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THROMBOXANE-MEDIATED INJURY FOLLOWING RADIATION

Annual Summary Report

John C. Rose, M.D.

August 31, 1984

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) The hypothesis under investigation is that moderate levels of radiation exposure result in endothelial and other tissue damage which, in turn, increases <u>in vivo</u> synthesis of thromboxane A2 (TXA2) and/or decreases synthesis of prostacyclin (PGI2). It is proposed that this altered arachidonate metabolism results in vasoconstriction and decreased peripheral blood flow, thereby leading to further tissue damage which is proportional to the degree of radiation exposure. The observations described in this first annual report demonstrate that whole body gamma irradiation results in an indomethacin sensitive increase in urine TXB2 four to 120 hours after 10 Gy and four to 12 hours after 20 Gy whole body irradiation. Exposure to 20 Gy also increased <u>in vivo</u> synthesis of the PGI2 hydrolysis product, 6-keto PGFla. These studies also showed that radiation-induced increases in TXB2 are due to altered extrarenal synthesis, while the altered 6-keto PGFla levels appear to be due to changes in intrarenal arachidonate metabolism. The projected studies for the second year will include identification of the organs and tissues involved in			
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19. Abstract (Continued)

radiation-induced alterations in urine cyclooxygenase product synthesis using regional shielding techniques. A systematic evaluation of the role of the kidney in elaborating altered urine arachidonate metabolite levels will be conducted using the isolated perfused rat kidney preparation. The role that free radicals play in altered cyclooxygenase product synthesis will be determined by using free radical scavengers after irradiation, and by the use of hydrogen peroxide injections to simulate radiation-induced peroxide formation in non-irradiated rats. Finally, the effect of ionizing radiation and the observed alterations of arachidonate metabolism on vascular reactivity will be assessed in order to determine the degree to which cyclooxygenase pathway products play a role in the pathogenesis of acute radiation injury.




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Foreword

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

BACKGROUND:

Observations on the cellular and tissue effects of ionizing radiation are numerous but the molecular manifestations of radiation injury are poorly understood. Radiation injury is associated with the formation of free radicals and lipid peroxides (1). Several groups of investigators have demonstrated a pivotal role for peroxides in the acceleration of cyclooxygenase activity (2-5) and the inhibition of prostacyclin synthesis (6-9). Since cyclooxygenase activity may be one of the rate limiting steps in arachidonate metabolism, and since the cyclooxygenase products have powerful vasoactive and platelet aggregatory properties (10-22), it is possible that these arachidonate metabolites play an important role in radiation-induced vascular injury.

In recognition of this possibility, Eisen and Walker (23) showed that exposure of mice to 7 Gy x-irradiation resulted in increased pulmonary and splenic PGE-like activity and increased synthesis of PGF_{2a}. Other studies have shown that hepatic and cerebral PGE-like activity and PGF_{2a} synthesis increased after 5 to 7.5 Gy (24). Irradiation of mice with 9 Gy gamma irradiation resulted in a significantly increased *in vitro* synthesis of PGE₂ and PGF_{2a} by hepatic microsomes and homogenates of brain and testis (25,26). These and other studies demonstrated that *in vitro* synthesis of the classical products of the cyclooxygenase pathway was increased following exposure to ionizing radiation (23-27).

The effect of ionizing radiation on TXA₂ synthesis is less clear. Maclouf et al (28) demonstrated a significant increase in TXB₂ release by splenic microsomes isolated from rats exposed to 9.0 Gy whole body irradiation. Steel and Catravas (29) showed increased TXB₂ release from guinea pig lung parenchymal strips one to three hours post irradiation. On the other hand, Steel et al (30) failed to show an increase in TXB₂ synthesis by guinea pig lung airway tissue following irradiation. Similarly, Allen et al (31) could not demonstrate an alteration in TXB₂ release by platelets irradiated *in vitro*.

Recently, several *in vivo* studies on TXB₂ synthesis have been performed. Schneidkraut et al (32) showed that urine TXB₂ levels were elevated four to 12 hours after 10 Gy whole body gamma irradiation. These results were subsequently confirmed by Donlon et al (33) who demonstrated a significant increase in TXB₂ excretion rate 12 and 36 hours after 9 Gy whole body irradiation of rats. These studies indicate that *in vivo* synthesis of TXA₂ is increased acutely after whole body irradiation. Furthermore, the increase in urine TXB₂ levels appears to be a result of altered arachidonate metabolism by diverse tissues but the increased release of this cyclooxygenase product is not a function of all tissues.

The effect of ionizing radiation on PGI₂ synthesis is also controversial. Allen et al (31) showed that PGI₂ synthesis by umbilical artery rings was depressed following irradiation. Baluda et al (34) also demonstrated that PGI₂-like activity was decreased in the abdominal aorta of guinea pigs following whole body gamma irradiation. Similarly, Tsao et al (35) determined that PGI₂-like activity of rat lungs was depressed up to 50% 24 hours post irradiation. On the other hand, Hahn et al (36) showed that cultured pulmonary endothelial cells have increased release of PGI₂ following *in vitro* irradiation. This finding was confirmed by Eldor et al (37) who determined that the increase in PGI₂ release from irradiated endothelial cells was due to increased substrate availability and not increased enzyme activity.

Several groups have suggested that the altered synthesis of PGI₂ is dependent on the time between irradiation and determination of arachidonate metabolism (35,38). These investigators indicate that PGI₂ synthesis is initially increased following irradiation and is progressively inhibited (38). Conversely,

Tsao et al (35) states that irradiation results in an initial decrease in PGI₂ synthesis followed by a progressive increase in the release of this arachidonate metabolite over the next six months. These contradictory reports clearly underscore the need to continue a systematic study of the effect of radiation exposure on TXA₂ and PGI₂ synthesis.

Schneidkraut et al (32) suggest that *in vivo* PGI₂ synthesis is increased four hours after whole body gamma irradiation but is unchanged from 12 hours to 5 days post irradiation. Recent studies have indicated that urine 6-keto PGF_{1a} may not reflect systemic synthesis of prostacyclin (39,40). Several investigations showed that injection of either PGI₂ or 6-keto PGF_{1a} results in a rapid conversion of either arachidonate metabolite to 6,15 diketo 13,14 dihydro-prostaglandin Fla as well as other metabolites (40-42). In addition, other studies suggest that PGI₂ is metabolized by the kidney to the 6,15 diketo metabolite (43). These studies indicate that the *in vivo* measurement of PGI₂ synthesis is complex and, at present, poorly understood. The uncertainty as to how to measure *in vivo* PGI₂ synthesis has prompted us, with the agreement of Dr. Theoharides, to omit 6-keto PGF_{1a} measurements from further studies.

Prostaglandins probably play a role in the pathogenesis of radiation injury. Radiation-induced esophagitis in the opossum was attenuated by treatment with indomethacin or aspirin before radiation exposure, and was potentiated by pretreatment with 16, 16 dimethyl prostaglandin E₂ (44-47). The precise role prostaglandins play in radiation-induced tissue injury remains to be determined.

APPROACH TO THE PROBLEM

Irradiation and Urine Sampling: The first series of studies performed were designed to determine if whole body gamma irradiation altered *in vivo* arachidonate metabolism. These studies also aimed to determine the threshold irradiation dose and time course of altered cyclooxygenase product release. Male Sprague-Dawley rats (200-250 g) were anesthetized with sodium pentobarbital (30 mg/kg i.p.) before irradiation in order to maintain a consistent pattern of exposure in the radiation chamber. The rats were exposed to either sham irradiation or 2, 10, or 20 Gy gamma irradiation in a ventro-dorsal orientation to a 7.4×10^{13} becquerel ¹³⁷Cs radiation source (Best Industries Small Animal Irradiator, Arlington, VA). The rate of delivery was previously calibrated at 0.92 Gy per minute.

Rats were re-anesthetized 2,4,12,24,72, or 120 hours after irradiation and urine samples taken. The bladder was exposed by a 1 cm midline abdominal incision and the urine was drained from the bladder via syringe. A 0.2 ml aliquot was removed from each sample for an osmolarity determination (Advanced Digimatic Osmometer, Needham, MA) and the remainder of the sample was frozen at -20° C. The urine samples were thawed as a group and assayed for TXB₂ and 6-keto-PGF_{1a} by radioimmunoassay.

Indomethacin Pretreatment: These experiments were performed to determine if urine TXB₂ and/or 6-keto PGF_{1a} levels reflected cyclooxygenase activity. A solution of indomethacin was prepared to a final concentration of 2.0 mg/ml by dissolving 50 mg indomethacin in 25 ml sodium carbonate (2.94 mM) buffered isotonic saline. Rats were anesthetized with ether and injected i.v. with indomethacin (5.0 mg/kg) or with an equivalent volume of sodium carbonate buffered saline. One hour after indomethacin or vehicle injection, the animals were re-anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and subjected to either 20 Gy gamma irradiation or sham irradiation. Urine samples were taken 4 hours later and assayed for TXB₂ and 6-keto-PGF_{1a} by radioimmunoassay.

Isogravitometric cross perfusion: These experiments were designed to determine whether urine TXB2 and/or 6-keto PGFla reflect primarily renal or extrarenal synthesis. The cross perfusion experiments were performed on indomethacin pretreated rats (20 mg/kg i.v. 24 hours before cross perfusion) which were cross perfused with (1) indomethacin pretreated rats (indomethacin), (2) sham irradiated animals (control), or (3) animals exposed to 20 Gy gamma irradiation 4 hours before cross perfusion (irradiated). Urine samples were taken only from the indomethacin pretreated animals of the cross perfused pair prior to attachment to the cross perfusion system and after 60 min of cross perfusion.

The rats were anesthetized with sodium pentobarbital intraperitoneally and the trachea was intubated. The common carotid artery and jugular vein were also catheterized and the urinary bladder exposed. The animals were then injected with sodium heparin (1000 USP units/kg, i.v.) prior to attachment to the cross perfusion device.

The cross perfusion system consisted of 10 ml syringe reservoirs, to which was attached low resistance (PE240) polyethylene tubing. The reservoir was placed 2-3 cm above a two pan balance on which the anesthetized, catheterized rats were placed. The height of the reservoirs above the animals resulted in a calculated venous pressure of 1.5-2.2 mm Hg and an estimated flow rate of 0.4 ml per minute. The tubing and reservoirs were filled with isotonic saline containing 3% bovine serum albumin to maintain a normal blood volume. The carotid artery catheter was attached to the outflow tubing running to the reservoir and the jugular vein catheter was attached to the inflow tubing running from the reservoir (Fig. 1). The tubing and reservoirs were allowed to fill with blood before the two circulations were linked.

Immediately before the circulations were connected, the bladder was drained and the urine discarded. The inflow line from the left reservoir was then attached to the jugular vein catheter of the rat on the right pan and vice versa. The relative weights of the rats were balanced using the scale weights. After this time, and until the end of the cross perfusion, the relative weights of the cross circulating rats were maintained by adjusting the flow of blood into the reservoir by the screw clamps on the outflow lines (48).

Radiation Shielding: These investigations sought to determine whether radiation-induced increases in urine TXB2 and 6-keto PGFla concentrations were due to altered abdominal or extra-abdominal synthesis of these cyclooxygenase products.

Male Sprague-Dawley rats (200-250 g) were anesthetized with sodium pentobarbital i.p. and the tracheas were cannulated. The body volume of these animals was determined by immersion and water displacement. The volume of animal not exposed to ionizing radiation due to the radiation shield was calculated from the width of the shield and the circumference of the animal. The efficiency of the radiation shield was also assessed using lithium iodide thermoluminescent crystals. These studies showed that radiation levels were decreased by 92% on the ventral surface and 94% on the dorsal surface of the animal. Since the radiation dose penetrating the shield with a 20 Gy exposure was at most 1.6 Gy, and since previous studies indicated that this radiation dose would not affect arachidonate metabolism, the penetrating dose of radiation was not included in the calculation of whole body irradiation with the abdomen shielded. These studies indicate that the radiation shield protected 25% of the rat body volume. Therefore, in order to obtain a comparable whole body irradiation dose with the abdomen shielded compared to unshielded, the exposure level of the unshielded rats was reduced by 25% to 15 Gy.

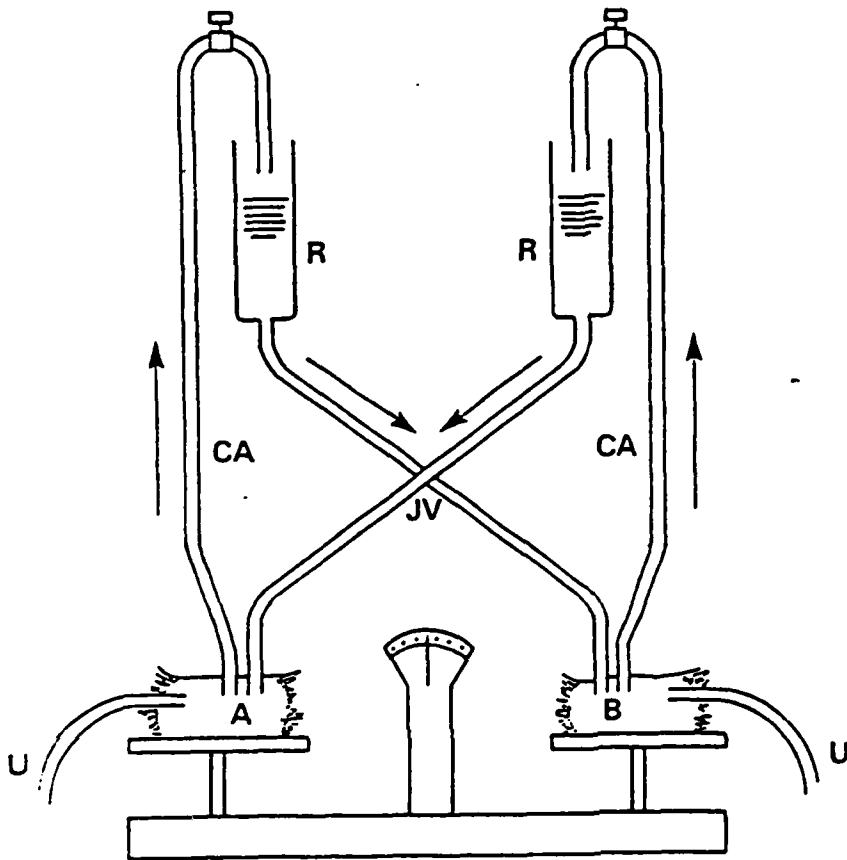


Figure 1: Schematic diagram of the isogravitometric cross perfusion system. A catheter from the carotid artery (CA) of rat A runs to the reservoir (R) and then into a catheter implanted in the jugular vein (JV) of rat B and vice versa. Urine was collected from the indomethacin pretreated rat of the cross perfused pair prior to attachment to the cross perfusion system and after one hour of cross circulation.

Rats of comparable weight were anesthetized with sodium pentobarbital and exposed to a radiation dose of 20 Gy with the abdomen shielded or to 15 Gy gamma irradiation with the abdomen unshielded. Controls were anesthetized and exposed to sham irradiation. Four hours after irradiation or sham irradiation, the animals were re-anesthetized and the urine collected as previously described. The urine samples were frozen at -20°C immediately after removal from the animal.

Radioimmunoassay: Urine TXB2 and 6-keto-PGF $_{1\alpha}$ concentrations were determined by radioimmunoassay using the methods of Granstrom and Kindahl (49,50). An aliquot of urine (20 μl) was added to 300 μl radioimmunoassay buffer (RIA buffer), 80 μl gelatinized radioimmunoassay buffer (gel buffer), 100 μl labelled ligand, and 100 μl antibody. The RIA buffer consists of 1.55 g Trisma 7.0 (Sigma Chemical Co., St. Louis, MO), and 9.0 g NaCl dissolved in 1 liter distilled water. To this was added 1.0 ml of 2.0 M MgSO_4 and 1.0 ml of 0.2 M CaCl_2 . Gel buffer consists of RIA buffer to which gelatin was added (0.25 g gelatin per liter RIA buffer). The urine-RIA buffer-Gel buffer-ligand-antibody mixture was incubated overnight at 4°C and the unbound labelled ligand precipitated by the addition of 0.9 ml dextran-coated charcoal (0.3 g Dextran 70 and 3.0 g charcoal suspended in 1 liter RIA buffer). The supernatant left after centrifugation at $1000 \times g$ was transferred to liquid scintillation vials to which 5.0 ml fluor was added (Atomlight, New England Nuclear, Boston, MA).

The specificity of the assay was investigated by determining the cross reactivity of the TXB2 and 6-keto-PGF $_{1\alpha}$ antibodies with other cyclooxygenase products. The cross reactivity of the TXB2 antibody was less than 0.3% with PGA $_2$, and less than 0.1% with PGF $_{2a}$, PGE $_2$, and 6-keto-PGF $_{1\alpha}$. The TXB2 antibody showed a high cross reactivity, approximately 60%, with 2,3 dinor TXB2. Consequently, the TXB2 levels determined refer to a mixture of TXB2 and its 2,3 dinor metabolite. The 6-keto-PGF $_{1\alpha}$ antibody showed 10% cross reactivity with PGE $_2$, 7.2% cross reactivity with PGF $_{2a}$, and less than 1% cross reactivity with either 6-keto-PGE $_1$ or PGF $_{1\alpha}$.

The sensitivity of the radioimmunoassay was evaluated on the basis of the avidity of the antibody for either TXB2 or 6-keto-PGF $_{1\alpha}$. Both the TXB2 and 6-keto-PGF $_{1\alpha}$ antibodies showed an avidity for their antigens that was comparable to that seen by other investigators (12) ($1.1 \times 10^{10} \pm 4.2 \times 10^9 \text{M}^{-1}$ and $4.2 \times 10^{10} \pm 2.4 \times 10^{10} \text{M}^{-1}$ respectively).

Precision was assessed by calculation of the coefficient of variation of each point on the standard curves run during these experiments. For TXB2, the coefficient of variation for the standard curves ($n=19$) ranged from 0.274 to 0.299. For the 6-keto-PGF $_{1\alpha}$ assay, the coefficient of variation ranged from 0.255 to 0.288 ($n=17$).

The accuracy of the two assays was determined by adding known amounts of TXB2 and 6-keto-PGF $_{1\alpha}$ to urine samples from 20 Gy irradiated or non-irradiated rats. The correlation coefficients of the two curves were determined. For the TXB2 assay, control urines to which four known concentrations of TXB2 had been added showed a correlation coefficient of 0.938 while urine samples from irradiated rats showed a correlation coefficient of 0.967 ($n=8$ and 6 animals respectively). For the 6-keto-PGF $_{1\alpha}$ assay, control urines with four known concentrations of 6-keto-PGF $_{1\alpha}$ demonstrated a correlation coefficient of 0.965 and the samples from irradiated rats also had a correlation coefficient of 0.965 ($n=8$ and 4 animals respectively). In addition, the slopes of the binding curves from control and irradiated rats were parallel, indicating that urine from irradiated rats behaves in a similar fashion as control urine.

Statistical Analysis: The effects of ionizing radiation on urine TXB2 and 6-keto PGF1a levels were analyzed using the unpaired Student's t-test because each time point and dose had a parallel control with which it could be compared. The effect of indomethacin pretreatment on urine cyclooxygenase products concentrations with and without irradiation was also analyzed by the unpaired Student's t-test, as was the effect of abdominal shielding on urine TXB2 and 6-keto PGF1a. Cross perfusion data were analyzed using the paired Student's t-test with the pre-cross perfusion samples being compared to the 60 min post cross perfusion samples. In all studies, significance was set at $p < .05$.

Results

Animals exposed to whole body irradiation at a dose of 2 Gy showed no change in urine TXB2 at 2 to 120 hours post irradiation (Table 1). Increasing the exposure level to 10 Gy significantly ($p < .05$) increased urine TXB2 at 4, 12, 24, 72, and 120 hours post irradiation but not at 2 hours after exposure (Table 1). Rats exposed to 20 Gy had increased urine TXB2 concentrations of 233.1% and 105.3% ($p < .05$) at 4 and 12 hours post irradiation respectively, but not at any other observation time (Table 1). Urine samples could not be taken 120 hours after 20 Gy as this dose of radiation was an LD₁₀₀ dose by 96 hours post irradiation. Urine TXB2 in control urines for each radiation dose and at each observation time were not significantly different. Urine volumes in irradiated and non-irradiated rats were also not significantly different at all doses tested and all observation times.

Rats exposed to 2 or 10 Gy gamma irradiation demonstrated no change in urine 6-keto-PGF1a concentrations at any observation time. Elevating the exposure dose to 20 Gy increased urine 6-keto-PGF1a levels by 154.9% ($p < .05$) at 4 hours post irradiation. This same dose of radiation did not change 6-keto-PGF1a concentrations at 2, 12, 24, or 72 hours after exposure (Table 2).

In another series of experiments rats were pretreated with indomethacin 1 hour before sham irradiation or irradiation with 20 Gy. Four hours after sham irradiation, urine TXB2 levels in indomethacin injected animals were suppressed 99.9% ($p < .05$) compared to sham irradiated vehicle injected rats (Table 3). Animals pretreated with indomethacin prior to irradiation showed a 94.2% ($p < .05$) decrease in urine TXB2 compared to vehicle injected irradiated rats (Table 3). Indomethacin pretreatment also resulted in a significant (97.7% and 81.6%, $p < .05$) suppression of urine 6-keto-PGF1a levels in irradiated and non-irradiated rats respectively (Table 3).

Urine osmolarity was determined with each dose and at each observation time following irradiation. At none of the doses of radiation used and at none of the observation times was there a significant alteration in urine osmolarity in irradiated rats compared to non-irradiated controls. Linear regression analysis of all the control urine values failed to show a correlation between either TXB2 or 6-keto-PGF1a concentrations and urine osmolarity ($n=176$).

Indomethacin pretreated rats which were cross perfused with similar animals showed no change in urine TXB2 levels. In contrast, urine TXB2 of indomethacin pretreated rats, when cross perfused with either sham irradiated (control) or 20 Gy irradiated (irradiated) rats, increased significantly (Table 4). Indomethacin pretreated rats cross perfused with either control or irradiated animals showed no increase in urine 6-keto-PGF1a values (Table 4).

Abdominal shielding did not attenuate radiation-induced increases in urine TXB2 concentrations (Fig. 2). Shielding of the kidneys did prevent the increase in urine 6-keto PGF1a concentrations seen 4 hrs after 20 Gy whole body irradiation. Urine volumes in rats irradiated with the kidneys shielded or unshielded were not

Table 1
Effect of Ionizing Radiation on Urine TXB2 Levels

	2 ^a	4	12	24	72	120
Control	3.21 +0.96	2.75 +0.85	2.25 +0.41	2.61 +1.26	2.50 +0.58	3.13 +1.26
2 Gy	2.94 +0.82	3.16 +0.71	2.36 +0.58	3.74 +1.10	1.98 +0.58	3.27 +1.21
Control	2.64 +0.58	2.58 +0.41	1.87 +0.22	4.01 +0.55	2.61 +0.30	3.05 +0.80
10 Gy	3.13 +0.80	5.00* +0.38	5.55* +0.58	9.26* +2.03	7.50* +1.18	6.43* +1.24
Control	3.32 +0.60	1.51 +0.30	1.70 +0.50	2.69 +0.47	2.25 +0.58	N.D.
20 Gy	2.09 +0.55	5.03* +0.82	3.49* +0.58	3.38 +0.52	1.13 +0.14	†

Rats were exposed to graded doses of whole body irradiation at various times before urine samples were taken directly from the bladder. The urine samples were assayed for TXB2 by radioimmunoassay. Data are expressed as mean \pm SEM in nanomoles per liter of urine. N = 6-10 animals per group. ^aTime after irradiation expressed in hours.

N.D. - Not done; † - Expired prior to 120 hrs. post irradiation.

*p<.05

Table 2

Effect of Ionizing Radiation on Urine 6-keto-PGF_{1a} Levels

	2 ^a	4	12	24	72	120
Control	7.55	16.43	15.55	9.89	15.88	8.38
	<u>+1.40</u>	<u>+3.49</u>	<u>+3.54</u>	<u>+2.50</u>	<u>+5.88</u>	<u>+2.75</u>
2 Gy	8.19	12.06	13.98	12.20	17.50	6.40
	<u>+1.90</u>	<u>+3.27</u>	<u>+3.93</u>	<u>+3.13</u>	<u>+4.67</u>	<u>+1.10</u>
Control	10.80	6.07	6.98	10.28	7.91	11.04
	<u>+3.49</u>	<u>+1.15</u>	<u>+2.34</u>	<u>+2.75</u>	<u>+2.25</u>	<u>+2.42</u>
10 Gy	9.50	8.10	25.08	17.72	20.91	12.20
	<u>+4.23</u>	<u>+1.59</u>	<u>+8.24</u>	<u>+5.38</u>	<u>+6.59</u>	<u>+2.97</u>
Control	5.44	4.72	8.54	4.86	5.88	N.D.
	<u>+1.92</u>	<u>+1.35</u>	<u>+1.81</u>	<u>+1.29</u>	<u>+1.68</u>	
20 Gy	8.02	12.03*	7.69	4.20	2.97	†
	<u>+1.26</u>	<u>+1.98</u>	<u>+1.81</u>	<u>+0.74</u>	<u>+1.07</u>	

Rats were exposed to graded doses of radiation and urine samples were removed directly from the bladder at various times after exposure. Urine samples were analyzed for 6-keto-PGF_{1a} by radioimmunoassay. Data are expressed as mean \pm SEM in nanomoles per liter urine. n = 6-9 animals per group. ^aTime after irradiation expressed in hours. N.D. - Not done; † - Expired prior to 120 hrs. post irradiation.

*p<.05

Table 3

Effect of Indomethacin Pretreatment on Urine TXB2 and 6-keto-PGF1a Values
in Irradiated and Non-irradiated Rats

	Non-Irradiated		Irradiated	
	TXB2	6-keto-PGF1a	TXB2	6-keto-PGF1a
Buffer	2.56	4.45	5.06	12.25
	<u>+0.74</u>	<u>+0.71</u>	<u>+0.77</u>	<u>+ 2.97</u>
Indomethacin (5 mg/kg)	0.002*	0.82*	0.29*	0.28*
	<u>+0.002</u>	<u>+0.52</u>	<u>+0.16</u>	<u>+ 0.22</u>

Rats were pretreated with 5 mg/kg indomethacin i.v. or an equivalent volume of sodium carbonate buffered saline 1 hr before exposure to 20 Gy whole body irradiation or sham irradiation. Four hours after radiation exposure, urine samples were taken and analyzed for TXB2 and 6-keto-PGF1a by radioimmunoassay. Data are expressed as mean \pm SEM in nanomoles cyclooxygenase product per liter urine.

n = 6 animals per group. *p<.05

Table 4

Determination of the Source of Urine Thromboxane B2 and 6-keto-PGF1a

	Pre Cross Perfusion		Cross Perfusion (60 min)	
	TXB2	6-keto-PGF1a	TXB2	6-keto-PGF1a
Indomethacin	0.10 <u>+0.06</u>	0.23 <u>+0.13</u>	0.32 <u>+0.17</u>	0.41 <u>+0.19</u>
Control	0.03 <u>+0.03</u>	0.18 <u>+0.12</u>	2.14* <u>+0.89</u>	0.68 <u>+0.60</u>
20 Gy	0.002 <u>+0.002</u>	0.10 <u>+0.06</u>	0.52* <u>+0.19</u>	0.42 <u>+0.14</u>

The renal or extrarenal release of TXB2 and 6-keto-PGF1a into urine was evaluated using an isogravitometric cross perfusion system. Rats were injected with 20 mg/kg indomethacin 24 hrs before cross perfusion with either indomethacin pretreated rats, sham irradiated rats, or animals exposed to 20 Gy whole body irradiation 4 hours before cross perfusion. Urine TXB2 and 6-keto-PGF1a values were determined by radioimmunoassay and are expressed as mean \pm SEM in nanomoles cyclooxygenase product per liter urine. n = 7 pairs of animals per group.

*p<.01

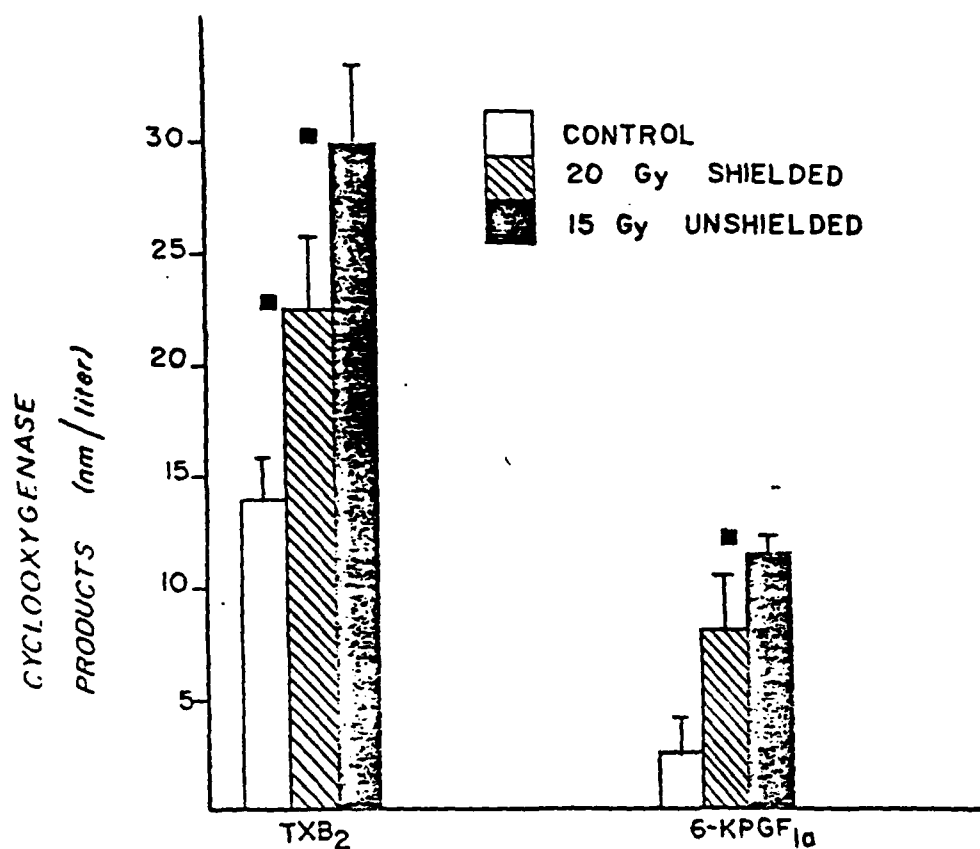


Figure 2

The effect of abdominal shielding on urine TXB₂ and 6-keto PGF_{1α} concentrations four hours after equivalent doses of whole body gamma irradiation. Data are expressed as mean \pm SEM in nanomoles cyclooxygenase product per liter urine.

N = 6-7 animals per group. p < .05.

significantly different from each other nor from control urine volumes.

Discussion

This series of investigations demonstrated that whole body gamma irradiation increased urine TXB2 concentrations while urine volumes remain unchanged. These results extend previous in vitro studies in which whole body ionizing radiation increased TXB2 synthesis by spleen microsomes (28) and lung parenchyma (29). Allen et al (31) showed no change occurring in TXB2 synthesis when platelets were irradiated with 20 Gy in vitro. The results of these workers may be related to the short time interval between irradiation of platelets and the measurement of TXB2 release. Our observations indicate that, at this level of radiation exposure, no change in urine TXB2 is seen before 4 hours post irradiation. Furthermore, other in vitro experiments using lung parenchyma demonstrated that exposure of guinea pigs to 30 Gy irradiation resulted in no change in TXB2 release before 3 hours post irradiation (29). These data indicate that in vivo release of TXB2 follows a comparable dose and time course as in vitro studies. In addition, our experience suggests there is a delay of several hours before irradiation-induced alterations in arachidonate metabolism are observed.

The present study confirms and extends previous work from this laboratory in which whole body irradiation resulted in altered in vivo synthesis of TXB2 (32). These results were also recently confirmed by Donlon et al (33) in which rats were exposed to either 1 or 9 Gy gamma irradiation and urine TXB2 was measured. Those studies showed significant elevations in urine TXB2 at both 1 and 9 Gy. These data indicate that alterations in TXA2 synthesis occur with a dose of radiation between 2 Gy and 10 Gy.

Exposure of rats to 20 Gy whole body gamma irradiation increased urine 6-keto-PGF1a levels at 4 hours. These results are in agreement with in vitro studies on pulmonary endothelial cells (36) and rabbit abdominal aorta (38). However, the time course of in vivo release of 6-keto-PGF1a appears to be different from that seen in rabbit aorta. Sinzinger et al (38) showed increased 6-keto-PGF1a in the incubation medium within 1 hour after exposure to 20 Gy. In addition, these investigators showed increased release of 6-keto-PGF1a with as little as 10 Gy gamma irradiation. Some of the differences in the time course and dose of radiation needed to increase 6-keto-PGF1a levels in the present study as compared to the work of Sinzinger et al may be due to a species difference. On the other hand, the 4 hour delay between irradiation and observable changes in urine 6-keto-PGF1a compared to the 1 hour delay between irradiation and altered in vitro release may reflect the time necessary for altered extrarenal PGI2 synthesis to result in increased urinary 6-keto-PGF1a concentrations. This appears unlikely however, since the isogravitometric studies suggest that urine 6-keto-PGF1a is synthesized primarily in the kidney. We have observed that, in order to alter PGI2 synthesis at 4 hours post irradiation, a threshold dose of radiation between 10 and 20 Gy is necessary. At all other sampling periods, 6-keto-PGF1a was not affected by radiation exposure.

Since the increase in urine TXB2 concentrations at 4 hours post irradiation seem related to the level of radiation exposure, it is possible that urine TXB2 may be used as a biological dosimeter. There has been an intensive search for urinary biological dosimeters. Gerber (51) has reviewed the correlation between taurine, deoxycytidine, pseudouridine, creatine, and 5-hydroxy-indol acetic acid levels and whole body irradiation dose. Each of these agents is characterized by a rapid rise in urinary concentration which is temporally similar to the increased TXB2 synthesis. In addition, changes in urinary concentration of each of these substances was related to the radiation exposure level. The limiting factor for the use of these metabolites as dosimeters is the dose range over which the urinary concentration of these substances is related to the exposure level. All of these

agents tended to lose their linear relationship to dose at irradiation levels above 3 to 6 Gy. Since the present study suggests a relationship between radiation dose and urine TXB2 levels in a range of 10-20 Gy, it is possible that the concentration of urine TXB2, coupled with one or several of these other urinary substances, may be used to evaluate whole body radiation exposure levels over a wide range of radiation doses.

The isogravitometric cross perfusion experiments provide evidence that urine TXB2 is normally derived primarily from an extrarenal source, agreeing with studies in which urine thromboxane was used as an indicator of renal allograft rejection (52). In that study, one renal transplant patient developed deep vein thrombosis while having no other evidence of allograft rejection. The urine from this patient showed highly elevated levels of urine thromboxane. It seems likely that extrarenally synthesized TXB2 was removed from the circulation by the kidneys and excreted in the urine.

Isogravitometric cross perfusion experiments also demonstrated that urine 6-keto-PGF1a is normally synthesized principally by the kidney. This observation is in agreement with the studies of Sun et al (39). Infusion of 6-keto-PGF1a into rats for 14 days resulted in approximately 10% recovery of unchanged 6-keto-PGF1a but infusion of PGI2 resulted in no 6-keto-PGF1a recovered in the urine. Those studies imply that a metabolite other than 6-keto-PGF1a should be used to assess extrarenal PGI2 synthesis.

Abdominal shielding studies were performed to determine if radiation induced increases in urine arachidonate metabolite levels were due to altered synthesis by the abdominal organs. These studies showed that radiation-induced increases in urine TXB2 were due to increased extra-abdominal synthesis while radiation-induced increases in urine 6-keto-PGF1a concentrations were due to altered abdominal organ synthesis of this arachidonate metabolite.

Rats were pretreated with indomethacin prior to irradiation or sham irradiation in order to show that urine immunoreactive TXB2 and 6-keto-PGF1a levels are a reflection of *in vivo* cyclooxygenase pathway activity. In irradiated and non-irradiated rats, urine TXB2 and 6-keto-PGF1a levels were significantly reduced compared to vehicle-injected controls, indicating that urine TXB2 and 6-keto-PGF1a are a reflection of cyclooxygenase pathway activity.

Urine osmolarities in irradiated and non-irradiated rats were determined to evaluate the possibility that changes in urine osmolarity correlated with changes in urine TXB2 and 6-keto-PGF1a levels. Unlike PGE2 which has been shown to be directly correlated with urine osmolarity (53), TXB2 and 6-keto-PGF1a concentrations in urine were not correlated with urine osmolarity. Thus, it is likely that PGE2 is excreted differently by the kidney than either TXB2 or 6-keto-PGF1a.

We propose that radiation exposure increases arachidonate release from membrane phospholipids. In addition, free radicals formed following irradiation may act to increase cyclooxygenase activity. The combination of increased arachidonate release from membranes plus the increase in cyclooxygenase activity could result in increased cyclooxygenase product synthesis. High doses of radiation, while probably also resulting in increased arachidonate release, may act to initially stimulate and subsequently inhibit cyclooxygenase pathway activity due to the formation of stimulatory, followed by inhibitory concentrations of free radicals. Control of this altered arachidonate metabolism following irradiation, either via free radical scavengers and/or cyclooxygenase inhibitors, may ameliorate radiation-induced systemic injury.

Conclusions

The data presented in this first annual report indicate that:

1. Urine concentrations of TXB2 and 6-keto PGF1a are increased following whole body ionizing radiation exposure.
2. The alterations in in vivo synthesis of TXB2 and 6-keto PGF1a are due to increased cyclooxygenase pathway activity.
3. The changes in urine arachidonate metabolite concentrations following radiation exposure are not related to changes in urine volume or urine osmolarity.
4. Urine levels of TXB2 in non-irradiated rats are due primarily to extrarenal synthesis while urine 6-keto PGF1a concentrations are normally of renal origin.
5. The radiation-induced increases in urine TXB2 levels are due to alteration in extra-abdominal synthesis while radiation-induced increases in urine 6-keto PGF1a concentrations are due to increased abdominal organ synthesis.

RECOMMENDATIONS**Specific Aims for the Next Year**

- A. To determine the source of altered in vivo cyclooxygenase pathway products in the urine of irradiated animals.
 - 1. More extensive and detailed regional shielding studies will be performed including renal and thoracic shielding.
 - 2. The isolated perfused rat kidney preparation will be used to determine if irradiated kidneys synthesize more TXB2 than normal kidneys thereby contributing to the increased cyclooxygenase pathway product levels seen in urine.

- B. To determine the effect of free radicals on the production of TXA2.
 - 1. Free radical concentrations will be reduced by using the free radical scavengers cysteamine or reduced glutathione. The effect of the free radical scavengers on in vivo TXB2 synthesis will be compared to the radioprotectant, WR2721.

- C. To determine the effect of radiation on vascular function and cyclooxygenase pathway product synthesis.
 - 1. The developed tension of isolated abdominal aortic ring segments will be determined in response to the TXA2 mimic, U46619.
 - 2. The in vitro synthesis of TXB2 and 6-keto PGF1a will be determined in abdominal aortic rings following irradiation.

PROJECTED METHODS

1. Urine Sampling: The urine sampling procedures will be modified in order to determine both urine cyclooxygenase pathway product concentration as well as rate of release. Both sham irradiated and irradiated rats will be anesthetized one hour before the urine sample is to be taken. The bladder will be exposed, drained of urine, and the urine discarded. At the predetermined sampling time, the bladder will again be drained of urine and the volume noted. The urine sample will then be immediately frozen at -20° C until the urine concentrations of TXB2 can be determined by radioimmunoassay. By knowing the cyclooxygenase pathway product concentration and the volume of urine produced per hour, the rate of arachidonate metabolite release will be determined.

2. Regional Shielding: Male Sprague-Dawley rats (200-250 g) will be anesthetized (30 mg/kg sodium pentobarbital i.p.) and exposed to 20 Gy whole body irradiation with the abdominal or thoracic cavity shielded, or will be exposed to 15 Gy whole body irradiation without any shielding. The urine concentrations and rate of arachidonate metabolite release will be determined in the abdominal shielded, thoracic shielded, and unshielded animals and compared to those levels of arachidonate metabolite production seen in sham irradiated controls.

Interpretation: If renal shielding results in a reduction in the urine concentration and rate of release of TXB2 compared to sham irradiated levels, while thoracic shielding does not affect TXB2 levels, it will be concluded that TXB2 is primarily synthesized by the shielded area of the abdomen. Should thoracic shielding result in a reduction in the urine concentrations and rate of release of TXB2 to control levels, it will be concluded that the lungs and/or liver are the primary source of urine TXB2. A reduction in TXB2 in both thoracic and abdominal shielded groups will be taken to mean that radiation-induced alterations in this cyclooxygenase product is of diverse origin involving organs of both the thoracic and abdominal cavities. Should any of the shielding experiments result in an attenuation or abolishment of altered urine cyclooxygenase pathway product levels, the isolated renal perfusion studies will be performed.

3. Isolated Perfused Kidney Experiments; Methodology: Male Sprague-Dawley rats (200-250 g) will be subjected to 20 Gy or sham irradiation as previously described. Four hours later, the animals will be re-anesthetized, the abdominal cavity opened, and the abdominal aorta above the renal arteries isolated. A loose ligature of umbilical tape will be placed around the abdominal aorta between the renal arteries and the diaphragm. Tight ties will be placed around the superior mesenteric artery and the coeliac artery. A loose tie of umbilical tape and one of 3-0 silk will also be placed around the inferior vena cava between the renal veins and the diaphragm. Two ligatures of 3-0 silk will be placed around the abdominal aorta and the vena cava together, cephalad to the iliolumbar vessels. The rat will then be injected with heparin (1000 USP units/kg) immediately before one ligature on the aorta at the iliolumbar vessels is tightened. The umbilical tape ligature on the aorta below the diaphragm will be tightened next. A small incision will be made in the abdominal aorta between the iliolumbar arteries and the renal arteries. A polyethylene catheter (PE 50 tubing) will then be passed into the aorta and tied in place by the second 3-0 silk ligature around both the aorta and vena cava. The catheter will be attached to the outflow from a pulsatile pump and heater arranged in series. The kidney will be perfused with Krebs-Ringers bicarbonate containing 5.6 mM glucose and 3% bovine serum albumin at a flow rate of 3.0 ml/min and a temperature of 37° C.

Immediately after the perfusion has begun, the umbilical tape ligature on the vena cava below the diaphragm will be tightened. An incision will be made in the vena cava above the renal veins and a catheter (PE 240 tubing) inserted into this

vessel. The catheter will be tied into the vena cava with the 3-0 silk ligature. The bladder will then be drained of urine by direct puncture as previously described and the urine discarded.

The renal bed will be perfused for 1 hour and the urine formed will be collected as previously described. The urine sample will be frozen immediately at -20° C. Urine samples will be thawed as a group and assayed for TXB2 by radioimmunoassay. A 2.0 ml aliquot of renal venous effluent will also be saved and assayed for TXB2 by radioimmunoassay.

The viability of the perfused renal bed will be assessed by determining sodium and potassium concentrations in the urine with a flame photometer (N.I.Lab. Flame Photometer, National Instrument Laboratories, Inc., Rockville, MD) and evaluating the sodium and potassium clearances in the perfused kidneys. The sodium and potassium clearances in the perfused kidneys will be compared to that seen in the intact irradiated and sham irradiated rat. The glomerular filtration rate (GFR) and the renal plasma flow (RPF) will also be determined in the perfused kidneys and in the intact irradiated and sham irradiated rat. The GFR will be determined from the clearance of radiolabelled inulin and the RPF will be determined from the clearance of radiolabelled sodium p-aminohippurate.

Interpretation: If the sodium and potassium clearances, plus the GFR and RPF in perfused kidneys fall within the normal range when compared to intact animals, it will be concluded that the perfused kidneys are viable. If the urine and/or venous effluent show an unchanged arachidonate metabolite concentration, labelled TXB2 will then be added to the perfusate in order to determine if this arachidonate metabolite is being cleared faster by the irradiated kidney. Should the irradiated kidney excrete TXB2 faster than the non-irradiated kidney, it will be inferred that the increased concentration of this arachidonate metabolite seen following irradiation may not be due to altered renal or extrarenal synthesis.

If the irradiated kidneys show increased levels of TXB2, this will suggest that the irradiated kidney is in part responsible for the increased urine concentrations of this arachidonate metabolite. Radiolabelled TXB2 will then be added to the perfusate to see if renal clearance of these cyclooxygenase pathway products is also affected. Also, unlabelled sodium arachidonate will be added to the perfusate to determine if the increase in arachidonate metabolite concentration is due to increased metabolite availability or increased cyclooxygenase activity.

4. Free Radical Scavengers - Cysteamine: Rats will be subjected to graded doses of whole body irradiation and the $LD_{50/30}$ determined. Rats will then be exposed to the $LD_{50/30}$ irradiation dose 30 min after being injected with varying doses of the radioprotectant, cysteamine. The efficacy of several doses of this radioprotectant will be evaluated and the dose of cysteamine that significantly reduces radiation-induced mortality will be used in the free radical scavenger study.

Rats will be injected with the previously determined dose of cysteamine or an equivalent volume of vehicle 30 min before exposure to 20 Gy gamma irradiation or sham irradiation. Urine samples will be obtained, as previously described, 4 hours post irradiation. The urine samples from the cysteamine injected-irradiated rats (protectant-irradiated) will be compared to vehicle injected-irradiated rats (control-irradiated) or to cysteamine injected-non-irradiated animals (protectant-sham) or to vehicle injected-non-irradiated rats (control-sham).

5. Free Radical Scavengers - Glutathione: Rats will be injected with the dose of glutathione that significantly protects against radiation-induced mortality when given 30 min before being exposed to 20 Gy gamma irradiation. Urine samples will be taken 4 hours post irradiation and assayed for TXB2 as previously described. The

urine concentrations of these arachidonate metabolites in glutathione-injected-irradiated rats (protectant-irradiated) will be compared to three other groups, namely, 1) glutathione-injected-non-irradiated rats (protectant-sham), 2) vehicle-injected-irradiated rats (control-irradiated), and 3) vehicle-injected-non-irradiated rats (control-sham).

6. Radioprotectant - WR2721: This section will determine if the radioprotectant WR2721 will alter cyclooxygenase product synthesis when injected at a dose capable of attenuating the radiation-induced mortality rate.

Rats will be injected i.v. with varying doses of WR2721 30 min prior to whole body gamma irradiation. The dose of WR2721 capable of affording optimal radioprotection, as assessed by a shift in the LD50/30 mortality curve, will be determined.

In another group of animals, the optimal dose of WR2721 will be injected i.v. 30 min before the animals are exposed to a level of radiation which significantly alters TXB2 synthesis. Urine samples from WR2721-injected or vehicle-injected irradiated rats will be taken at 4 hours after exposure and assayed for TXB2 by radioimmunoassay.

The urine levels of TXB2 in rats injected with WR2721 plus irradiation will be compared to those levels seen in vehicle-injected plus irradiated rats. The urine cyclooxygenase product levels in these two groups will also be compared to the concentration and rate of release of TXB2 from WR2721-pretreated-sham irradiated and vehicle-injected-sham irradiated animals.

Interpretation: If the protectant-irradiated rats show decreased cyclooxygenase product levels when compared to control-irradiated rats and the same cyclooxygenase product levels as protectant-sham and control-sham groups, it will be concluded that radiation-induced free radical formation is responsible for the altered cyclooxygenase product synthesis seen after irradiation.

Should a decrease in cyclooxygenase product synthesis by protectant-irradiated rats be seen when compared to control-irradiated rats, and should the levels of these arachidonate metabolites be elevated when compared to protectant-sham and control-sham groups, it will be concluded that free radical formation plays some role in altered cyclooxygenase product synthesis but that it is not totally responsible for these altered arachidonate metabolite levels. Additional studies will then be performed using higher doses of the protectant to see if the partial attenuation of altered arachidonate metabolite synthesis is a dose phenomenon.

Protectant-irradiated rats having cyclooxygenase product levels the same as control-irradiated rats and more than the control-sham and protectant-sham groups, will indicate that free radicals do not play a role in the altered cyclooxygenase product synthesis.

If the protectant-irradiated rats have cyclooxygenase product levels that are greater than control-irradiated and if control-irradiated rats have cyclooxygenase product levels that are greater than or equal to protectant-sham animals and if control-sham arachidonate metabolite levels are less than the protectant-sham group, these data will suggest that the radioprotectant is stimulating arachidonate metabolism itself. These results would further indicate that radiation-induced free radical formation either does not play a role in altered cyclooxygenase product formation or is acting to prevent still further increases in arachidonate metabolite synthesis caused by some other radiation-activated mechanism.

7. Vascular Reactivity to U46619: Male Sprague-Dawley rats (200-250 g) will be exposed to 20 Gy whole body gamma irradiation or sham irradiation as previously described. Four hours later, the rats will be re-anesthetized and a segment of abdominal aorta between the diaphragm and renal arteries isolated and removed. The abdominal aortic segment will be cleaned of all adherent fascia and the lumen rinsed of clotted blood. A 2 mm wide segment of the abdominal aorta will be fashioned into a vascular ring.

The ring of aortic tissue will be suspended by hooks fashioned from 30 gauge needles in a water jacketed tissue bath. The tissue bath will be filled with Krebs-Ringers bicarbonate, aerated with 95% O₂ and 5% CO₂, and warmed to 37° C. A one gram preload tension will be applied and the aortic ring will be allowed to equilibrate for 30 min.

After the equilibration period, graded concentrations of the TXA2 mimic, U46619, will be added cumulatively to the tissue bath. The tension developed by the aortic rings in response to 5 x 10⁻⁹ to 5 x 10⁻⁶ M final concentrations of U46619 will be recorded. The tension developed by irradiated aortic tissue will be compared to sham irradiated aortic rings.

Interpretation: An increased responsiveness of irradiated aortic rings to U46619 compared to controls will indicate that vascular responsiveness to cyclooxygenase pathway product vasoconstrictors is augmented by ionizing radiation. The vascular reactivity studies will then be repeated using a non-arachidonate metabolite agonist to determine if this phenomenon is or is not specific for prostaglandins. In addition, aortic ring segment synthesis of TXB2 and 6-keto PGF_{1α} will be determined. Finally, the time between irradiation and vascular reactivity studies will be varied in order to assess the time of onset, and duration of this altered vascular reactivity response to U46619.

Data showing irradiated aortic rings to be less responsive to U46619 than controls will suggest that ionizing radiation affects arachidonate metabolite binding to, or signal transduction in vascular smooth muscle. These results may also indicate that irradiation affects the contractile mechanism of vascular smooth muscle. In order to determine if the contractile mechanism is inhibited by ionizing radiation exposure, vascular responsiveness to norepinephrine will be tested four hours after 20 Gy whole body irradiation or sham irradiation. If vascular reactivity of irradiated vessels is decreased with this and other unrelated vasoconstrictors, it will be concluded that radiation damages the contractile mechanism of vascular smooth muscle. The duration between irradiation and vascular reactivity determination will be varied in order to evaluate the time of onset and duration of altered vascular contractility.

Should aortic rings show decreased responsiveness to U46619 compared to control but unchanged responsiveness to other vasoconstrictors, these results would infer that the U46619 receptor interaction is decreased. In order to determine if the receptors are down regulated in this case, a determination of vascular synthesis of TXB2 and 6-keto PGF_{1α} will be made. If vascular reactivity of U46619 is decreased and TXB2 levels are increased, it will be hypothesized that the TXA2 is down regulating the receptors thereby decreasing the responsiveness to U46619. If the vascular reactivity to U46619 is decreased and the 6-keto PGF_{1α} levels are increased, it will be concluded that the decreased responsiveness to U46619 may be due to the vasodilatory effect of increased PG₁₂ synthesis.

Should exposure of irradiated vascular segments to U46619 result in an unchanged developed tension when compared to non-irradiated aortic rings, it will be concluded that either ionizing radiation does not affect vascular reactivity to cyclooxygenase pathway products or there is a release of vasodilator substances that

counter-balances the vasoconstrictor effects of this TXA2 mimic. If this response is seen, it may also be concluded that altered responses to U46619 occur before four hours and are over by then, or occur after four hours post irradiation. The time between irradiation and vascular reactivity responses to U46619 will be varied to see if this latter possibility is true. Also, vascular synthesis of TXB2 and 6-keto PGFla will be performed to see if there is a decrease in TXA2 synthesis or an increase in PGI2 synthesis four hours post irradiation.

8. Vascular Synthesis of Arachidonate Metabolites: Rats will be anesthetized, irradiated with 20 Gy whole body irradiation and the abdominal aortic segments removed as previously described. The aortic ring will be fashioned from a 2 - 3 mm segment of the isolated aorta and transferred to 0.5 ml Krebs-Ringers bicarbonate which has been pre-warmed to 37° C. The aortic ring will be incubated in 0.5 ml Krebs-Ringers bicarbonate for 15 min. After the incubation period, the incubate will be frozen at -20° C and the aortic ring weighed. Both the wet and dry weight of the tissue will be determined. The incubates will be thawed as a group and the TXB2 and 6-keto PGFla concentrations determined by radioimmunoassay. The TXB2 and 6-keto PGFla synthesis will be expressed as nanomoles per milligram tissue. The arachidonate metabolite released from irradiated tissue will be compared to the synthesis by sham irradiated controls.

Interpretation: An increase in both TXB2 and 6-keto PGFla synthesis by irradiated aortic rings, would infer that ionizing radiation increases cyclooxygenase pathway enzyme activity or substrate availability. The vascular rings from irradiated and sham irradiated rats will then be incubated with sodium arachidonate and the cyclooxygenase pathway product synthesis determined. An increased TXB2 and 6-keto PGFla release by irradiated vascular tissue incubated with arachidonate would suggest that cyclooxygenase pathway enzyme activity is increased. If incubation with arachidonate results in unchanged or decreased TXB2 and 6-keto PGFla synthesis compared to controls, it will be concluded that ionizing radiation has no effect on cyclooxygenase pathway enzyme activity but increases substrate availability by increasing arachidonate release from membrane phospholipids.

Should TXB2 synthesis increase and 6-keto PGFla release decrease in irradiated vessels compared to controls, it will be inferred that irradiation inhibits prostacyclin synthetase activity but increases arachidonate release and/or oxygenation by cyclooxygenase. A determination of classical prostaglandin synthesis by vascular tissue will be performed to confirm this hypothesis.

If TXB2 synthesis is decreased and 6-keto PGFla release is increased in irradiated vessels compared to controls, it will be concluded that irradiation inhibits thromboxane synthetase but increases arachidonate release and/or cyclooxygenase activity. In order to test this hypothesis, irradiated and non-irradiated aortic rings will be incubated in Krebs-Ringers bicarbonate and the vascular synthesis of PGF2a and PGE2 determined.

A decreased release of TXB2 and 6-keto PGFla from irradiated vascular tissues would indicate that arachidonate release from membrane phospholipids is inhibited, or cyclooxygenase activity is decreased, or prostacyclin and thromboxane synthetase activity are both reduced. Irradiated vessel rings will then be incubated with exogenous arachidonate and the synthesis of TXB2 and 6-keto PGFla by irradiated and control aortic rings determined. If TXB2 and 6-keto PGFla release from irradiated rings is still less than control rings, it will be hypothesized that ionizing radiation reduces cyclooxygenase and/or both prostacyclin and thromboxane synthetase activities.

If both TXB2 and 6-keto PGFla release from irradiated aortic rings is unchanged from that seen with control vessels, it will be inferred that ionizing radiation does not affect vascular cyclooxygenase product synthesis or that the alterations in arachidonate metabolism occur before or after four hours post irradiation. The time between irradiation and vascular incubation will then be varied in order to determine if alteration in arachidonate metabolism occur following whole body gamma irradiation.

9. Statistical Analysis:

- A. The regional shielding studies involve a comparison of three groups to one control group, therefore, these data will be analyzed using the analysis of variance followed by a Dunnett's test.
- B. The effect of whole body irradiation on renal synthesis of TXB2 involves the comparison of this arachidonate metabolite concentration in the urine of irradiated perfused kidneys to the levels of this same cyclooxygenase product in sham irradiated perfused organs. These data will be evaluated by the unpaired Student's t-test.
- C. The effect of free radical scavengers on TXB2 synthesis in irradiated and non-irradiated rats will be compared to the urine concentrations of this cyclooxygenase pathway product in vehicle-injected irradiated and vehicle-injected sham irradiated controls. Since comparison of four groups is necessary, an analysis of variance followed by a Newman-Keuhl's test will be performed for statistical analysis.
- D. The vascular reactivity studies in irradiated and sham irradiated aortic segments will have the EC50 determined by probit analysis (54). The EC50 for U46619 in irradiated compared to non-irradiated vascular tissue will be compared by the unpaired Student's t-test.
- E. Vascular synthesis of TXB2 and 6-keto PGFla by irradiated and non-irradiated aortic tissue will be assessed by the unpaired Student's t-test.

In all experiments, the confidence interval will be set at 95%.

SIGNIFICANCE

The projected studies for the second year of this contract are logical continuations of the experiments performed during the first year. The projected studies will:

1. Determine the roles that organs of the abdominal cavity and thoracic cavity play in radiation-induced increases in urine cyclooxygenase pathway product concentrations.
2. Determine the role that the kidney plays in altering urine and vascular concentrations of arachidonate metabolites following whole body gamma irradiation.
3. Determine the effect of ionizing radiation on vascular synthesis of cyclooxygenase pathway products.
4. Determine the effect of whole body irradiation on vascular reactivity to vasoactive substances.

These experiments will provide necessary data for the evaluation of the effect of ionizing radiation on in vivo arachidonate metabolite synthesis. In addition, these studies will examine the degree of vascular impairment occurring following radiation exposure, and provide clues to the pathogenesis of several of the components of acute radiation injury.

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