X-radiation effect on the vascularization of submaxillary gland

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Abstract

The effect on the vascularization of the submaxillary gland of the adult Wistar rat of 20, 40, 80, 160 Gy of X-rays applied locally has been quantitatively assessed in the present study. The radioinduced damage to the vasculature was analyzed by perfusion with a silicone elastomere, Microfil, and stereological analysis of vascular volume and length. Vascular length did not vary significantly 7 days post-irradiation whereas vascular volume increased by about 100%. This vasodilatation could be caused by altered vasomotoric response induced, in turn, by the liberation from damaged cells of inflammatory mediators. The ATPase technique, which labels all blood vessel walls, revealed an unaltered vascular pattern 7 days after irradiation which would indicate that damage at this time is functional and not morphological.

Key words: salivary glands - vascularization - radiation damage.

EFECTO DE RADIACION SOBRE LA VASCULARIZACION DE LA GLANDULA SUBMAXILAR.

Resumen

Se ha analizado en este trabajo, el efecto de 4 diferentes dosis de radiación X, sobre la vascularización de la glándula submaxilar de la rata Wistar adulta. Las dosis empleadas localmente han sido de 20, 40, 80, 160 Gy y su efecto ha sido estudiado por perfusión de un elastómero siliconado (Microfil) y la medición estereológica del volumen vascular y la longitud vascular. Si bien ésta última no presentó diferencias significativas 7 días después de la irradiación, el volumen vascular se vió incrementado alrededor de un 100%. Esta vasodilatación podría estar causada por una respuesta vasomotora inducida por mediadores de la inflamación. La técnia de ATPasa utilizada como marcador vascular no demostró alteraciones del patrón vascular, con lo cual se confirmaría que el daño 7 días post-irradiación es funcional y no morfológico.

Palabras claves: glándulas salivales - vascularización - daño por irradiación.

Introduction

It is a well known fact that vascular damage is a major component of late radiation lesions. Fibrosis of arteries and arterioles and collagen deposition in capillary lumens impairs nutrition and induces irreversible alterations in all the tissues studied. (1-4).

The acute phase of radiation injury has been reported to consist mainly of increased permeability which in turn causes oedema and fibrin deposition (5, 6).

The degree to which vascular damage contributes to overall parenchymal injury varies with organs. Acute radiation injury in liver, brain or myocardium would be largely due to microvascular lesions (6, 7).

However, it has been reported that major parenchymal damage in irradiated salivary glands exists without concomitant permeability changes and altered capillary ultrastructure.

In view of these findings and the hypothesis that the vascular network of the irradiated gland may be damaged at the level of vessels of larger diameter, a quantitative analysis of overall vascular alterations in the locally irradiated submaxillary gland of the rat has been performed in the present study.

Materials and Methods

Twenty adult male rats weighing 200-250 g were used throughout. The area located 2 to 3 mm from the clavicle up to the line that passes between the maxillary angles, was irradiated with doses of 20, 40, 80 and 160 Gy of X rays. The animals were placed ventral side up in a lead cage that shielded the body. The head of the rat was shielded by another lead cage smaller than the first one and a small window exposed the area to be irradiated. Single doses were delivered by a deep therapy X-ray Philips machine at 220 KV 14 mA with added filtration of 2 mm A1, STD 22.8 cm. Animals were irradiated under Nembutal anesthesia. Five animals were irradiated with each dose and 5 animals were sham-irradiated. Five days post-irradiation, the animals were perfused with a silicone compound (low viscosity, silicone elastomere, Microfil, Canton Biomedical Products) This method has been extensively used to study the vascular network of various normal organs and experimental pathological models (10-13).

The technique involves prior anesthesia, careful cannulation of the abdominal aorta, perfusion with 1 ml of heparinized physiological solution and, finally, perfusion with the silicone compound.

Perfusions were performed at a constant rate (1-7 ml/min) employing a syringe pump (Lage model 255/1) during 10 minutes (3.25 ml/kg). After 8 minutes of perfusion the lower cava vein was sectioned in order to allow drainage of the material, and both the aorta and the vein were clamped. Thereafter, all rats were kept at 4° C for 48 hours. After careful dissection of the SBM gland, they were rendered transparent by subjecting them to increasing concentrations of ethyl-alcohol and then to methyl-salicylate solutions (25% upto 90%). Glands were then kept during 24 hours in absolute alcohol and the final clearing was achieved in absolute methyl-salicylate. Thus the injected vascular network was rendered visible against a transparent background. Three longitudinal slices were obtained from each gland, and the central slice was used for stereological analysis by direct projection in an electronic image processor (Septum Kontron Messgeräte, Zeiss). In each slice 3 square areas were evaluated which correspond to a volume of 3291 mm^3. This volume was evaluated considering the magnification of the projection and the thickness of the tissue which contained the blood vessels visible on each projection. This thickness, in turn, was calculated considering the focal distance of the objective taken as the average of 10 measurements.

Each sample was focused only once after undertaking projection. Parameters analyzed were: 1) Total vascular length (mm/mm^3) and 2) Vascular volume (mm^3/mm^3). In addition, other groups of sham-irradiated and irradiated glands were fixed in formaldehyde 6% in order to perform the ATPase technique (14) for demonstration of blood vessels according to the studies of Dotto et al. (15)

Results

Microscopic studies revealed that perfusion with the silycone elastomere afforded a good vascular filling which, in turn, rendered possible the measurement of vessels whose diameter was greater than 7 μ m. Both control and irradiated glands exhibited an overall pattern similar to that described by other authors, a central blood vessel with its ramifications towards the periphery (Suddick and Dowd, 1969 among others). Figure 1 shows that in control SBM glands the vascular length was found to be 8.75 ± 0.83 mm/mm^3 The different irradiation doses did not significantly alter vascular length.

Vascular volume, however, was significantly higher for irradiated glands as compared to controls (C = 0.18 ± 0.03; 20 Gy = 0.33 ± 0.05; 40 Gy = 0.36 ± 0.05; 80 Gy = 0.35 ± 0.02; 160 Gy = 0.38

± 0.04), but no significant differences were found between the averages obtained for each dose. In Figure 2 these parameters can be subjectively observed.

The ATPase technique, which labels all existing blood vessel walls, and evaluation of the projections as described above, afforded values of vascular length which were similar for irradiated and control glands.

Discussion

Radioinduced damage to the vascular system conditions tissue lesions. Thus, many studies have been performed on the effect of radiation on the morphology and function of the vascular network, (7,17-19)

Reinhold et al. (20), on reviewing the subject, suggested that the primary radiation lesion would be endothelial cell sterilization, cell damage and depolymerization of membranes, thus causing vasodilation (erythema), the liberation of inflammatory mediators and increased permeability.

X-ray induced alterations of the vascularization of SBM glands, for which the radioinduced damage to the parenchyma is well known, (21-23) was studied using intravascular injections of Microfil MV orange. This method renders possible the quantitative stereological analysis of the structure of the vascular network as far as venules and arterioles. Changes in the microvascular network, however, can only be inferred.

Experimental results reveal a radioinduced increase in vascular volume of upto 100% and unaltered vascular length. This vasodilation would be caused, at this level, by altered vasomotoric responsiveness induced, in turn, by the liberation from damaged cells, of inflammatory mediators (20). Acute damage to the microvasculature and ensuing increased permeability has been described for several organs (24). Hurley et al. (25), however, reported unaltered vascular morphology and permeability by colloidal carbon labelling in the acute phase (up to 8 days) of radiation damage. These results may be biased however, by the difficulty with which changes in permeability can be observed with this method at light microscopy level due to the gland's vascular pattern and the fact that vascular filling is extremely inhomogeneous at an ultrastructural level and difficult to correlate with overall degree of permeability.

Microvascular alterations may thus exist in the SBM gland although the injection of Microfil orange does not afford information on this point. However, the ATPase technique performed in the present study reveals no radioinduced changes in vascular pattern, which would imply that the changes observed by perfusion with Microfil MV orange are functional and not morphological as the ATPase technique labes all existing blood vessel walls.

It may be argued, however, that radiation may damage blood vessel walls causing blood vessels, in turn, to dilate only under perfusion pressure. However, colloidal carbon and peroxidase labelling at light mocroscope level (7-26) and ultrastructural studies (7) do not reveal early morphological changes of the blood vessel wall. In the light of this knowledge the results afforded by the present work favour the hypothesis of functional and not morphological injury.

Proximal vasodilation is probably accompanied by capillary damage and ensuing tissue nutritional alterations.

Vascular damage is not dose-dependent under the conditions of the present study. However, a dose response pattern has been reported for various radiation models such as the acanthotic response of rat skin (27-28), hair follicle damage (29) and vascular alterations (17-26-30-31). It may be argued that the high doses employed in the present study have surpassed the doses for which blood vessels retain their capacity for vasomotoric response.

Thus further studies with doses below 20 Gy would be necessary to investigate the existence of a threshold dose and the dose-response function if such a function does in fact exist.

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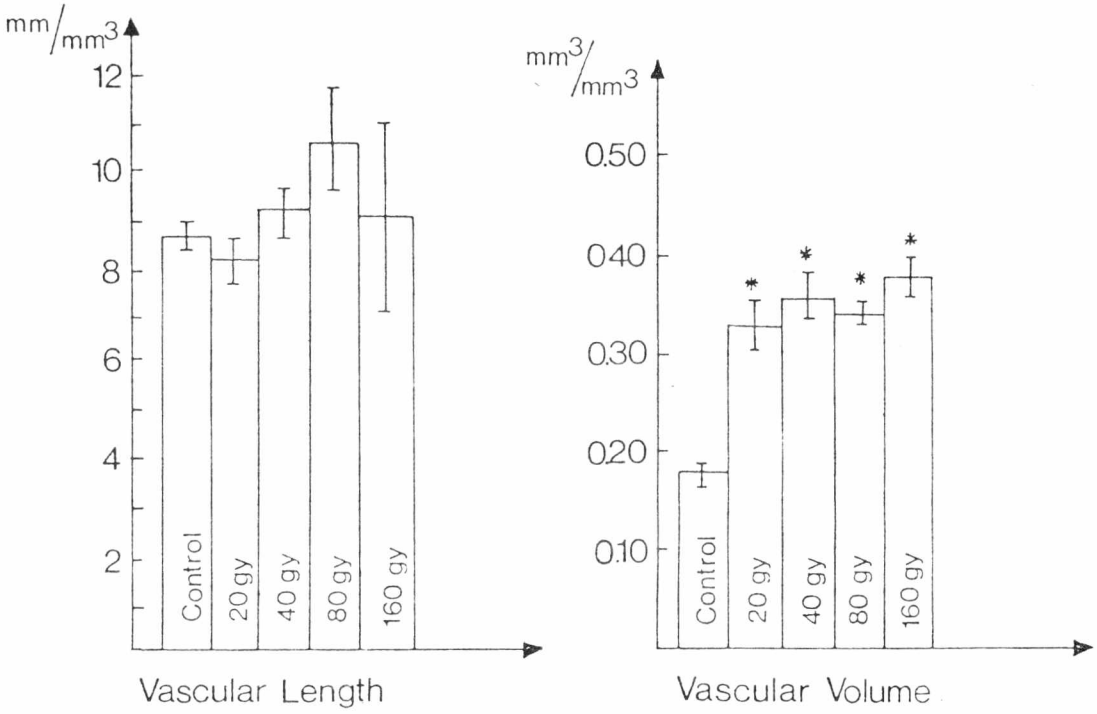
References

1. AARNOUDSE M.W., LAMBERTS H.B. Arterial wall damage by X-rays and fast neutrons. Int. J. Radiat. Biol. (1977) 31: 87-94.
2. OHUCHI K., CHANG L.F., TABACHNICK J Radiation fibrosis of guinea pig skin after Beta irradiation and an attempt at its suppression with proline analogs. Rad. Res. (1979) 79: 273-288.
3. SAMS A. Histological changes in the larger blood vessels of the hind limb of the mouse after X-irradiation. Int. J. Radiat. Biol. (1964) 9: 165-174.
4. ULLRICH R.L., CASARETT G.W. Interrelationship between the early inflammatory response and subsequent fibrosis after irradiation exposure. Rad. Res. (1977) 72: 107-121.
5. REINHOLD H.S., KEYEUX A., DUNJIC A., JOVANOVIC D. The influence of radiation on blood vessels and circulation. XII Discussions and Conclusions. Curr. Top. in Rad. Res. (1974) 10: 185-198.
6. LAW M.P. Radiation induced vascular injury and its relation to late effects in normal tissues. Advances in Rad. Res. (1981). 37-70.
7. SHOLLEY M.M., SODICOFF M., PRATT N.E. Early radiation injury in the rat parotid gland. Lab. Investig. (1974) 31: 340-354.
8. CUTRIGHT D.E., BHASKAR S.N.A. A new method of demostrating microvasculature. Oral Surg. Oral Path. Conf. (1967) 24: 422-426.
9. HUNSUCK E.E. A method of quantitatively analyzing the micocirculatory architecture of the mandible: Preliminary report. Oral Surg. (1968) 26: 449-452.
10. REYNOLDS D.G. Silicone rubber technique for microvascular studies. Lab. Mgmt. (1968) 6: 24-12.
11. CUTRIGHT D.E. The proliferation of blood vessels in gingival wounds. J. Periodont. (1969) 40: 137-141.
12. BRADY J.M. CUTRIGHT D.E. A new technique of measuring blood vessel volume applied to the mandible and humerus of the rat. Anat Rec. (1970) 168: 381-392.
13. HOCK J. Vascular morphology in noninflammed healed gingiva of dogs. J. Clin. Periodont. (1979) 6: 37-44.
14. WACHSTEIN M. MEISEL E. Histochemistry of hepatic phosphatases at a physiologic pH, with Special reference to the demostration of bile canaliculi - Am. J. Clin. Path. (1957) 27: 13-23.
15. DOTTO C A., MAYO J., CABRINI R.L., ITOIZ M.E., CARRANZA F. A quantitative study of vascular response in irradiated wounds. Surg., Gynec. Obstetr. (1970) 130: 875-878.
16. SUDDICK R.P., DOWD F.J. The microvascular architecture of the rat submaxillary gland-possible relationship to secretory mechanisms. Archs. Oral Biol. (1969) 14: 567-576.
17. NARAYAN K., CLIFF W.J. Morphology of irradiated vasculature: a combined in vivo and electron microscopic study. Am. J. Pathol. (1982) 106: 47-62.
18. EASSA H., CASARETT G. Effecto of EACA on radiation induced increase in capillary permeability. Radiology (1973) 106: 679-688.
19. KEYEUX A., DUNJIC A., ROYER E., JOVANOVIC D., VAN DE MERCKT J. Late functional and circulatory changes in rats after local irradiation. Int. J. Radiat. Biol. (1971) 20, 7-25.
20. REINHOLD H.S., KEYEUX A., DUNJIC A., JOVANOVIC D. The influence of radiation on blood vessels and circulation XII Discussions and Conclusions. Curr. Top. in Rad. Res. (1974) 10: 185-198.
21. VAN DER BREND H.A.S., SPARROW N., MOORE V. Effect of X-radiation on salivary gland growth in the rat. I Effect of single doses on post-natal differentiation and growth of acinar and duct components. Int. J. Radiat. Biol. (1966) 16: 241-266.
22. SHAFER W.G. The effect of single and fractionated doses of selectively applied X-irradiation on the histologic structure of major salivary gland of the rat. J.Dent. REs. (1953) 32: 796-806.
23. ESPINAL E.G., DE REY B.M., CABRINI R.L. Radiation effects on submandibular gland of the rat. Stereological and ultrastructural study. Strahlentherapie (1983) 159: 290-295.
25. HURLEY J.V., HAM K.M., RYAN G.G. The mechanism of the delayed response to X-irradiation of the skin of hairless mice and rats. Pathology (1969) 1: 13.
26. SCHWINT A.E., ITOIZ M.E., CABRINI R.L. A quantitative histochemical technique for the study of vascularization in tissue sections using horseradich peroxidase. Histochemical Journal (1984) 16: 907-911.
27. DE REY B.L.M., KLEIN -SZANTO A.J.P. A histometric study of acute radiation effects on rat tail epidermis. Strahlentherapie (1972) 143: 699-704.
28. KLEIN-SZANTO A.J.P , CABRINI R.L. Ultrastructural stereology of X-irradiated epidermis. Rad. Res. (1979) 77: 103-116.
29. KLEIN-SZANTO A.J.P., DE REY B.L.M. Surface architecture of X-irradiated hair follicles. J. Cutaneous Pathology (1978) 5: 184-192.
30. HAMPTON J.C. Acute radiation effects in kidney. Rad. Res. (1972) 52: 316-323.
31. HOPEWELL J.W. Early and late changes in the functional vascularity of the hamster cheek pouch after local X-irradition. Rad. Res. (1975) 63: 157-164.



bar = s.d

p = 99 %

FIGURA 1.

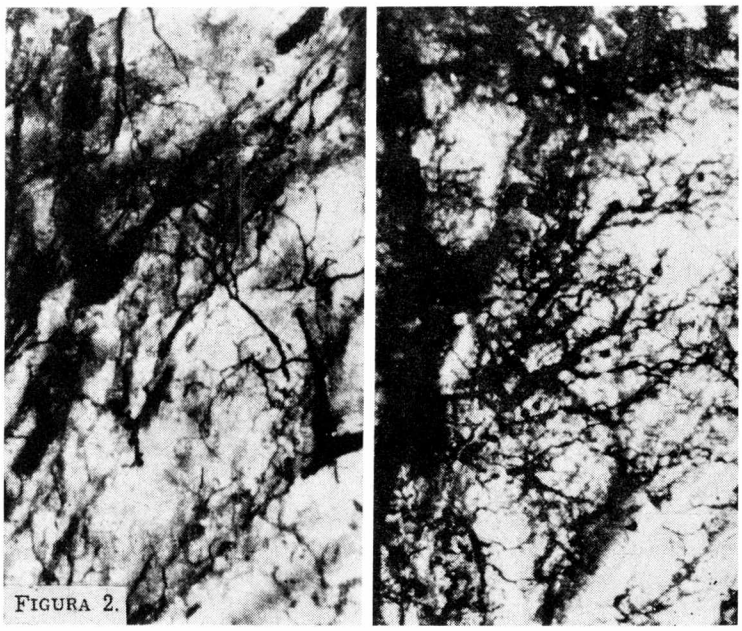


FIGURA 2.