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THE EFFECTS OF RADIATION ON THE CONTRACTILE ACTIVITY OF GUINEA PIG MESENTERIC LYMPHATICS

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ABSTRACT

In order to assess the effects of irradiation on lymphatic function, the contraction frequency and maximum and minimum diameters of guinea pig mesenteric collecting lymphatic vessels were measured in vivo *4 hours after 1000 rads of abdominal irradiation. The mean contraction frequency for lymphatics from irradiated guinea pigs (7.6±0.7) was significantly higher than for normals (non-irradiated) (4.7±0.7) during an initial control observation period, but there was no difference in maximum or minimum diameter between the two groups during this period. Topical application of 10^{-4}M noradrenaline (NA) significantly increased contraction frequency in both groups; lymph vessel diameter significantly decreased after NA in irradiated, but not in normal guinea pigs. Intravenous infusion of calcium dobesilate (200 mg/kg) caused a significant increase in the contraction frequency of lymphatic vessels in both normal (to 9.4±1.5) and irradiated (to 9.8±1.2) animals, but diameter was not significantly altered. Thus, lymphatic vessels from irradiated guinea pigs were still responsive to exogenous stimuli 4 hours post-irradiation and were initially pumping more actively than those from normal guinea pigs, presumably in response to radiation-induced edema. They also exhibited a supersensitivity to the vasoconstrictive effects of NA, perhaps due to an alteration of the pacemaker or smooth muscle cells by irradiation.*

X-ray and gamma irradiation have been shown to cause acute edema and an inflammatory response in the bowel wall of experimental animals (1,2) and may also contribute to chronic secondary limb edema in humans (3). Chronic edema in humans may result from radiation-induced fibrotic changes in surrounding tissues (over several years), leading to compression, distortion, and obstruction of local lymphatic vessels, and hence, swelling of the affected region (3,4). Damage to lymphocytes by radiation was cited as the most probable cause for the fibrosis found in intestinal tissue of dogs exposed to 32 Gy of Cobalt radiation (5). The acute response to abdominal irradiation, however, is a hyperpermeability of intestinal blood capillaries, with subsequent edema of the surrounding tissue spaces for up to 24 hours post-irradiation (6). We have shown that the most significant increase in interstitial volume of the bowel wall (as indexed by C^{14}-inulin accumulation) was at 4 hours post-irradiation (2). Thus, the focus of this study was to examine the effects of this immediate edema, as the acute effects of irradiation on lymphatic vessels of the mesentery are not known. If the lymphatic vessels are unaffected by irradiation, it is presumed that they will increase their activity in order to remove the excess fluid that accumulates in the edematous bowel wall. However, if blood vessel permeability is compromised by irradiation, it is possible that the lymphatic vessels may also be directly affected and function suboptimally as a consequence.

The mesenteric collecting lymphatics are responsible for propelling lymph derived from the bowel wall back to the systemic venous circulation. These pre-nodal collecting vessels are large and exhibit spontaneous contractility. Because the lymphatics are prominent and accessible, much information has been gathered about them, and parameters such as contraction frequency, intraluminal lymphatic pressure and vessel diameter can be measured to assess their functional activity. Previous investigations have used these indicators to assess the response of the mesenteric "lymph pump" to various types of edema and drug treatments (7-10). The aim of this study was to measure contraction frequency and diameter of mesenteric lymphatic vessels in order to assess the response of the lymphatics to abdominal irradiation and to the application of lymphatic stimulants.

Various substances, such as benzopyrones and calcium dobesilate, stimulate the lymphatic system and can reduce the severity of high protein edemas (11). Such substances can be used as tools to assess the integrity of certain components of lymphatic function. The classic neurotransmitter noradrenaline (NA) has been shown to stimulate lymphatic vessels by increasing their contraction frequency while increasing their resistance to flow (12-14). NA acts through both alpha-1 and alpha-2 adrenoreceptors on lymphatic smooth muscle cells to stimulate contractile activity; therefore, it can be used to test the responsiveness of the receptor-smooth muscle component of the lymphatic vessel wall. Calcium dobesilate (doxium; calcium dihydroxy-2,5 benzenesulphonate) shows clinical similarities to the benzopyrones; that is, it reduces the volume of swelling associated with lymphedema in humans (11). Piller and Browning (8) examined the effects of doxium on the pumping of mesenteric lymphatics in guinea pigs and found that acute IV administration of 200 mg/kg caused a statistically significant decrease in lymphatic vessel contraction frequency. It was also found to significantly increase the time the lymphatic vessel spent at maximal luminal closure during the contractile cycle; that is, it appeared to make the vessel contractions stronger (8). NA and calcium dobesilate, both shown to affect lymphatic vasculature, were employed to detect any potential differences between lymphatic vessels from irradiated and non-irradiated experimental animals.

MATERIALS AND METHODS

Surgical Preparation

Guinea pigs (250-650g) were housed in an environmentally controlled room and allowed free access to food and water up to two hours before experimental use. They were anesthetized with intramuscular (IM) injections of diazepam (Kabi Pharmacia; 0.5 ml/kg of 5 mg/ml), ketamine (Apex Lab.; 0.36 ml/kg of 100 mg/ml) and xylazine (Bayer; 0.5 ml/kg of 20 mg/kg); IM supplements of one-half of the initial dosage of ketamine and xylazine were administered as needed, approximately every hour. A tracheostomy was performed to facilitate spontaneous respiration and the right carotid artery and jugular vein were cannulated for measurement of arterial blood pressure and infusion of drugs, respectively. The animal's core body temperature was maintained at 37.5°C.

A loop of small intestine and adjacent mesentery were exteriorized via a lateral laparotomy and placed in a temperature controlled bath maintained at 37.5°C. The mesentery was draped over a pedestal, with a 21 mm diameter glass viewing window. The bath was continuously perfused with Phosphate Buffered Saline (PBS; 0.14M NaCl. 7.5mM Na_2HPO_4, 2.5mM $NaH_2PO_4.H_2O$, pH 7.4, 37.5°C) at a rate of 1.0 ml/min. The exteriorized bowel and portions of the mesentery not under view were covered with transparent plastic film (Suran, USA). Mesenteric lymphatic vessels were observed from a distance of 1 mm to 30 mm proximal to the mesenteric margin of the small intestine. Only lymphatic vessels that exhibited spontaneous contractility were chosen for observation.

in Vivo Measurements

A Zeiss ACM microscope with a Leitz 10x lens was used to visualize the mesenteric lymphatic vessels. The microscopic field was imaged by a video camera (National WV-1550E/A) and recorded on a video tape recorder (National AG-6200) for subsequent analysis. Contractions, i.e., an obvious and sustained decrease in diameter, were observed from the videotape, and their frequency was measured in beats per minute; vessel diameter was measured at minute intervals from the freeze frame images and averaged. All diameter measurements were taken at points between lymph valves, which best represented the average diameter of lymph vessels viewed.

Experimental Protocol

Guinea pigs were randomly divided into two groups: normal (i.e., not irradiated) and irradiated. Lymphatic vessels from each animal were observed either; a) for a control period; b) after topical application of noradrenaline (NA); and/or c) after IV infusion of calcium dobesilate. Control measurements of contraction frequency and diameter were made to establish baseline values. Topical application of noradrenaline (30 µl of 10^{-4} M NA), or of 30 µl of PBS alone, was delivered by a glass micropipette placed directly above the lymphatic under view. In some guinea pigs, an IV infusion of calcium dobesilate (200 mg/kg in a 100 mg/ml solution), or equivalent volumes of PBS alone, was administered over a 30 second interval.

Irradiation

Guinea pigs were anesthetized with IM injections of ketamine (0.18 ml/kg) and xylazine (0.25 mg/kg) and exposed to 1000 rads of deep X-ray irradiation in an unfractionated dose of 160 rad/min for 6.25 minutes. X-rays were generated at 100Kv, 5.5 mA from a Phillips X-ray head (Type 9811/201/0001, tube 4512/140/05921) and exited through a 1 mm beryllium window positioned 21 cm above the mid abdominal horizontal plane. X-rays were delivered to a localized area of the abdomen directly under a 6.5 cm diameter circular hole in a 3 mm lead shield designed to protect the rest of the supine animal's body from irradiation. *In vivo* microscopy measurements of mesenteric lymphatic vessel contraction frequency and diameter were made as for the normal group, at 4 hours post-irradiation [a time known to be associated with bowel wall edema (1,2)].

Data Analysis

Data were analyzed by two way repeated measures ANOVA, one way ANOVA, or paired or normal t-tests, as appropriate. Results are presented as mean ± standard error (SE).

RESULTS

Blood Pressure

The mean arterial blood pressure for normal guinea pigs was 74.2±2.1 mmHg and was not significantly different from irradiated guinea pigs (77.3±3.3 mmHg; two sample t-test, t_5=2.2, p=0.45). The IV infusion of calcium dobesilate had no effect on blood pressure in normal animals, but caused a transient (for one minute) increase in the irradiated group immediately following the infusion of the drug.

Baseline Measurements

Examples of lymphatic contractions are given in *Fig. 1*. The contraction frequency of all lymphatic vessels declined significantly over the first 10 minutes of observation [2-way ANOVA, time (0-10 min.) vs. groups,

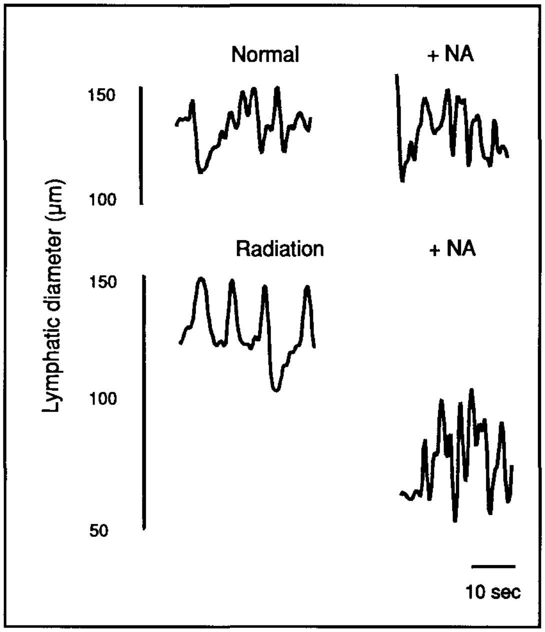

Fig. 1. Diameter (µm) of individual in vivo lymphatic vessels from normal and irradiated guinea pigs before and immediately after topical application of noradrenaline (NA, 30 µl of 10^{-4}M).

$F_{(9,100)}$=3.6, p <<0.01], but stabilized thereafter. This effect was consistent across both normal and irradiated groups [interaction effect, $F_{(9,100)}$=1.2, p=0.32]. The contraction frequency of lymphatic vessels in irradiated animals (7.6±0.7, n=6) was significantly higher than in non-irradiated animals (4.7±0.7, n=6) during the first 10 minutes of observation [2-way ANOVA, $F_{(1,100)}$=63.2, p <<0.01]. This difference in contraction frequency, however, diminished over time of observation.

Both maximum and minimum diameters remained constant for at least 10, and often up to 30 minutes after commencing observations. There was no significant change in maximum and minimum diameters over the first 10 minutes of observation for both groups combined [2-way ANOVA; for maximum diameter, effect of time $F_{(9,100)}$=0.06, p=1.00; for minimum diameter, effect of time $F_{(9,100)}$=0.35, p=0.95]. There was no significant difference in maximum and minimum diameters between normal and irradiated groups [2-way ANOVA; maximum diameter $F_{(1,9)}$=2.75, p=0.10, minimum diameter $F_{(1,9)}$=2.92, p=0.09] over the control observation period.

Noradrenaline (NA)

Topical application of 30 µl of 10^{-4} M NA caused an immediate and significant increase in contraction frequency of lymphatic vessels in both groups *(Fig. 2a)*. The maximum increase in contraction frequency caused by NA was not significantly different in normal (7.0±1.7) compared to irradiated animals (7.2±1.1, two sample t-test, t_5=2.2, p=0.9). NA commonly caused different sections of the lymphatic vessel to contract in an erratic and unconcerted fashion *(Fig. 1)*. Topical application of 30 µl of PBS had no significant effect on contraction frequency or vessel diameter.

NA had no significant effect on maximum or minimum diameter in the normal group, but caused a significant decrease in maximum and minimum diameters of the irradiated group within 1 minute of application which then returned to baseline values within 3 minutes *(Fig. 2b)*.

Calbium Dobesilate

IV infusion of 200 mg/kg calcium dobesilate increased lymphatic contraction frequency in both groups *(Fig. 3)*. In vessels from non-irradiated animals, there was a significant increase in contraction frequency within 3 minutes after calcium dobesilate infusion, which reached a peak of 9.4±1.5 at 6 minutes post calcium dobesilate treatment. The vessels of irradiated animals showed a significant increase at 5 minutes post calcium dobesilate infusion, which reached a peak of 9.8±1.2 at 9 minutes. Contraction frequency remained elevated for the remainder of the observation period (up to 30 minutes after infusion). Calcium dobesilate had no effect on lymphatic diameter. No significant change in contraction frequency or vessel diameter occurred in either group as a result of IV infusions of equivalent volumes of PBS alone.

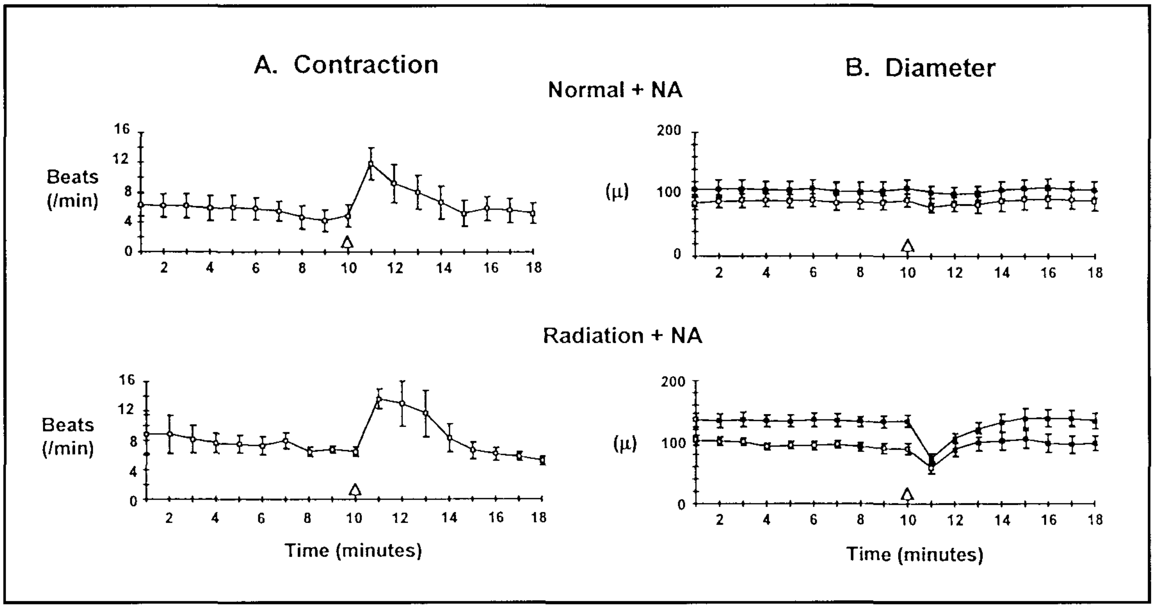

Fig. 2. A. Topical application of NA (30 μl of 10^{-4}M) significantly increased contraction frequency within one min in vessels from both normal and irradiated guinea pigs (paired t-test for pre NA vs. 1 min post NA; t_5=4.7, p=0.003; t_5=6.8, p<<0.01, respectively). B. Maximum (■) and minimum (□) lymphatic diameters were not affected by NA in normal guinea pigs, but decreased in irradiated animals within one min of application (paired t-test for pre NA vs. 1 min post NA; t_5=4.4, p=0.004; t_5=2.8, p=0.004, respectively).

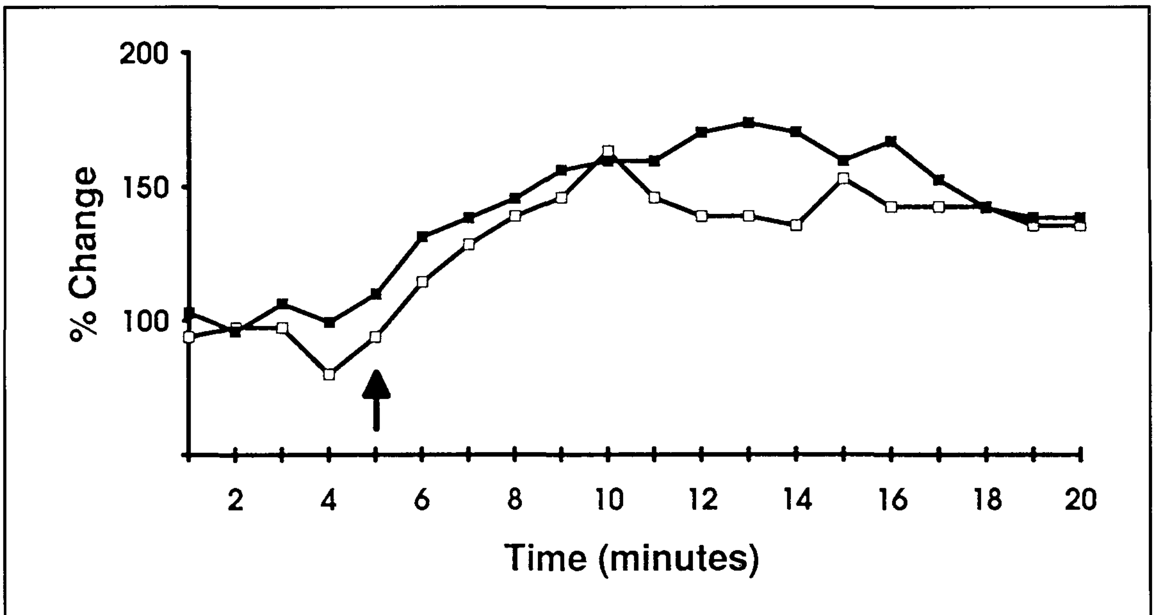

Fig. 3. IV administration of calcium dobesilate (200mg (↑) significantly increased contraction frequency within 3 min in normal guinea pigs (□); paired t-test for pre calcium dobesilate vs 3 min post calcium dobesilate; t_6=2.7, p=0.02), and 5 min in irradiated guinea pigs (■; t_4=2.8, p=0.02), but had no significant effect on lymphatic diameters.

DISCUSSION

As a consequence of abdominal irradiation, the small intestine undergoes an inflammatory response with an associated edema (1,2,6). Buell and Harding (1), demonstrated an increased macromolecular permeability of blood capillaries in the small intestine after abdominal irradiation, and Lee et al (2) found a significantly increased interstitial volume at 4 hours post-irradiation (using C^{14}-labeled Inulin as an index for interstitial volume). In this study, mesenteric lymphatic contractile activity was increased at 4 hours post abdominal irradiation, as indicated by the significantly higher mean contraction frequency of lymphatic vessels in irradiated guinea pigs. Benoit et al (9) demonstrated that the characteristic response of mesenteric collecting lymphatic vessels to an acute period of induced edema (via hemodilution) was to increase their contraction frequency and stroke volume. This response had the effect of increasing the rate of lymph flow from the bowel back to the vascular system via the thoracic duct (10). The increased contraction frequency of the collecting lymphatic vessels of the mesentery after irradiation is consistent with the behavior of lymphatic vessels responding to edemagenic stress. There was no significant difference in lymphatic vessel diameter between the two groups, but the vessels were contracting at a significantly higher rate after irradiation, presumably in response to excess interstitial fluid. This contractile activity suggests that the mesenteric lymphatic vessels were still capable of responding to an edematous workload 4 hours post-irradiation.

The contraction frequency of collecting lymphatic vessels is significantly increased by exogenous topical applications of NA (12). NA also causes lymph vessel constriction, as evidenced by increases in the perfusion pressure of lymph vessels during intralymphatic infusion of NA (15). McHale and Roddie (12) have shown that the dose-response curve for the stimulatory effects of NA on lymph vessel contractility is an inverted U-shape. In other words, there is an optimal dose (10^{-5} M) which produces the peak contraction frequency. Above and below this dosage the increase in contraction frequency is not as great, with very high doses (10^{-2} M) actually halting vessel contraction altogether. Topical application of NA significantly increased contraction frequency to the same degree in both normal and irradiated groups in this study. However, it significantly reduced maximum and minimum diameters in irradiated guinea pigs only, having no significant effect on vessel diameter in normal (non-irradiated) guinea pigs. Given that the smooth muscle cells lining the wall of collecting lymphatics have alpha adrenoreceptors which respond to NA (12), it could be that the receptor-smooth muscle complex is affected by irradiation, making them supersensitive to the vasoconstrictive effects of NA. For example, an alteration in receptor-mediated contractility could shift the inverted U-shaped dose response curve to the right, making the relatively high dose of 10^{-4} M NA induce stronger contractions in irradiated guinea pigs than in normals.

Exogenous application of NA in this study resulted in an increase in contraction frequency in both groups, but it also caused different adjacent sections of the lymphatics to contract in an erratic and unconcerted fashion. By histochemical (16) and electrophysiological (17) methods, the area of collecting lymphatic vessels directly around the lymphatic valve often lacks any kind of musculature. If there is a discontinuity of myoepithelium from one lymphangion to the next, then it is unclear how an electrical impulse or signal from a pacemaker cell is transmitted from one lymphangion unit to another (17). It has recently been suggested that contiguous short segments (of at least 80 mm) of bovine lymphatics may have their own independent pacemakers capable of mutual entrainment (18). If these pacemakers respond to exogenous NA, as appears to be the case (15), disruption of entrainment between segments may occur, resulting in erratic and uncoordinated contractions, as found in this study.

IV infusion of calcium dobesilate significantly elevated lymphatic contraction frequency in both normal and irradiated guinea pigs, which implies that the component of the mesenteric lymphatic system that responds to calcium dobesilate is still intact 4 hours after irradiation. The increase in contraction frequency caused by calcium dobesilate in this study was contrary to other findings (8), in which contraction frequency was significantly reduced after IV infusion of 200 mg/kg calcium dobesilate. Given that calcium dobesilate increases thoracic duct lymph flow in dogs and guinea pigs (19), one of its actions must be to accelerate lymph production or collector lymph flow, albeit by means which have yet to be established. One possibility involves an increase in lymph vessel contraction frequency, a phenomenon associated with increased lymph flow (10).

Lymphatic vessel diameter remained relatively constant throughout our experiments, with the only significant response being a constriction after topical application of NA in irradiated animals. Moderate changes in diameter of any given lymphangion segment will alter the amount of lymph traveling through that segment at a given time; formulas exist to derive 'lymph flow' from contraction frequency and changes in lymph vessel diameter [or 'stroke volume'; e.g. (9)]. Such calculations of lymph flow were not made in this study, because lymph flow (evident as the movement of chylomicra or particulate matter) was occasionally observed in lymph vessels which were not consistently contracting. Moreover, a lack of lymph flow was also observed in lymph vessels that were contracting normally (possibly due to back pressure from downstream vessels). Occasionally, lymph flow appeared to reverse and become retrograde. Thus, lymph flow did not consistently appear to be directly related to lymphatic vessel contraction.

In summary, the contraction frequency of mesenteric lymphatic vessels in guinea pigs four hours post irradiation was significantly increased over lymphatic vessels in non-irradiated guinea pigs, presumably in response to radiation induced intestinal edema. That lymphatic vessels from irradiated guinea pigs responded to edema and to lymphogogues implies that they were still functional 4 hours post irradiation.

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