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MEASUREMENT OF CANINE INTESTINAL CAPILLARY BLOOD FLOW IN THE TERMINAL PHASE OF THE GASTROINTESTINAL RADIATION SYNDROME

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13. ABSTRACT

The objective of this study was to test a method of measuring the distribution of intestinal capillary blood flow. Adult male beagles were divided into two groups, one of which was sham irradiated while the other was subjected to 1500 rads of whole-body pulsed gamma-neutron radiation. Seventy-two hours postirradiation, radioactive-tagged microspheres were injected directly into the left ventricle of the heart. After 1 minute, the circulation was stopped; the intestine was removed; and the amount of radioactivity was measured in the mucosal and muscularis parts of the upper, middle, and lower small intestine. Data were compared with those from sham irradiated control animals. The results showed that the intestinal capillary blood flow (in percent of cardiac output) of the irradiated dogs was higher than that of the nonirradiated dogs. Although the weight of the mucosal layer of the small intestine decreased significantly postirradiation, its relative capillary blood flow remained at the preirradiated level, indicating increased capillary blood flow per unit weight of mucosa. The method was successfully applied to the measurement of mucosal flow as a function of cardiac output and can be applied to the study of the pathophysiology of a variety of stresses including irradiation.

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I. INTRODUCTION

In shock, one of the major hemodynamic compensatory mechanisms is increased peripheral resistance due to vasoconstriction,[10] the distribution of which varies in different species.[5] For instance, vasoconstriction in the small intestine of dogs is disproportionately increased during hemorrhagic or endotoxin shock, and intestinal ischemia is frequent.[1,2] Conversely, intestinal ischemia itself is able to induce shock in dogs.[11,15] Shock ending in cardiovascular collapse is a characteristic feature of the gastrointestinal syndrome,[9] but the role of these vascular changes in the progressive deterioration of the gut is not known.

The objective of this study, therefore, was to measure the presence or absence of intestinal ischemia by measuring the distribution of small intestinal capillary blood flow in the terminal phase of the gastrointestinal syndrome (GI-S), and to test, thereby, the applicability of the tagged microsphere method of study to problems of this type.

II. MATERIALS AND METHODS

Eleven male beagles, 12-14 months old and weighing 11.0 ± 1.0 kg, were divided into two groups: (1) five in the sham irradiated control group and (2) six in the irradiated group. The dogs were housed individually and fed food and water ad libitum until the night before irradiation or sham irradiation.

At approximately 1 hour before irradiation, the animals were placed in Lucite restraining cages and transferred to the exposure room of the AFRRI-TRIGA reactor where they received 1500 rads (midline tissue dose) of pulsed mixed gamma-neutron radiation. The dogs were positioned on an isodose exposure curve about the reactor

core with the center line of the restraining cages 200 cm from the vertical core center line. The AFRRI-TRIGA reactor and exposure facilities have been described elsewhere.[19] The midline tissue dose for each exposure was calculated as the product of two factors: tissue kerma, free-in-air, times 0.81. The variation in tissue kerma, free-in-air, from position to position in each exposure group was less than 4 percent from the mean. Approximately 60 percent of the tissue kerma, free-in-air, was from gamma radiations having an effective energy between 1 and 2 MeV. Approximately 10 percent was from neutrons with energies greater than 3 MeV, 10 percent from neutrons with energies between 1.5 and 3 MeV, 10 percent from neutrons with energies between 0.01 and 1.5 MeV, and 10 percent from slower neutrons. About 80 percent of the dose was delivered in less than 70 msec. The method for dosimetry has been delineated by Fitchford and Thorp.[17]

Sham irradiated dogs were positioned in the reactor exposure room in the same manner and handled, after sham exposure, in the same way as irradiated dogs.

After exposure, the dogs were returned to their cages. During the postirradiation period, all animals were free to consume food and water ad libitum. Prior to the experimental procedure, food was withheld from both groups overnight (16 hours).

The fractional distribution of regional capillary blood flow of the small intestine was determined at 72 hours postirradiation, using a modification of the technique of Grim and Lindseth.[7] Anesthesia was induced by intravenous injection of 30 mg/kg pentobarbital. Following a brief rest period of about 30 minutes, microspheres* tagged with 50 μCi of ytterbium-169, and 15 ± 5 μm in diameter, were injected through a

* 3M Company, Nuclear Products for Medicine, St. Paul, Minnesota

catheter inserted via the femoral vein into the left ventricle. The position of the catheter was verified by obtaining the ventricular pressure curve. One minute after injection, the heart was stopped by the intracardiac injection of saturated KCl. The abdomen was opened and the whole small intestine was removed, washed through a cut along the mesenterium and blotted on filter paper. The mucosal layer was carefully separated from the muscularis by scraping. The small intestine was divided in three equal parts approximating the duodenum, jejunum and ileum. Each section was weighed, cut into small pieces and placed into a counting tube. The samples were counted in a Nuclear-Chicago dual channel gamma analyzer.

The fractional distribution of cardiac output (C.O.) to the different segments of the small intestine was calculated by the following equation according to Wagner et al.:[20]

$$\text{percent C.O.} = \frac{q}{Q} \times 100$$

where q = intestinal cpm and Q = total injected cpm. The microspheres used in this study were large enough to be trapped completely in any capillary of the small intestine, yet were small enough to pass through any open arteriovenous shunts. Therefore the measurement reflects the actual capillary or nutrient blood flow only.

To evaluate and confirm the radiation-induced intestinal injury, the following supportive studies were undertaken: (1) regular histological sections and (2) Microfil* preparations of the intestinal vasculature.

For statistical analysis of the data obtained, the mean and standard deviations were calculated and a t-test was performed.

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* Canton Bio-Medical Products, Boulder, Colorado

III. RESULTS

At 72 hours postirradiation, the fractional distribution of cardiac output in the small intestine of irradiated animals was greater than that in the sham irradiated controls (Table I). A similar change can be seen after calculating the small intestinal capillary blood flow according to its regional distribution; namely, in the mucosa and in the muscularis layers.

Table I. Percent Distribution of Cardiac Output in Small Intestine of Dog

Tissues	Percent distribution of cardiac output		
	Control sham irradiated	72 hours postirradiation	p value
Mucosa	1.92 ± 0.51	3.54 ± 1.52	< 0.05
Small intestine without mucosa	2.26 ± 0.61	3.40 ± 0.65	< 0.05
Total small intestine	4.18 ± 1.00	6.94 ± 2.01	< 0.05

The mucosal layer of the small intestine was considerably decreased in weight as a result of both cellular destruction and massive water loss. In contrast, a small decrease was measured in the muscularis (Table II). Histological sections confirmed mucosal cell destruction. Despite the epithelial cell destruction, the villous capillary network still appeared to be intact and continuous, using the technique of perfused Microfil (Figure 1). There was no evidence of capillary rupture and extravasation of the plastic material. Only a few small areas of the villous capillary network were not filled completely due to plug formation and/or actual capillary destruction.

Table II. Weight Changes Induced by 1500 Rads Whole-Body Gamma-Neutron Radiation at 72 Hours Postirradiation

	Sham irradiated	Irradiated	p value
Body weight (kg) Initial	11.64 ± 0.72	11.72 ± 1.40	>0.1
Body weight (kg) Final	11.55 ± 0.46	10.42 ± 1.43	>0.1
Mucosal part of small intestine (g)	96.33 ± 10.68	26.73 ± 9.65	>0.001
Muscularis part of small intestine (g)	173.88 ± 13.36	151.82 ± 11.57	>0.05

Figure 1. Microfil cast of the intestinal vascular bed showing a cross section of an irradiated dog's ileum at 72 hours postirradiation. Note the lack of filling in the villi capillaries without extravasation of the plastic material.

The ratio of the weight of the mucosal layer to total intestinal weight in the duodenum, jejunum, and ileum was 12.67, 15.46, and 16.11 percent at 72 hours postirradiation in contrast to 35.35, 33.92, and 36.67 percent in the sham irradiated controls (Table III). The corresponding regional distribution of the mucosal capillary blood flow (calculated in percentage of the total capillary blood flow in a particular segment) did not show any significant alteration after radiation injury (Table III). Despite the

Table III. Percent Distribution of Mucosal Weight and Capillary Blood Flow in Different Segments of Small Intestine of Dog at 72 Hours Postirradiation

		Percent mucosal weight and capillary blood flow (CBF) of an intestinal segment		p value
		Sham irradiated	Irradiated	
Duodenum	weight	35.35 ± 3.16	12.67 ± 3.71	< 0.001
	CBF	50.25 ± 7.99	49.62 ± 10.72	> 0.1
Jejunum	weight	33.92 ± 5.43	15.46 ± 4.48	< 0.01
	CBF	36.25 ± 11.25	50.68 ± 12.01	> 0.1
Ileum	weight	36.67 ± 1.00	16.11 ± 6.93	> 0.001
	CBF	44.03 ± 5.73	43.52 ± 5.81	> 0.1

remarkable decrease of the mucosal mass of the small intestine, the blood flow of the mucosal and muscularis layers of the duodenum, jejunum and ileum in the irradiated dogs exhibited the same proportional blood flow distribution as in the sham irradiated controls. This is a reflection of the relative hyperemia of the mucosal part of the small intestine during the terminal phase of the GI-S.

IV. DISCUSSION

One of the predominant pathological alterations during the 4-day period of the GI-S is the structural breakdown of the small intestine.[16,18] It was generally accepted

until recently that the denudation of the intestinal epithelium leads directly to a loss of fluid and electrolytes. This concept was challenged by Lushbaugh[14] who stated that "Intestinal mucosal loss would seem to have little relation to the underlying lesion which must be located in the vascular system." Indeed, radiation-induced morphological and functional blood vessel alterations have been observed about the 3rd day postirradiation.[4,13] However, during the first 2 days postirradiation, hemodynamic homeostasis is remarkably well maintained.

Circulatory deterioration begins about the 3rd day postirradiation and ends in circulatory collapse.[3,14] Hemodynamic alterations of the terminal phase of the GI radiation injury resemble those of severe hemorrhagic shock. A decreasing baroreceptor stimulation causes increased sympathetic activity to both heart and systemic resistance vessels, resulting in improved cardiac output and systemic blood pressure by peripheral vasoconstriction.[10] The magnitude and distribution of the peripheral resistance in shock is characteristic of the particular species. It is known that, in the dog, a disproportionately increased small intestinal vasoconstriction may be manifested.[1,2] This compensatory hemodynamic regulation might play a role in the vicious cycle of shock because intestinal ischemia itself can lead to circulatory collapse.[11] It was previously reported that at 72 hours postirradiation, under the same experimental condition as described here, the cardiac output was decreased about 40 percent below the control, nonirradiated values.[12] However, since in a compensatory manner the total peripheral resistance increased, the mean blood pressure remained basically unaltered. Whether the progressively increased peripheral vasoconstriction reflected a disproportionately increased intestinal resistance similar to that in other shock

states is a question that remains unanswered. It should be emphasized that such increased vasoconstriction would be detrimental to the high nutritional requirements of the intestinal mucosa at 72 hours postirradiation[8] which requires adequate capillary blood flow for regeneration.

The Microfil technique indicates virtual continuity of the capillary network at 72 hours postirradiation. Therefore, the applied microsphere technique is a better indicator of capillary blood flow than the utilization of the ^{86}Rb extraction technique[6] due to the possible radiation-induced increased capillary permeability.

The microspheres (15 ± 5 μm in diameter) that were used in this study were large enough to be trapped completely in the intestinal capillaries, yet small enough to pass through any arteriovenous shunt. Thus, the method described should give an accurate estimate of the relative capillary blood flow if the following pitfalls are avoided: (1) loss of microspheres from some impaired capillaries into the lumen or the perivascular space and (2) recirculation if radiation impairs the lung microsphere-trapping capacity. In this study, these errors were ruled out in the following way: the lumen washout and the arterial blood did not show radioactivity, and there was no evidence for extravasation of microspheres by microscopic examination.

Cardiac output was not measured in this study; thus the microsphere technique permitted only the measurement of the relative intestinal capillary blood flow. The data show that the relative capillary blood flow of the small intestine at 72 hours postirradiation was higher, compared with the control values. Even if we take into account the considerably large decrease of cardiac output as measured in a previous study under identical experimental conditions, it appears that the intestinal capillary blood

flow was not significantly altered in the terminal phase of the GI-S. In addition, despite significant intestinal weight loss, the ratio of mucosal to muscularis capillary blood flow was unchanged. However, although mucosal blood flow does not decrease as much as cardiac output, no statement can be made as to the adequacy of the blood supply. Nevertheless, it has been clearly demonstrated that this method of studying blood supply changes is applicable to the problem of determining the vascular factors in postirradiation pathophysiology.

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