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A quantitative investigation of microvascular changes in the thyroid gland after infrared (IR) laser radiation

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Summary. We present an ultrastructural study of thyroid capillaries in which 50-day-old rats Wistar rats, were irradiated with an infrared (IR) laser, (total dose, 46.80 J/cm^2), the tissue quantified 1 day after ending treatment and a quantitative capillary analysis carried out by light and electron microscopy. Light microscopy was used to calculate capillary volume density revealing a significant increase in the irradiated rats. The quantitative measurement of parameters by electron microscopy required a two stage analysis: Level I, Electron Microscopy (Magnification x5,000); and Level II, Electron Microscopy (Magnification x26,000). At Level I, the following parameters were measured in each capillary: capillary area, capillary diameter, luminal area, luminal diameter, endothelial area, nuclear area and mean endothelial thickness. At Level II, pinocytotic vesicle diameter and their numerical density in endothelial cells were evaluated. Electron microscopic analysis revealed an increased luminal area in the capillaries of the irradiated rats. They also presented a decrease in endothelial cell thickness and vesicular diameter and an increase in vesicle numerical density. This latter increase is indicative of presumptive changes in capillary permeability, but the possible functional significance of these morphological changes in the endothelial cells requires further investigation.

Key words: Thyroid capillaries, Light microscopy, Electron microscopy, Morphometry, Stereology

Introduction

Evaluation of changes in the thyroid gland secretory activity has been made possible thanks to the morphological studies of thyroid capillaries carried out under both normal and pathological conditions. We have learned, for instance, about vascularization changes produced by hyperplasia-type cellular growth disorders. In hyperplasia caused by the administration of TSH (Fujita, 1988), goitrogenic substances (Smeds and Wollman, 1983; Wollman et al., 1990) or laser radiation (Parrado et al., 1990; Lerma et al., 1991; Pérez de Vargas et al., 1992), the capillary network presents signs of dilation and a greater number of mitoses in endothelial cells and pericytes (Erickson and Wollman, 1980). Studies carried out by electron microscopy have also shown an increase in organelles associated with biosynthesis activities, especially of RER, and in endothelial cell pores (Fujita, 1988).

Taking as a reference point the research available on vascularization in highly vascularized organs, such as the brain (Anwar et al., 1992; Meier-Ruge et al., 1992), skeletal muscle (Mathieu-Costello, 1991; Diamuro et al., 1992; Poole and Mathieu-Costello, 1992), the myocardium (Poole et al., 1992; Rakusan et al., 1994; Villar, 1995) and the lungs (De Fouw, 1984; Shumko and De Fouw, 1987), we undertook a quantitative study of thyroid capillaries under normal physiological conditions and after exposing the gland to IR laser radiation. In previous studies the "vascular effect" of IR laser radiation has been described as: dilation of arterioles, capillaries and venules, acceleration of blood flow, decrease in capillary pressure, reabsorption of interstitial fluid and also vascular regeneration (Gorisch and Boerger, 1980; White et al., 1986; Fulga et al., 1995).These effects are connected with the clinical efficacy of IR laser in inflammatory and regenerative conditions (Chelyshev and Kubitsky, 1995; Lowe et al., 1995). Our aims in this report were to determine, using objetive parameters, the underlying morphological factors of the "vascular effect" of IR laser radiation and to assess possible alterations in the thyroid capillary walls.

Materials and methods

Animals

Fifty-day-old albino Wistar rats weighting 200-250 g were used in our study. Six rats were irradiated for 15 days with an IR laser and sacrificed 24 hours later while six other animals served as sham-irradiated controls. The animals were subjeted to a day/night cycle by exposing

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them to artificial light from 08:00 to 20:00 hours. They were kept at a constant temperature of 23° ± 1°C and fed on Pruteen pellets.

Laser radiation protocol

A commercial IR laser (UEDA-SPACE Italy) was used, emitting light at a wavelength of 904 nm, with a pulse duration of 200 nsec. and operated at 800 Hz. The cross-sectional area of the optic fibre was 0.0961 cm^2 and the total dosage applied over the course of 15 days was 46.80 J/cm^2 (daily dosage 3.12 J/cm^2, daily exposure 2.5 minutes). These parameters have been found to be optimal for producing changes in the thyroid structure (Pérez de Vargas et al., 1992). Transmitted energy densities were measured before and after treatment with a conventional power meter. The animals were spot irradiated over the anterior surface of the neck at a previously shaved predetermined skin area overlaying the thyroid gland (Parrado et al., 1990).

Morphological methods

The animals were anesthetized with an 8% chloral hydrate solution at a dosage of 4.5 ml/Kg weight administered intraperitoneally and perfused through a cannula inserted into the ascending aorta with phosphate-buffered saline containing 0.025% heparin followed by 2.5% glutaraldehyde in 0.12M phosphate-buffer (pH 7.3) at 37 °C, perfused at the rate of 21 ml/min for 30 min, as a fixing medium. The perfusing fluid was administered by means of a roller pump (WATSON-MARLOW, type 101 U, London, UK). The thyroid glands were removed under a dissecting lens immediately after sacrifice, between 8:00 am and 9:00 am. Each lobule was cut into 6 sections through its longitudinal axis, making a total of 12 blocks per gland (Conde et al., 1991). Each block was then fixed by inmersion in 2.5% glutaraldehyde at 0 °C over a period of 3 hours. Subsequently, the blocks were rinsed several times with buffer and postfixed in buffered 1% osmium tetroxide (0.12M phosphate-buffer, pH 7.3). Finally, they were dehydrated in a graded series of ethanol, infiltrated with propylene oxide, and embedded in EPON-812. Semithin sections of 1 μm thickness were cut, using an LKB ultramicrotome, stained with 1% toluidine blue and then examined by light microscopy (Delverdier et al., 1991).

Once the light microscopic analyses were concluded, and the area to study selected, ultrathin silver sections (50 nm) were cut, stained with uranyl acetate and lead citrate and finally examined under a JEOL 100 CX electron microscope.

Stereological methods

Light microscopy

Capillary volume density (V_v C) was assessed by point counting carried out at x63 magnification using Weibel's method (Weibel, 1979). The microscope field image was projected onto a screen containing a 225 point test grid. The distance *d* between each point was 1 cm and capillary volume density was defined as the ratio between the number of grid points superimposed on the outline of the capillary and the total number of grid points.

The samples were selected according to stratified sampling procedures (Weibel, 1979, 1989). Six thyroid glands were examined in each group (control and irradiated with IR laser) by selecting ten blocks (5 per lobule, thus a total of 60 blocks). Only one section from each block was taken (amounting to 60 per group), and from each section, two fields were selected, always corresponding to the top right-hand corner and the bottom left-hand corner (n=120 per group). Finally, a 225 point grid was superimposed, computing a total of 27,000 points per group.

Electron microscopy

For electron-microscopic studies 6 thyroid glands were examined in each group (control and irradied with IR laser) by selecting 4 blocks (2 per lobule, thus a total of 24 blocks per group). An ultrathin section was obtained per block making 24 sections in all. From each section 5 micrographs at x5000 were taken, 120 per group (Level I) and another 5 at x26000, 120 per group, to study pinocytotic vesicles (Level II).

All micrographs were magnified 3.2 times using a DURST BIMACAP L 900 enlarger and the parameters computed with an automatic image analyzer (VISILOG SOFTWARE, version 3.6).

Level I (x 5000)

A quantitative study of the capillaries was carried out to determine the following parameters: capillary area, capillary diameter, luminal area, luminal diameter, endothelial area, nuclear area and mean endothelial thickness.

Capillaries were identified as the smallest microvessel profiles devoid of smooth muscle, with luminal diameters of 3-10 μm. The x5000 micrographs showed a cross section of a single capillary, located close to the follicular epithelium. All of them showed the nucleus of the endothelial cell in the section.

Level II (x 26000)

In order to objectively assess endothelial cell activity, the numerical density of pinocytotic vesicles was calculated (N_V PV), applying the Floderus formula (Weibel, 1979):

$$N_V = \frac{N_A}{t + D - 2h}$$

Where: N_A= Number of vesicles per μm^2 in the

endothelial cell cytoplasm; t= mean thickness of the section (50 nm); D= mean diameter of the vesicles; h= diameter of small cap sections lost from the vesicle count

The x26000 micrographs were obtained at random from the cytoplasmic areas of the endothelial cells in the same capillaries used for the Level I.

Statistical methods

The data obtained from the quantitative examinations was analyzed using the Kolmogorov-Smirnow test followed by the Student t-test. All data is presented as mean±SE.

Results

Light microscopy

Capillary quantification showed a capillary volume density of 0.056±0.001 $\mu m^3/\mu m^3$ in the control group, which was significantly lower ($p \leq 0.001$) than in the irradiated group (0.068±0.002 $\mu m^3/\mu m^3$). Thus, a 22.3% increase in volume density occurred when the thyroid gland was irradiated (Table 1).

Electron microscopy

Level I (x 5000)

There was no significant difference between the capillary areas of control and irradiated animals (35.76±1.33 μm^2 for the controls and 39.12±1.17 μm^2 for the irradiated group). Similarly, the capillary diameter did not increase significantly (control 6.67±0.12 μm and irradiated 7.01±0.10 μm) (Table 1).

Our study of the luminal capillary area showed this parameter to be smaller in the control group (17.93±0.9 μm^2) than in the irradiated one (22.33±1.14 μm^2) ($p \leq 0.01$). Similar results were obtained from the luminal diameter, smaller in the control (4.65±0.13 μm) as compared to irradiated animals (5.33±0.12 μm, $p \leq 0.006$). On the other hand, no significant differences were observed between the endothelial and nuclear areas of the control group (7.88±0.29 μm^2, 9.95±0.49 μm^2, respectively) and those of the irradiated group (8.11±0.25 μm^2, 8.68±0.48 μm^2, respectively).

The mean endothelial thickness of capillaries in the irradiated group was significantly smaller than in the control group ($p \leq 0.02$), being 0.88 ± 0.03 μm, and 1.01 ± 0.03 μm respectively.

Level II (x26000)

The pinocytotic vesicle diameter was 74.47±1.38 nm in the control group, whereas it was significantly smaller in the irradiated group (70.90±1.24 nm) ($p \leq 0.001$). Pinocytotic vesicle numerical density, however was significantly greater ($p \leq 0.001$) in the irradiated group (329±15/μm^3) as compared to the control (206±12/μm^3) (Table 1).

Discussion

The aim of this experimental work was to use objetive criteria to determine the possible morphological alterations caused in the vascular structure of the thyroid gland by IR light. The main effect of laser on the thyroid gland has already been described by Gorish and Boerger (1980), White et al. (1986) and Lerma et al. (1991). They observed some hyperplasia and stroma edema when the thyroid gland was irradiated with low-intensity laser light. These alterations were also studied and described in some of our previous works where we used irradiation dose of 46.80 J/cm^2 (Parrado et al., 1990; Pérez de Vargas et al., 1992). We concluded at that time that the alterations might be related to the laser effect on the synthesis of nucleic acids (Hefetz et al., 1990; Friedman et al., 1991; Rimoldi et al., 1991).

This quantitative study with rats irradiated with a dose of 46.80 J/cm^2 laser light and sacrificed 24 hours after treatment was designed to provide the objetive data about laser effects on thyroid gland microcirculation absent from previous works. Volume density of capillaries, capillary and luminal areas, capillary and luminal diameter, endothelial and nuclear areas and

Table1. Quantitative analysis of the thyroid capillaries.

	CONTROL	IRRADIATED	P
Capillary volume density ($\mu m^3/\mu m^3$)	0.056±0.001	0.068±0.002	0.001*
Capillary area (μm^2)	35.76±1.33	39.12±1.17	0.082
Luminal area (μm^2)	17.93±0.90	22.33±1.14	0.010*
Capillary diameter (μm)	6.67±0.12	7.01±0.10	0.08
Luminal diameter (μm)	4.65±0.13	5.23±0.12	0.006*
Endothelial area (μm^2)	7.88±0.29	8.11±0.25	0.58
Nuclear area (μm^2)	9.95±0.49	8.68±0.48	0.09
Mean endothelial thickness (μm)	1.01±0.03	0.88±0.03	0.02*
Pinocytotic vesicle diameter (nm)	74.47±1.38	70.90±1.24	0.001*
Numerical density of pinocytotic vesicles (μm^{-3})	206±12	329±15	0.001*

Data is mean±SE.*: asterix represents the significant differences.

capillaty thickness were determined. Equally, a quantitative analysis of the pinocytotic vesicles was performed. The various parameters used in the thyroid capillary study provided data on organ and tissue oxygenation, and therefore, on their metabolism and function (Cruz-Orive and Weibel, 1990).

Light microscopy analysis showed acute vasodilation of arterioles, capillaries and venules 24 hours after irradiation. A signifcant increase was found in capillary volume density in irradiated animals $0.68\pm0.002\ \mu m^3/\mu m^3$, as compared to $0.56\pm0.001\ \mu m^3/\mu m^3$ in the controls, which confirmed the vasodilation observed in the morphological analysis. These values are similar to the ones found in striated glycolitic fibers and higher than those in striated aerobic fibers (Mathieu-Costello, 1991).

In a previous work where we used, the same dose, we concluded that the IR light produces a significant increase in the volume of the follicular epithelium and the follicle. The colloid substance and the activation index did not change. The glandular stroma decreases proportionally to the increase in the follicular volume density (Pérez de Vargas et al., 1992). These morphological changes may be connected to the increased density of the capillaries.

Electron microscopy extended the data obtained by light microscopy. Thus, examination of the areas revealed the luminal area to be significantly greater in irradiated animals ($22.33\pm1.14\ \mu m^2$), as compared to controls ($17.93\pm0.9\ \mu m^2$) ($p\leq 0.01$), while no alteration was detected in capillary, endothelial and nuclear areas. Diameter analysis also supported the results provided by previous analysis: luminal diameter was significantly higher in the irradiated group ($5.33\pm0.12\ \mu m$) as compared to the control group ($4.65\pm0.13\ \mu m$), but the capillary diameter was not significantly different ($6.67\pm0.12\ \mu m$ in controls and $7.01\pm0.10\ \mu m$ in irradiated animals). Finally, endothelial mean thickness presented a significant decrease ($0.88\pm0.033\ \mu m$) relative to controls ($1.01\pm0.03\ \mu m$) ($p\leq 0.02$).

The analysis of these quantitative data revealed an increase in luminal capillary without a concomitant increase in the capillary area, although the thickness of the endothelial cells appeared reduced. These changes may well be the basis for the vascular effect caused by IR laser, induced directly by the radiation (Gorish and Boerger, 1980), or indirectly by vascular metabolites (Karu, 1989; Hanke et al., 1991).

In order to establish the laser effect on the capillary wall it was necessary to examine endothelial cells and evaluate their pinocytotic vesicles. Therefore, the mean diameter in pinocytotic vesicles was calculated. This showed a statistically significant decrease ($p\leq 0.001$) in irradiated animals (70.90±1.24 nm) in comparison to the controls (74.47±1.38 nm). In addition, the numerical density of pinocytotic vesicles, was estimated, which presented a significant ($p\leq 0.001$) increase in the irradiated group ($329\pm15\ /\mu m^3$) as compared to control animals ($206\pm12/\mu m^3$).

When our results were compared to others concerning highly vascularized organs, it was found that pinocytotic vesicles in lung capillaries had a greater diameter (81 nm) and numerical density ($424/\mu m^3$) (De Fouw, 1984) than in the thyroid gland. Equally, according to Johanson (1979), vesicle numerical density in muscles is greater (between 600 to $800/\mu m^3$).

Quantitative data about the pinocytotic vesicles seems to suggest increased permeability determined by an increase in numerical density to compensate for the reduction in size of each vesicle. In several pathologies, increased numerical density has been related to alterations in permeability in: brain capillaries after a high blood pressure episode (Nag et al., 1979), glioma vessels (Takanato et al., 1991), experimental autoimmune encephalomyelitis (Claudio and Brosnan, 1992); retinal capillaries in diabetic rats (Dosso et al., 1990); or acute lung edemas (De Fouw, 1984).

The morphological data obtained from thyroid capillaries irradiated with laser seems to confirm the presence of a "vascular effect", which, according to our own studies, is characterized by an increase in capillary volume density and luminal diameter, together with an increase in pinocytotic vesicles. These findings may have importance to our further understanding of laser effects on the microvasculature and its putative clinical efficacy in inflammatory and regenerative conditions.

References

Anwar M., Weiss J. and Weiss H.R. (1992). Quantitative determination of morphometric indices of the total and perfused capillary network pig brain. Pediatr. Res. 32, 542-546.

Chelyshev Y.U. and Kubitsky A.A. (1995). Effect of infra-red low-power laser irradiation on regeneration of myelinated axons. Laser Med. Sci. 10, 273-277.

Claudio L. and Brosnan C.F. (1992). Effects of prazosin on the blood-brain barrier during experimental autoimmune encephalomyelitis. Brain. Res. 542, 233-243.

Conde E., Martín-Lacave I., González-Cámpora R. and Galera H. (1991). Histometry of normal thyroid gland in neonatal and adults rats. Am. J. Anat. 191, 384-390.

Cruz-Orive L.M. and Weibel E.R. (1990). Recent stereological methods for cell biology: a brief survey. Am. J. Physiol. 258, PL148-156.

De Fouw D.O. (1984). Vesicle numerical densities and cellular attenuation: Comparisons between endothelium and epithelium of the alveolar septa in normal dog lungs. Anat. Rec. 209, 77-84.

Delverdier M., Cabanie P., Roome N., Enjalbert F. and Van Haverbeke G. (1991). Quantitative histology of the rat thyroid. Influence of histologic techniques on the morphometric data. Anal. Quant. Cytol. Histol. 13, 110-114.

Diamuro J., Balnave R.J. and Shorey C.D. (1992). Effect of anabolic steroids and high intensity exercise on rat skeletal muscle fibres and capillarization. A morphometric study. Eur. J. Appl. Physiol. 64, 204-212.

Dosso A., Runger-Brandle E., Rohner-Jeanrenaud F., Ionescu E., Guillaume-Gentil C., Jeanrenaud B. and Leuenberger P.M. (1990). Ocular complications in the old and glucose-intolerant genetically obese (fa/fa) rat. Diabetologia 33, 137-144.

Erickson L.E. and Wollman S.H. (1980). Increase in the rough endoplasmic reticulum in capillary endothelial cells and pericytes in hyperplastic rat thyroid glands. Endocrinology 107, 732-737.

Friedman H., Lubart R. and Laulicht I. (1991). A possible explanation of laser-induced stimulation and damage of cell cultures. J. Photochem. Photobiol. B. Biol. 11, 87-95.

Fujita H. (1988). Functional morphology of the thyroid. Int. Rev. Cytol. 113, 145-185.

Fulga C., Fulga I.G. and Predescu M. (1995). Effect of laser therapy on experimental inflammation in rats. Rom. J. Intern. Med. 33, 257-61.

Gorish W. and Boerger K.P. (1980). Laser related heat effects on blood vessels. In: Laser in biology and medicine. Hillenkamp F., Pratesi R. and Scchi C.A. (eds). Plenum Press. New York. pp 99-109.

Hanke H., Haase K.K., Hanke S., Oberhoff M. Hassenstein S., Betz E. and Karsch K.R. (1991). Morphological changes and smooth muscle cell proliferation after experimental excimer laser treatment. Circulation 83, 1380-1389.

Hefetz Y., Dunn D.A., Deutsch T.F., Buckley L., Hillenkamp F. and Kochevar I.E. (1990). Laser photochemistry of DNA: two-photon absortion and optical breakdown using high-intensity, 532-nm radiation. J. Am. Chem. Soc. 112, 8528-8532.

Johanson B.R. (1979). Size and distribution of endothelial plasmalemmal vesicle of microvasculature in cat skeletal muscle. Microvasc. Res. 17, 107-117.

Karu T. (1989). Photobiology of Low-Power laser therapy. Health Phys. 56, 691-704.

Lerma E., Hevia A., Rodrigo P., González-Cámpora R., Armas J.R. and Galera H. (1991). The effect of He-Ne laser radiation on the thyroid gland of the rat. Int. J. Exp. Pathol. 72, 379-385.

Lowe A.S., Walsh D.M., Baxter G.D. and Allen J.M. (1995). Low-intensity laser irradiation (830 nm) reduces skin blood flow in humans. Laser Med. Sci. 10, 245-251.

Mathieu-Costello O. (1991). Morphometric analysis of capillary geometry in pigeon pectoralis muscle. Am. J. Anat. 191, 78-84.

Meier-Ruge W., Brude A. and Theodore D. (1992). Histochemical and morphometric investigation of the pathogenesis of acute brain infraction in primates. Acta Histochem. Suppl. 42P, 59-70.

Nag S., Robertson D.M. and Diusdale H.D. (1979). Quantitative estimates of pynocytosis in experimental acute hypertension. Acta Neuropathol. 146, 107-116.

Parrado C., Peláez A., Vidal L. and Pérez de Vargas I. (1990). Quantitative study of the morphological changes in the thyroid gland following IR laser radiation. Laser Med. Sci. 5, 77-79.

Pérez de Vargas I., Parrado C, González V., Vidal L. and Rius F. (1992). Histological study of the thyroid gland following 904 nm laser radiation. In: Optical methods of biomedical diagnostics and therapy. Tuchin V. (ed). SPIE Proocedings series. Washington. Vol. 1981. pp 267-270.

Poole D.C. and Mathieu-Costello O. (1992). Capillary and fiber geometry in rat diaphragm perfusion fixed in situ at different sarcomere lenghts. Appl. Physiol. 73, 151-159.

Poole D.C., Batra S., Mathieu-Costello O. and Rakusan K. (1992). Capillary geometrical changes with fiber shortening in rat myocardium. Circ. Res. 70, 697-706.

Rakusan K., Cicutti N. and Flanagan M.F. (1994). Changes in the microvascular network during cardiac growth, development, and aging. Cell. Mol. Biol. Res. 40, 117-22.

Rimoldi D., Miller A.C., Freeman S.E. and Samid S. (1991). DNA damage in culture human skin fibroblasts exposed to excimer laser radiation. J. Invest. Dermatol. 96, 898-902.

Shumko J.Z. and De Fouw D.O. (1987). The response of subpleural pulmonary capillary endothelium to hidrothorax in rats. Histol. Histopathol. 2, 79-87.

Smeds S. and Wollman S.H. (1983). SH-thymidine labeling of endothelial cells in thyroid arteries, veins and lymphatics during thyroid stimulation. Lab. Invest. 48, 225-291.

Takanato S., Yoshii Y. and Nose T. (1991). Ultrastructure of glioma vessels. Morphometric study for vascular permeability. No To Shinkei 43, 49-56.

Villar V.C. and Mandarim de Lacerda C.A. (1995). Myocardial microvasculature in fetuses and neonates of rat. Stereological study. Ital. J. Anat. Embryol. 100, 211-228.

Weibel E.R. (1979). Point counting methods. In: Stereological methods for biological morphometric. Vol 1. Academic Press Inc. London. pp 109-136.

Weibel E.R. (1989). Measuring through the microscope: development and evolution of stereological methods. J. Microsc. 155, 393-403.

White R.A., Abergel R.P., Lyons R., Klein S.R., Kopchok G., Dwyer R.M. and Vitto J. (1986). Biological effects of laser welding on vascular healing. Laser Surg. Med. 6, 137-141.

Wollman S.H., Herverg J.P. and Tachiwaki O. (1990). Histologic changes in tissue components of the hyperplastic thyroid gland during its involution in the rat. Am. J. Anat. 189, 35-45.

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