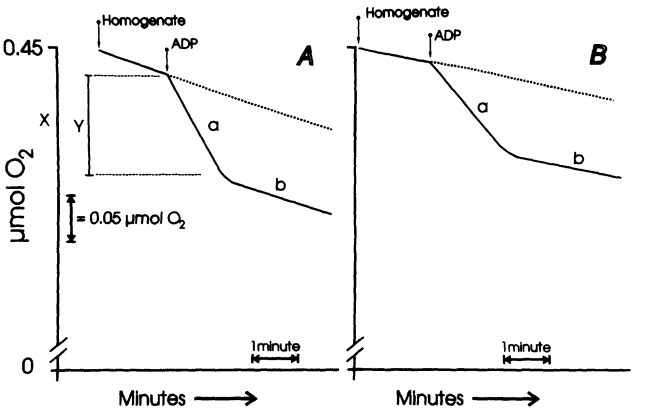

FIG. 1. Schematic representation of the polarographic registration of oxygen consumption in a myocardial homogenate 6 months after treatment of a control heart (A) and an irradiated heart (B). Note that slopes a and b in (A) are steeper than those in (B). This indicates that the rate of state 3 and state 4 respiration in the control heart is faster than that in the irradiated heart. The rate of oxygen consumption in the presence of ADP (slope a) in (A) is 26.1 μmol/min/g protein compared to 20.0 μmol/min/g protein in (B). The P/O ratio is calculated as follows: μmol of ADP added (0.56 in our experiments)/μatoms oxygen used ($Y/X \times$ the number of oxygen atoms in the medium). The P/O in (A) is 2.8, compared to 3.1 in (B). RCI (respiratory control index) is the ratio of the respiratory rate in the presence of ADP (slope a) and the respiratory rate after ADP has been phosphorylated (slope b). The RCI in (A) is 6.0, compared to 6.5 in (B).

β-hydroxybutyrate: 1 *M* succinate, 1 *M* pyruvate, or 1 *M* glutamate + 100 m*M* malate. By adding 100 μl of ADP solution (5.6 m*M*) state 3 respiration was initiated.

The following parameters of oxidative phosphorylation were obtained: the rate of mitochondrial oxygen consumption in the presence of ADP (state 3, which is the active respiration) and after ADP has been used (state 4, which is the resting respiration); P/O ratio (number of molecules of ADP phosphorylated to ATP per atom of oxygen consumed); Respiratory Control Index (RCI, which equals state 3 respiratory rate/state 4 respiratory rate). The P/O is a quantitative measurement of the degree of coupling of oxidation to phosphorylation whereas the RCI is a qualitative measurement of the integrity of the mitochondria. Curves for oxygen consumption using β-hydroxybutyrate as a substrate are shown in Fig. 1. Oxygen consumption is expressed as μmol oxygen/min/g of tissue protein, determined according to Bradford (*20*).

Enzyme Activities

In a separate experiment aliquots of the homogenates prepared at 6 months after treatment were used to measure enzyme activities. Mitochondrial citrate synthase (*21*), mitochondrial and cytoplasmic creatine kinase (Boehringer testkit), and cytoplasmic lactate dehydrogenase (Boehringer testkit) were measured spectrophotometrically. Cytochrome *c* oxidase was measured polarographically (*22*). Enzyme activities were related to protein.

Statistical Analysis

The data presented are expressed as means ± SEM. Statistical differences between groups were calculated by analysis of variance (ANOVA) according to Wallenstein *et al.* (*23*) using SPSS software (SPSS-PC+, SPSS Ltd., Chicago, IL). Comparisons between groups were made groupwise and per time. Also, the effect of time on the measurements within a group,

TABLE I
Concentration of Adenosine Nucleotides (μmol/g Dry Weight) in Normal and 20-Gy-Irradiated Rat Hearts

Time after treatment	ATP	ADP	AMP	Sum[a]	n[b]
Controls					
24 h	10.7 ± 1.4[c]	8.6 ± 0.6	4.3 ± 0.5	23.6 ± 1.5	9
2 months	9.0 ± 0.7	10.3 ± 0.8	8.4 ± 1.3	27.6 ± 1.2	10
4 months	16.7 ± 1.0	7.0 ± 0.4	2.1 ± 0.4	25.9 ± 0.6	7
6 months	13.2 ± 1.4	6.9 ± 0.8	2.5 ± 0.3	22.6 ± 1.8	9
8 months	10.9 ± 0.9	9.2 ± 0.6	5.1 ± 0.9	25.1 ± 1.4	7
12 months	13.8 ± 0.9	7.4 ± 1.1	3.6 ± 0.6	24.8 ± 2.2	7
16 months	10.0 ± 1.2	9.9 ± 0.5	5.9 ± 0.8	25.8 ± 0.3	10
Irradiated					
24 h	10.3 ± 1.5	8.7 ± 0.6	4.4 ± 0.7	23.4 ± 1.2	10
2 months	11.2 ± 0.7	10.0 ± 1.0	5.7 ± 0.7	26.8 ± 1.2	10
4 months	9.4 ± 0.7[d]	6.0 ± 0.5	2.6 ± 0.4	17.9 ± 1.2[d]	9
6 months	9.3 ± 1.0[d]	5.2 ± 0.7	2.9 ± 0.4	17.4 ± 1.5[d]	9
8 months	10.4 ± 0.9	8.2 ± 0.4	4.4 ± 0.4	23.0 ± 1.3	10
12 months	11.8 ± 1.1	6.6 ± 0.3	3.4 ± 0.9	21.7 ± 1.0	8
16 months	8.6 ± 1.4	7.0 ± 0.7[d]	4.6 ± 0.6	20.2 ± 1.5[d]	8

[a] Sum of ATP, ADP and AMP.
[b] *n*, number of animals evaluated.
[c] Values represent mean ± SEM.
[d] Significantly lower than in the age-matched controls ($P < 0.05$).

irradiated or control, was tested. Results were considered significant at a value of $P < 0.05$.

RESULTS

Local heart irradiation with a dose of 20 Gy did not lead to cardiac failure or cardiac-related death during the observation period, and the animals did not show any sign of congestive heart failure. Irradiated animals showed a slightly impaired growth rate resulting in lower body weights and reduced left ventricular weights at 16 months after treatment. Average body weight of control animals ranged from 290 ± 6 to 460 ± 30 g; average body weight of irradiated animals ranged from 280 ± 4 to 390 ± 14 g; average left ventricular weight of control animals ranged from 700 ± 14 to 940 ± 37 mg; and average left ventricular weight of irradiated animals ranged from 700 ± 13 to 860 ± 56 mg. The left ventricular weight/body weight ratios of irradiated and nonirradiated animals, which ranged from 2.0 ± 0.1 to 2.5 ± 0.1 mg/g, were not significantly different, even up to 16 months after treatment. Seven animals (from both the control and the irradiated group) were excluded from the experiments because of spontaneously developing mammary tumors (mean latent period 7.5 months) outside the treatment area.

In Table I the myocardial concentrations of ATP, ADP, and AMP and the total adenosine nucleotide concentrations in irradiated rats and age-matched controls are presented. After short intervals (24 h and 2 months) irradiation did not affect the adenosine nucleotide concentrations. After long intervals between irradiation and evaluation (≥4 months) the concentration of adenosine nucleotides in irradiated hearts was lower than in the age-matched controls. The concentration of ATP at 4 and 6 months after treatment in the irradiated hearts was significantly lower than in the control hearts. However, at these times the values for the controls were elevated compared to those at other times. At 8, 12, and 16 months the ATP concentration in the irradiated hearts was also lower than in the control hearts, but this difference was not significant. For ADP the trend was similar to that observed for myocardial ATP concentration. From 4 months onward ADP concentrations in irradiated hearts were slightly lower than in control hearts. Only at 16 months after irradiation was this difference significant. The AMP concentration in the irradiated hearts was lower than in the control hearts at 2, 8, and 16 months after treatment, although at 4 and 6 months after irradiation AMP levels were slightly elevated. At 24 h and 2 months the sum of the adenosine nucleotide concentrations (ATP + ADP + AMP) after irradiation was not different from the values found in age-matched controls. The sum of the adenosine nucleotide concentrations of irradiated hearts was significantly ($P < 0.05$) lower compared to control hearts at 4, 6, and 16 months after treatment whereas at 8 and 12 months after treatment this difference did not reach significance.

The effects of irradiation and time on the rate of state 3 oxygen consumption using β-hydroxybutyrate as a substrate per gram of protein are shown in Fig. 2. Between 2 and 12 months after irradiation the rate of mitochondrial oxygen consumption in ventricular homogenates from irradiated hearts was significantly lower than that from age-matched controls. At 16 months after treatment the rate of state 3 respiration was lower in irradiated hearts than in control hearts, but this difference was not significant. Twenty-four hours after irradiation the rate of state 3 mitochondrial oxygen consumption was not different from that in control hearts. In the period of study, the rate of state 3 oxygen consumption fell progressively with time ($P < 0.001$) both in control hearts and in irradiated hearts.

Respiratory control indices (RCI = state 3 respiratory/state 4 respiratory) of irradiated hearts using β-hydroxybutyrate as a substrate were not significantly different from those of control hearts (Fig. 3). The RCI values in both groups decreased from a value of 8 measured at 24 h to a value of 4 at 16 months after treatment. The time-dependent decrease during the period of study was significant in both groups ($P < 0.005$).

The P/O ratio did not differ between irradiated and control hearts during the period of study up to 16 months (Ta-

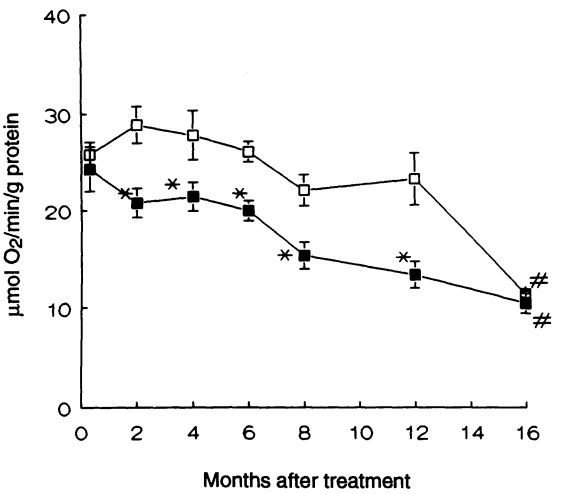

FIG. 2. State 3 oxygen consumption using β-hydroxybutyrate as a substrate in left ventricular homogenates of rat hearts at 24 h and 2, 4, 6, 8, 12, and 16 months after treatment. (■) Irradiated hearts, (□) control hearts. Each point represents the mean of 10 animals ± SEM. *Significantly different per time ($P < 0.05$). #Significant decrease over the period of study ($P < 0.001$).

ble II). Aging had no significant effect on the P/O ratio from both groups of hearts. A value of approximately 3 was obtained for the P/O ratio, which corresponds with the theoretical value if β-hydroxybutyrate is used as a substrate.

The rate of oxygen consumption was also evaluated at one time (6 months after treatment) using three other substrates (see Table III). Like with the substrate β-hydroxybutyrate, active respiration (state 3) in irradiated hearts appeared to be depressed using glutamate/malate or succinate as a substrate. Using pyruvate, the active respiration of irradiated hearts was not different from that of age-matched controls.

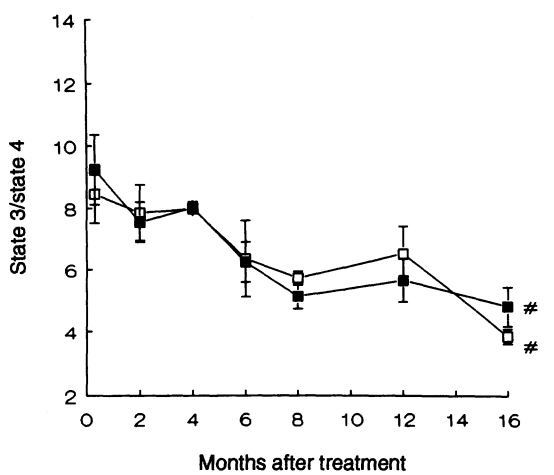

FIG. 3. Respiratory control index (state 3 respiration/state 4 respiration) in left ventricular homogenates of rat hearts at 24 h and 2, 4, 6, 8, 12, and 16 months after treatment. (■) Irradiated hearts, (□) control hearts. Each point represents the mean of 10 animals ± SEM. #Significant decrease over the period of study ($P < 0.005$).

TABLE II

P/O Ratios Measured in Left Ventricular Homogenates of Control and Irradiated Hearts

Time after treatment	P/O Ratio	
	0 Gy	20 Gy
24 h	3.7 ± 0.19[a] (10)[b]	3.4 ± 0.18 (9)
2 months	2.8 ± 0.12 (10)	2.9 ± 0.09 (10)
4 months	3.2 ± 0.08 (9)	3.2 ± 0.09 (10)
6 months	3.1 ± 0.22 (9)	2.9 ± 0.71 (10)
8 months	3.4 ± 0.23 (5)	3.3 ± 0.09 (10)
12 months	3.0 ± 0.17 (8)	3.6 ± 0.56 (8)
16 months	2.9 ± 0.10 (9)	3.0 ± 0.10 (6)

Note. Oxidative phosphorylation was measured by using β-hydroxybutyrate as a substrate. Theoretical value of the P/O ratio with this substrate is 3. None of the differences per time were significantly different.

[a] Mean ± SEM.

[b] Number of animals evaluated.

The RCI values for irradiated hearts at 6 months after treatment using glutamate/malate, pyruvate, or succinate as a substrate did not differ from the values obtained for control hearts (Table III). Likewise, no difference in the P/O value between irradiated and control hearts was observed with these substrates. The P/O ratio for glutamate/malate and pyruvate was 3, whereas for succinate a P/O value of approximately 2 was calculated.

At this time myocardial enzyme activities were also measured (Table IV). Lactate dehydrogenase and creatine kinase activities were significantly lower in irradiated hearts than in control hearts. The activity of the mitochondrial enzymes cytochrome *c* oxidase and citrate synthase was

TABLE III

State 3 Oxygen Consumption and Respiratory Control Index (RCI) Measured in Left Ventricular Homogenates Using Different Substrates at 6 Months after Irradiation

Substrate	State 3		RCI	
	0 Gy	20 Gy	0 Gy	20 Gy
Glutamate + malate	21.15 ± 0.87	15.48 ± 0.88	7.7 ± 0.6	6.9 ± 1.2
Pyruvate	18.67 ± 0.94	18.33 ± 1.72	6.5 ± 0.6	5.6 ± 0.7
Succinate	35.45 ± 3.61	24.85 ± 2.14[a]	1.5 ± 0.1	1.5 ± 0.1
β-Hydroxybutyrate	26.12 ± 1.07	19.99 ± 1.04[a]	6.4 ± 1.2	6.3 ± 0.7

Note. State 3 oxygen consumption is expressed in µmol O_2/min/g tissue protein ± SEM. Each treatment group consisted of six rats.

[a] Significantly lower compared to control; $P < 0.05$.

TABLE IV
Effect of Local Heart Irradiation on Myocardial Enzyme Activities of Rats at 6 Months after Treatment

Enzyme	0 Gy	20 Gy
Cytochrome *c* oxidase	17.96 ± 1.44	11.42 ± 1.75[a]
Citrate synthase	(244.0 ± 28.6) × 10^{-3}	(215.2 ± 12.8) × 10^{-3}
Lactate dehydrogenase	36.10 ± 1.76	29.17 ± 1.44[a]
Creatine kinase	52.47 ± 3.12	38.86 ± 4.21[a]

Note. Results are expressed in nkat/mg tissue protein ± SEM. Each treatment group consisted of six rats.

[a] Significantly lower compared to control; $P < 0.05$.

also depressed, although the reduction of the latter enzyme was not significant.

DISCUSSION

The results of this study demonstrate that heart irradiation with 20 Gy leads to a decreased rate of mitochondrial oxygen consumption in left ventricular homogenates in the presence of ADP (state 3 respiration). In general, irradiation hardly changed myocardial ATP levels. Only at 4 and 6 months after irradiation were the ATP concentrations lower compared to the age-matched controls. This study further shows that several myocardial enzyme activities were depressed by irradiation.

Several mechanisms can be responsible for a decrease in the rate of oxidative energy production, including ineffective coupling of oxidative phosphorylation, reduction of the number of mitochondria, and a reduced number of functionally active energy-producing respiratory assemblies on the inner mitochondrial membrane. Uncoupling of the oxidative phosphorylation appears to be unlikely as we observed no difference in RCI and P/O between irradiated and control hearts during the period of study.

We observed that mitochondrial citrate synthase activity in the irradiated myocardium was not significantly different from that in control hearts at 6 months after treatment. This suggests that the number of mitochondria is unaffected by radiation treatment as in the heart this enzyme is present only in the mitochondrial matrix. This observation corresponds with the results of Cilliers *et al.* (*10*). After irradiation with 20 Gy they observed swelling and damage to the mitochondrial membranes, without reduction of the number of mitochondria in the hearts of Wistar rats. Maeda (*24*), however, observed an "early" increase (2 days after treatment) in the number of mitochondria and the formation of giant mitochondria in irradiated (30 Gy) rabbit hearts. In a later stage, (>2 weeks), severe myocardial fibrosis developed and mitochondrial function was decreased. Kimler *et al.* (*25*) reported a reduction in the number of mitochondria in hearts of Sprague–Dawley rats about 3 months after X irradiation of the thorax with 15 and 24 Gy. This reduction was dose-dependent.

Our observation that the cytochrome *c* oxidase activity was depressed by irradiation might indicate that radiation damaged the mitochondrial inner membrane. This enzyme is involved in electron transport through the respiratory chain. Disturbances in electron transport inevitably lead to a decreased rate of oxidative phosphorylation.

So far very few studies have been performed which focus on oxidative phosphorylation in various tissues after local irradiation. Cohan *et al.* (*26*) observed a decrease in RCI and P/O in brain mitochondria within 5 min after irradiation of the head of Sprague–Dawley rats with a single dose of 200 Gy. However, this dose is too high to be clinically relevant. Several studies using total-body irradiation (with doses up to 11 Gy) have shown an effect on mitochondrial function in different species of animals [see review in Ref. (*12*)]. In mitochondria from several tissues (the heart was not studied) a transient reduction of P/O and RCI was observed up to 48 h after treatment (*12, 27*). These results seem to differ from the results on heart mitochondria reported in the present study. This discrepancy may be explained by the fact that, after total-body irradiation, alterations of the mitochondrial metabolic capacity either are due to direct irradiation of the organ of interest or are secondary due to irradiation damage to other organs such as the thyroid or adrenal glands. For instance, an increased secretion of adrenocortical hormones after whole-body irradiation led to a depression of the oxidative phosphorylation in rat liver (*28*).

In addition to a radiation-induced change in the rate of energy production, an effect of age on the rate of energy production also was observed. In both irradiated and control hearts, mitochondrial energy production decreased with age. An effect of age on the mitochondrial state 3 respiratory rate in hearts of 25-month-old sedentary Sprague–Dawley rats compared to 9-month-old rats using glutamate–malate, succinate, and palmitoylcarnitine as substrate has been reported by Starnes *et al.* (*18*). They concluded that "older hearts" rely increasingly on the less efficient glycolytic pathway for their energy production.

Changes in oxidative energy production have been reported to be implicated in several heart diseases [see review in Ref. (*13*)]. Buchwald *et al.* (*15*) reported a decrease in cytochrome *c* oxidase activity in human hearts with dilated cardiomyopathy. Lindenmayer *et al.* (*29*) observed a significant decrease in the rate of state 3 respiration in guinea pigs and rabbits with experimentally induced heart failure. Reduced myocardial energy production of the hypertrophied failing heart is one of the important factors responsible for reduced contractility (*14, 29*).

The present data on myocardial adenosine nucleotide levels do not clearly demonstrate that irradiation with 20 Gy leads to a reduction of these compounds in spite of the fact that ATP concentrations measured at 4 and 6 months after treatment were significantly lower than in control animals. The concentrations of ATP, ADP, and AMP measured in native heart tissue from control animals ranged from 9.0 to 16.7, 6.9 to 10.3, and 2.1 to 8.4 μmol/g dry wt, respectively. These values do correspond with those observed in native hearts of WKY rats.[1] It appeared that values for ADP and AMP concentrations in native hearts are higher compared to values found in perfused hearts, whereas ATP concentrations do not differ.[1]

The observation that myocardial creatine kinase and lactate dehydrogenase activity were depressed in irradiated rat hearts at 6 months after treatment demonstrates that these hearts suffer not only from an altered energy metabolism but also from other injury. Depressed activities of these enzymes are characteristic for damaged heart tissue (*13*). Minor focal damage (about 5%) to the heart muscle of Sprague–Dawley rats 6 months after 20 Gy has been described by Schultz-Hector *et al.* (*9*). Therefore, an irradiation-induced myocardial atrophy cannot be ruled out.

In conclusion, the present data show that irradiation leads to a reduction of mitochondrial function. However, the effect of irradiation on the adenosine nucleotide concentration is ambiguous. Changes in myocardial energy production and use probably contribute to the depressed contractility of heart muscle observed after irradiation (*13*). However, other mechanisms such as less efficient conversion of metabolic energy to contractile work, changes in the handling of intracellular Ca^{2+} ions, and progressive cardiac atrophy cannot be excluded as mediators of irradiation-induced left ventricular dysfunction.

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