The Effect of Irradiation on the Structure of Vasculature of Experimentally Induced Rat Salivary Gland Carcinoma

Kang, Hyo Shik D.D.S.

Introduction

It is well known that the response of tumors to radiotherapy is closely related to the changes in vascular circulation during the course of treatment. An increase in vascular function and blood supply may enhance oxygenation of tumors and thus increase the radiosensitivity of surviving tumor cells to subsequent irradiation, whereas extensive radiation-induced vascular damage would result in poor oxygenation of the tumor, making the tumor cells radioresistant.

Up to date, a number of investigators have studied on the vascular damage in irradiated tumors of breast and liver, but reports on the salivary gland tumors were hardly seen. Recently, microangiography has been introduced in the study of normal vascular arrangement.[1,2,3]

Tumors of mice or rats extensively studied by microangiography have appeared to have the characteristic vascular arrangements.[4,5,6] But the changes of vascular arrangements in the salivary gland tumor of rats following irradiation were rarely reported.

On experimental tumor of the salivary gland, in 1942, Steiner[7] successfully induced squamous cell carcinoma and adenocarcinomas with injection of methylcholanthrene, 1,2,5,6-dibenzanthracene and 3,4-benzopyrene in the salivary gland of the mouse, albino rat, hamster and rabbit. Subsequently, more prompt and creditable methods were developed.[8,9] And made it easier to study the morphology of the tumor, the mechanism of carcinogenesis,[10,11] the histopathologic pattern, the influencing factors of tumorigenesis,[12,13,14] and the chemotherapeutic agents.[15,16,17]

Sugimura and Kawakatsu,[18] and Matsumura[19] studied histochemically the enzymatic pattern of DMBA (7,12-Dimethylbenzanthracene) induced carcinogenesis in mouse salivary gland and tried to clarify the progenitor cells of salivary gland tumors.

To clarify the radiation effect on microvasculature of DMBA induced rat salivary gland tumor, the author examined the histopathologic findings of microvasculature and microangiography, by barium perfusion and indian ink perfusion technique, in tumors and their surrounding tissues of non-irradiated and irradiated groups.

Materials and Methods

The experiment was divided into two schedules consisting of DMBA dosage dependent tumorigenesis (Table 1) and changes of microvasculature in the most appropriate tumorigenic group (5.0 *mg* DMBA powder inoculated group) (Table 2).

A) Carcinogenic effect of varying amounts of DMBA

This article had been presented to the 57th congress of Gifu Dental Society Japan on 20, June 1987.
This research was supported by the Inje Research and Scholarship Fund in 1986 and 1987.
*Department of Oral and Maxillofacial Surgery, Inje Medical College, Paik Hospital, Seoul

Table 1 : Distribution of animals used for histopathologic study of DMBA inoculation in the salivary gland

Group / Week	1	2	4	7	13	14	16	20	Total
Control	2	2	2	2	2	2	2	2	16
Group 1	3	3	3	3	3	3	3	3	24
Group 2	3	3	3	3	3	3	3	3	24
Group 3	3	3	3	3	3	3	3	3	24
Total	11	11	11	11	11	11	11	11	88

Table 2 : Distribution of animals used for the study of vascular changes in the irradiated and non-irradiated group

Group / Week		1	2	4	7	12	13	14	16	Total
Non-irradiat.	Barium						3	3	3	9
	Indian ink						3	3	3	9
Irradiated	Histo.					•	3	3	3	9
	Barium					•	3	3	3	9
	Indian ink					•	3	3	3	9
	Total						15	15	15	45

*point of irradiation

The Sprague-Dauley rats of 3 months, weighing 200 ~250 grams without distinction of male and female, were divided into 3 groups of 24 rats each and 1 control gfoup of 16 rats (Table 1). Depending upon the Cataldo, Shklar and Chauncey method.[20] 72 injection needles of 15 gauge were divided into 3 groups and each 24 needles were cut 2 *mm*, 4 *mm*, and 6 *mm* in lengths, and the cut needles were pakced with DMBA powder (7, 12-dimethylbenzanthracene, Kodak). The DMBA powder in each needle was 2.5 *mg* (group 1), 5.0 *mg* (group 2), and 7.5 *mg* (group 3), respectively.

The animals were anesthetized with thiopental sodium 2.5 *mg / kg* and incision was done 3 *cm* along the midline of the neck below the mandible and exposed the submandibular gland. Then, an aseptic 15 gauge cut needle packed with DMBA powder was inserted and inoculated into the submandibular gland, and the incision line was sutured with 3-0 silk. The control group was injured with 15 gauge needle injection without DMBA inoculation. The 3 experimental group animals and 1 control group animals were sacrificed at the 1,2,4,7,13,14,16,20th week (Table 1).

The enucleated submandibular glands were first fixed in 10% neutral buffered formaline, dehydrated, and embedded in paraffin. The 4-6 μ sections were stained with hematoxylin-eosin and were examined under light microscope.

B) Changes of vasculature by irradiation

i) Carcinogenesis of 5 mg inoculation

It was recognized that the most appropriate dosage of DMBA was 5.0 *mg* by the result of the above experiment. And so, 45 rats inoculated with 5.0 *mg* DMBA were adapted to indian ink perfusion and microangio-

graphic technique. The diameter of the tumor was measured every week by a vernier caliper and measured about 2 *cm* to 2.5 *cm* at the 13th week. The animals were divided into a non-irradiated group of 18 rats and an irradiated group of 27 rats (Table 2).

ii) Irradiation procedure

For irradiation, 27 rats were anesthetized with thiopental sodium 2.5 *mg/kg* and tied on an experimental plate. The tumor was given a single dose of 20 Gy using LINAC 4 *MeV* Mitsubishi unit with 3×3 *cm* at 80 *cm* SSD. The dose rate was 2.5 Gy per minute. The irradiation was performed at the 12th week and the animals were sacrificed at the 13th week (1 week post-irradiation), 14th week (2 weeks post-irradiation) and 16th week (4 weeks post-irradiation) (Table 2).

iii) Microangiographic Procedure

Materials for microangiographic studies were available in each three cases of irradiated and non-irradiated group. Under the anesthesia with thiopental sodium 2.5 *mg/kg*, explolapa was done, and the thoracic artery was catheterized with 23 gauge needle, and the inferior vena cava was washed with normal saline containing heparin,[21] and then, a 5% gelatin solution containing 20% micro-barium was manually injected into the left ventricle with gentle pressure until the concentration of the output solution was equal to that of the input solution. The excised tumor masses were immediately fixed in carnoy fixing solution for 1 week. The tumor masses were cut into 1 *mm* to 2 *mm* thick sections and were photographed with soft X-ray (OEG-50, Nachlett, U.S.A.) for microangiography.

The film used was Kodak 649.0 and it was developed with D-158 Kodak developing solution and examined microscopically by ×40, ×100.

iv) Indian ink perfusion procedure

The procedures of anesthesia and explolapa were the same as the microangiographic method (See iii). The 5% gelatin mixed with indian ink was injected into the left ventricle instead of micro-barium perfusion until the head of the rat became black in colour. The submandibular salivary glands were immediately enucleated and cooled in a refrigerator to make the gelatin hardened. The enucleated submandibular glands were dehydrated and put into xylene and salicylic acid for translucent sections.

The translucent sections of the submandibular gland containing tumor were cut 1 *mm* to 2 *mm* thick. The sliced sections were mounted on a slide glass and examined under light microscope.

The number of animals experimented in the indian ink perfusion procedure was 3 cases in each irradiated and non-irradiated group.

Result

On macroscopic examination, there were no specific gross changes in non-irradiated group throughout the experimental period. In group 1, there were no significant changes in the early period. In the first week, inflammatory swelling was noted in the inoculated site, and when incised, abscess formation was observed. In the 7th week, tumor mass with induration was recognized. When it was incised, greenish pus and whitish keratin-like material oozed out. In the later stages (16-20th week), large reddish blue exophytic tumor mass was formed but it was well encapsulated and there was no surface necrosis. In group 2, the findings were similar to group 1. But the tumor mass was larger than that of group 1. In group 3, there was no change up to the 8th week. In the 11th week, enlargement of the indurated mass was palpated. In the later stages (16-20th week), reddish black exophytic tumor mass was formed.

A) Histologic findings

i) Non-irradiated Group

In the DMBA inoculated groups, histopathologic features of group 1 (2.5 *mg*), group 2 (5.0 *mg*) and group 3 (7.5 *mg*) were basically similar to each other. The most representative features were recognized in group 2 (5.0 *mg* inoculated). Thus, the author mainly described the features of group 2, and compared them with those of group 1 and 3. And the author also shortly described

the features of group 1 and 3.

a) The lst week group; Severe inflammation and necrosis were generally recognized. Especially serous acinar cells were more affected than mucous acinar cells. Atrophy of serous aciner cells, ductal metaplasia, atrophy of granular convoluted tubule cells, loss of granule and ductal hyperplasia were observed (Fig. 1,2).

b) The 2nd week group; Histopathologic features of the lst week persisted. But more severe necrosis, ductal hyperplasia and squamous metaplasia were seen (Fig. 3,4).

c) The 4th week group; Histopathologic features of the lst and 2nd week persisted. The basal cell hyperplasia in the squamous cell islands and epithelial lining of cystic wall was observed with cellular atypism, pleomorphism and transformation into squamous cell carcinoma (Fig. 5).

d) The 7th week group; Infiltration of squamous cell carcinoma into adjacent connective tissue were observed. Partly, the epithelial lining of cyst wall showed mild atypism and epithelial hyperplasia (Fig. 6).

e) The 13th week group; The tumor cells showed infiltrative growth pattern. Surrounding stroma was edematous and showed rich and dilated blood vessels and infiltration of small round cells. There were also ductal proliferations in the stromal tissue (Fig. 7,12,14).

f) The 14th week group; Proliferations of neoplastic cells were more diffuse and edematous stromal connective tissues showed dilated capillaries, diffuse and mild infiltration of small round cells (Fig. 8,16,18).

g) The 16th week group; There were features of aggressive and infiltrative squamous cell carcinoma and massive infiltration of small round cells into stromal connective tissue (Fig. 5). Partly, cancer cell nests fell in necrosis and granulation tissue were formed around the necrotic tissue, which showed proliferation of capillaries and fibroblasts. Vascular lumina had slit-like appearances as they were compressed by tumor mass (Fig. 9,20,22).

h) The 20th week group; One case of collision tumors of fibrosarcoma and squamous cell carcinoma was seen with ductal structure (Fig. 10).

Briefly the lst group (2.5 *mg* inoculated) showed similar appearances to the 2nd group but presented more delayed process than that of the 2nd group. Especially, the squamous cell carcinomas appeared in the 7th week and a case of them showed the features of carcinosarcoma in the 20th week.

The 3rd group (7.5 *mg* inoculated) revealed the same features observed in the two previous groups, but the more generalized necrosis and delayed development of tumor were also recognized. In the 20th week, there were also the features of squamous cell carcinoma and appearances of fibrosarcoma.

ii) Irradiated Group

a) 1 week post-irradiation; The tumor tissue was more differentiated than that of the non-irradiated group. Infiltration of tumor cells was arrested and mild infiltration of small round cells was seen in stromas surrounding tumor mass consisted of dense collagenofibrous connective tissue. The number of blood vessels was also markedly decreased with the narrowing of the lumen and thickening of the arterial intima (Fig. 11,13).

b) 2 weeks post-irradiation; The neoplastic tissue was well differentiated and the stroma also consisted of dense collagen bundle with mild infiltration of small round cells. The degree of vascularity was markedly

Table 3 : Summaries of vascular changes in irradiated group.

Histologic Findings	1Wk	2Wk	4Wk
vasculitis	+	++	++
intimal swelling	+	++	++
subintimal fibrosis of artery	−	+	++
thrombosis	−	+	+
organization of thrombus	−	−	+
thrombophlebitis	−	+	++
decreased vascular lumen	+	++	+++

*Degree of changes − absent + mild ++ moderate +++ severe

decreased, and the vascular lumina were narrowed. There were also features of vasculitis and thrombosis with the thickening of the blood vessel wall (Fig. 15,17).

c) 4 weeks post-irradiation; The features in this stage were the same as those of 2 weeks post-irradiation, but the vascularity was decreased and showed severe sclerosis with dense collagen bundle. Some of the blood vessels showed organization and recanalization in some thrombosed arterioles. Some arterial walls showed thickening by intimal thrombosis (Fig. 21) (Table 3).

B) Vascular changes in the indian ink perfusion method

i) Non-irradiated Group

1) 13th week group; Blood vessels surrounding the tumor nest showed loose reticular pattern and irregular courses as the tumor cells proliferated. The luminal diameters of blood vessels were relatively constant but, partly, they showed some discontinuity (Fig. 25).

b) 14th week group; As they ran from the periphery to the inside of the neoplastic lesion, the vascular lumina were gradually narrowed, and ran perpendicular courses with the sharpening of terminal ends. The lumina of the main blood vessels were dilated (Fig. 26).

c) 16th week group; The findings were almost similar to those of the 14th week group.

ii) Irradiated Group

a) 1 week post-irradiation; Vascularity around the tumor mass was decreased with loose and beaded appearance. In some portions distant from the tumor mass, blood vessels running perpendicular courses toward the tumor mass were observed with relatively constant luminal diameter. Blood vessels in terminal end near the tumor mass showed beaded appearance and leakage of indian ink (Fig. 27).

b) 2 weeks post-irradiation; The number of blood vessels around tumor nests diminished and the blood vessels around the tumor mass were circularly arranged. Terminal ends of the blood vessels were blunted and showed less beading (Fig. 28).

Table 4 : Findings of Vasculature with Microangiography and Indian ink perfusion

Observation	Non-irradiated Group	Irradiated Group
terminal end	branching / reticular	straight / blunt
vascular lumen	smooth / tapering	bead / varicose
continuity	continuous	discontinuous / fragmentation
leakage (extravasation)	none	noted
density	dense reticular	loose

c) 4 weeks post-irradiation; The adjacent connective tissue surrounding the necrotic tumor tissue were hyalinized and showed sharp demarcation between the two lesions. And the vascularity of this hyalinized tissue was decreased remarkably. But the outside of the hyalinized zone consisted of granulation tissue with well vascularized, edematous, inflammatory infiltration, mainly plasma cells, and fibroplasia with scanty bizarre fibroblast (Fig. 19,21,23,24). Well vascularized neighboring areas also showed some varicosities, leakage of indian ink and fragmentation of capillaries (Fig. 29,30, Table 4).

C) Vascular changes in microangiographic method

i) Non-irradiated Group

a) 13th week group; Generally, blood vessels increased but they were not recognized in the necrotic portion of carcinoma, and the main artery was eccentrically displaced to the periphery of the necrotic portion. Vessels were slightly tortuous and irregular, and stretched from the periphery into the center of the tumor. Large stretched blood vessels were also observed, and the terminal ends of dilated capillaries were sharply tapered. In the tumor center, the capillary network was rarely observed, but tortuous and discontinuous blood vessels were observed (Fig. 31).

b) 14th week group; Tumor mass was more enlarged than that of the previous week. The density and

Table 5 : Vascular changes in Microangiographic method.

	Non-irradiated Group			Irradiated Group		
	13th Wk	14th Wk	16th Wk	13th Wk	14th Wk	16th Wk
Tortuousness	−	−	+	+	+	++
Beaded	−	−	+,−	−	++	++
Fragmentation	−	−	+	−	+	++
Leaking	−	−	+	−	++	+++
Narrowing of Blood vessel	+	+,−	+,−	+	++	+++

*Degree of changes − negative + mild
++ moderate +++ severe

number of blood vessels increased in comparison with those of the previous week. Branching pattern and luminal diameter decreased and the directional pattern of blood vessels was also irregular.

c) 16th week group; Blood vessels in the central necrotic portion of carcinoma disappeared. There was an increase in the number of blood vessels in the peripheral portions of tumor necrosis. And other features were almost the same as those of the previous 14th week.

ii) Irradiated Group

a) 1 week post-irradiation; The blood vessels were tortuous and the luminal diameters decreased. Partly, decrease in the intercapillary distance due to increase in the vascular density was observed. Directional irregularity, network formation, beading, fragmentation and abrupt decrease in diameter were also observed (Fig. 32,33).

b) 2 weeks post-irradiation; The blood vessels were smaller and tortuous, and showed decreased intervascular distance. But the outline of blood vessels was indistinct and ended abruptly. There were also leaking appearance and fragmentation of blood vessels (Fig. 34).

c) 4 weeks post-irradiation; The blood vessels appeared more tortuous, beaded and narrower. Fragmentation of blood vessels, and partial leakage of barium in carcinoma were seen. The peripheral vasculatures were dense and formed tortuous network. Leaking appearance was more severe than that of the previous group (Fig. 35,36). These results are summarized in Table 5.

Discussion

Submandibular gland is more sensitive in carcinogenesis than parotid and sublingual glands.[7] Chaudhry et al.[22] and Standish[23] reported that the easiness of tumor development in submandibular gland was due to well developed granular convoluted tubule cell.

The author induced carcinoma by inoculation of pure DMBA powder into submandibular gland of rat, and it was discovered that the amount of DMBA powder was not always in proportion to the development of carcinoma. And, the author found that histopathologic features were similar to those of many other researchers.[18,19]

Development of tumor was recognized at the 4th week in 4 *mm* (5.0 *mg*) group, at the 8th week in 2 *mm* (2.5 *mg*) group and at the 20th week in 6 *mm* (7.5 *mg*) group. The author found out that the suitable amount of DMBA powder for tumorigenesis was 5.0 *mg*, and large amount of DMBA delayed carcinogenesis by intensive necrosis as presented by Franseen.[24]

Standish[23] classified 5 stages of carcinogenesis into connective tissue reaction with giant cells and inflammatory change, parenchymal tissue reaction, epidermoid cyst formation, ductal obstruction, and squamous cell carcinoma stages. Cataldo and Shklar,[8]

classified 4 stages into tissue necrosis, ductal hyperplasia, squamous metaplasia, and squamous cell carcinoma. In their experiments, DMBA cytotoxicity on acinar cells caused the more sensitive atrophy in serous acinar cells than in mucous acinar cells, and showed ductal hyperplasia and squamous metaplasia.

Cataldo et al.[30] observed mitoses of acinar cells adjacent to the ductal cells and stated that these acinar cells were transformed into duct cells, and these duct cells underwent squamous metaplasia, and they considered the acinar cells as the progenitor cells. But, Bauer and Byrne[13] stated that acinar cells did not participate in carcinogenesis and concluded that pluripotential intercalated duct cells and myoepithelial cells were progenitor cells. Standish[23] considered progenitor cells to be granular convoluted cells, not acinar cells.

In this experiment, the author observed mitoses of myoepithelial cells, and acinar cells, but also observe them in basal cells of epidermoid cyst originated from duct cells and squamous epithelial islands. Thus, it is considered that progenitor cells are not myoepithelial cell or acinar cell as mentioned already by Matsumura,[19] and that the study by histochemical, immuno-histo-chemical method is necessary to clarify the origin of progenitor cells.

Sugimura and Kawakatsu,[18] and Matsumura[19] observed the distribution pattern of enzymes in DMBA induced mouse salivary gland tumors histochemically. The enzymatic behaviour in the epithelial cells during oncogenesis showed marked decrease in alkaline phosphatase, non-specific esterase and succinate dehydrogenase, but a marked increase in lactate, and glucose-6-phosphate dehydrogenase as in naturally occurring epithelial carcinoma.

Folkman and Cotran[27] had demonstrated that development of adequate vascular supply was critical to growth and development of the cancer as well as metastasis. And neoplastic cells elaborated a soluble tumor angiogenesis factor that promoted vascularization of the stroma and permitted the progressive growth of solid tumors. More recently Taylor and Folkman[26] reported that heparin promoted angiogenesis, and protamine (a heparin antagonist) not only inhibited angiogenesis but also suppressed tumor growth. In this experiment, the stromal vascularity was increased in histologic findings, indian ink perfusion and microangiogram with the development and growth of the tumor.

Benson[27] reported that vascular changes were prominent in all irradiated tissues, both in the normal or neoplastic. Endothelial cells were only moderately radioresponsive but, with the intensive therapy administered to tumors, radiational changes were almost always seen in the vasculature of the neoplasm itself and in the normal tissues interposed between the source of radiation and the neoplasm. During the immediate post-irradiation period, vessels might show only dilatation, accounting for the erythema of the skin.

Later on with higher dosages, a variety of regressive changes appeared, including endothelial cell swelling and vacuolation or even dissolution with total necrosis of the walls of small vessels (such as capillaries and venules). Affected vessels might rupture, yielding hemorrhage, or they might thrombose.

For reasons unknown, these vascular changes were peculiarly spotty in their distribution along the course of the vessels and so, in the same tissue section, some channels were affected and others spared. In some parts, the cancericidal effectiveness of radiation was attributable to such vascular damage. At a later stage, endothelial cell proliferation and collagenous hyalinization with thickening of the media were seen in irradiated vessels, resulting in markedly narrowing or even obliterating of the vascular lumina. The vascular changes of the irradiated group were the most prominent feature in this experiment. The vascularities were partly decreased and the lumina were narrowed accompanied with stromal sclerosis. And there were vasculitis, intimal swelling, and thrombosis causing narrowing of vascular lumen as the Benson's reports.[27] These findings support the spotty increase in intervascular distance, tortuousness, narrowing of the vascular lumina and leakage in indian ink perfusion and microangiographs.

The microangiography was developed by Barclay,[21] who first published the angiographic picture of the abdominal organs. But it attracted little attention. Recently, as the development of the photographic materials and technique made it possible to get the fine pictures, it has attracted some attention again.[1]

The conventional pathologic examination only showed the cross-sectional view of limited range, but the microangiogram clearly revealed the entire length of the vasculatures with its anatomical structures.

Though the author did not think the reticular structure of the stroma as a tumor proper, it must be a neoplastic tissue because of numerous capillaries with the reaction to the tumor cells.

In this experiment the vascular pattern of the tumor-bearing tissues, by indian ink perfusion method and microangiography by barium perfusion, were basically same in their appearances, except for the clearance of the outline of the vessel wall. Though the outline of the vessel wall was somewhat blurred, the detailed appearances of blood vessel such as bead, spiral or continuity were rather prominent in microangiograph.

Newly formed blood vessels of tumor usually went tortuous course and had no elastic tissue or smooth muscle in its wall.[1] Margulis[28] carried the first angiograph of the breast cancer of the experimental animal using mouse. He observed that the breast cancer had the tendency to form lobules, and showed somewhat hypervascularization, and parallel and spiral blood vessels which arose from peripheral blood vessels and infiltrated into the lobule.

Rubin et al.[1] classified the vascularities in experimental animal as three types with microangiogram; In the first type, the blood vessels mainly distributed in the periphery and some developed into the cancer. In the second type, there were only periphery located blood vessels without any central blood vessels. In the third type, there were only central blood vessels. And he reported that the breast cancer could be grouped into the first type.[1] Saeki et al.[29] also reported that the microangiographic findings in the breast cancer of the albino rat could be grouped into the first type. In this experiment, non-irradiated salivary gland tumor showed some degree of hypervascularization, central necrosis and peripherally located blood vessels which belongs to second type by the classification of Rubin et al.[1] and the findings were compatible with previous reports.[28,6,29]

Reinhold,[30] Hilmas et al.[31,32,33] and Kallman et al.[34] reported transient increase in blood flow and vascular volume, in the early stage of post-irradiation (radioisotope) of single and fractionated dose, and histopathological examination in animal experiments.

In the present study, the large distended blood vessels which were observed before irradiation, disappeared and the new, thin, tortuous blood vessels were observed in compact arrangement at 1 week after irradiation. It was compatible with histopathological findings. It was supposed that this change resulted in effective increase in blood flow, promoted the metabolism and reoxygenation of the tumor, and increased the radiation sensitivity.

This result might be a morphological evidence of the vascular changes with irradiation using microangiogram, which had been supposed by Hilmas et al.[31,32,33] and Kallman et al.[34]

The direct vascular changes caused by irradiation may be summarized as the vascular dilatation and permeability change in the early stage, vascular intimal wall change in the middle stage, and change in the large blood vessel wall in the later stage.[11] Although the exact mechanism of early vascular permeability change is still unclear, it seemed to result from the direct injury to the vascular wall.[35]

Song et al.[36,37] and Hilmas et al.[31,32,33] reported that the blood flow decreased with time, with tracing of blood flow to two weeks after irradiation. McAlister and Margulis[38] with angiogram, reported that the features of irregular decrease in vascular lumen, tortuous changes, and obliteration of blood vessels were the severest at 9-13 days after irradiation, and it returned to the features prior to irradiation. The post irradiation stromal hyalinization surrounding the tumor tissue was one of the promoting factor of the vascular disappear-

ance and narrowing.

It is generally known that the vascular changes after irradiation occur independently with changes of the tumor size. In the present study, tortuous deformities of blood vessels, decreased inner diameter, insulation and obliteration of blood vessels and reticular configuration of microvasculatures are observed at 4 weeks after irradiation in indian ink perfusion and microangiogram. These phenomena coincided with the thickening of blood vessel wall and the infarction histopathologically. And it seemed that the blood flow in the tumor (would be) decreased gradually with these changes.

References

1) Rubin, P., Casarett, G. and Kurohara, S.S., et al.: Microangiography as a technique: Radiation effect versus artifact. A.J.R., 1942; 48: 597-611.
2) Sutton, D.: Percutaneous arteriography with special reference to peripheral vessels. Brit. J.Radiol, 1955: 28: 13.
3) Wickbon. I.: Angiographic demonstration of tumor pathology. Acta. Radiol., 1933; 40: 529.
4) Kliegerman, M.M. and Henel, D.K.: Some aspects of the microcirculation of a transplantable experimental tumor. Radiology, 1961; 76: 810-817.
5) Margulis, A.R., and Murphy, J.O.: Ateriography in neoplasms of extremities. A.J.R., 1958; 80: 330.
6) Rubin. P., and Casarett, G.: Microcirculation of tumors. Part I: Anatomy, function, and necrosis. Clin. Radiol., 1966; 17: 220-229.
7) Steiner, P.E.: Comparative pathology of induced tumors of the salivary glands. Arch. Path., 1942; 34: 613-625.
8) Cataldo, E., and Shklar, G: Chemical carcinogenesis in the hamster submaxillary gland. J. Dent. Res., 1964; 43: 568-579.
9) Chauncey, H.H., Shklar, G. and Quintarelli, G.: Histochemistry of experimentally induced fibrosarcoma in submaxillary gland. J.Dent. Res., 1961; 40: 684.
10) Chaudhry, A.P., Reynolds, D.H., Gorlin, R.J., and Vickers, R.A.: Experimental carcinogenesis in submaxillary glands of hamsters. J. Dent. Res., 1961; 40: 426-432.
11) Shafer, W.G.: Experimentally salivary gland tumorigenesis. J.Dent. Res., 1962; 41: 117-124.
12) Asfahani, A.E., Higashi, G.J., and Ahmed, M.A.: Chemical carcinogenesis of submaxillary salivary gland in BALB/C mice and syngeneic passage of the tumor. Oral Surg., 1979; 48: 47.
13) Bauer, W.H., and Byrne, J.J.: Induced tumors of the parotid gland. Cancer Res., 1950; 10: 755-761.
14) Thomlinson, R.H.: Radiation and the vascularity of tumors. Br. Med. Bull., 1973; 29: 29-32.
15) Selye, H., Veilleux, R., and Cantin, M.: Excessive stimulation of salivary gland growth by isoproterenol. Science, 1961; 133: 44-45.
16) Shklar, G. and Turbiner, S.: Effect of flurouracil on carcinogenesis of rat submandibular gland. J.Dent. Res., 1971; 50: 985.
17) Shklar, G. and Cataldo. E.: Effects of a chemical carcinogen on the submaxillary gland of albino rats treated with isoproterenol. Cancer Res., 1966; 26: 1319-1323.
18) Sugimura, M., and Kawakatsu, K.: Histochemical studies of enzymatic patterns during experimental carcinogenesis in the mouse parotid gland. Archs Oral Biol., 1966; Vol. II: pp.1269-1291.
19) Matsumura, T.: Enzyme histochemistry of experimentally induced tumors in the mouse submaxillary and sublingual glands during carcinogenesis. GANN, June 1966; 57: 251-263.
20) Cataldo, E., Shklar, G. and Chauncey, H.H.: Experimental submaxillary tumors in rats; Histology and histochemistry. Arch. Path., 1964; 77: 305.
21) Barclay, A.E.: Micro-arteriography, A.J.R., 1948; 60: 1-12
22) Chaudhry, A.P., Liposky, R., and Jones, J.: Dose-response of submaxillary glands to carcinogen pellets in rats and hamsters. J.Dent. Res., 1966; 45: 1548.
23) Standish, S.M.: Early histologic changes in induced tumors of the submaxillary salivary glands of the rat. Am. J.Path., 1957; 33: 671-689.

24) Franseen, C.C., Aub, J.C. and Simpson, C.L.: Experimental Tumors in lymphnodes and in endocrine and Salivary glands. Cancer Res., 1941; 1: 489-493
25) Folkman, J., and Cotran, R.S.: Relation of vascular proliferation to tumor growth. Int. Rev. Exp. Pathol. 1972; 16: 261-274.
26) Taylor, S., and Folkman, J.: Protamine is an inhibitor of angiogenesis. Nature., 1982; 297: 307.
27) Benson, E.P.,: Radiation injury to large arteries. 3. Further examples with prolonged asymptomatic intervals. Radiology. 1973; 106: 195-206.
28) Margulis, A.R., Carlsson, R., and McAlister, W.H.: Angiography of malignant tumors in mice. Acta. Radiol., 1961; 56: 179-192.
29) Saeki, Y., Shimazaki, S., and Urano, M.: Radiation effect on the vascularization of a C3H mouse mammary carcinoma. Radiology 1971; 101: 175-180.
30) Reinhold, H.S.: Improved microcirculation in irradiated tumors. Europ. J. Cancer. 1971; 7: 273-280.
31) Hilmas, D.E., and Gillette, E.L.: Tumor microvasculature following fractional X-irradiation. Radiology 1975; 116: 165-169.
32) Hilmas, D.E., and Gillette, E.L.: Morphometric analysis of the microvasculature of tumors during growth and after X-irradiation. Cancer 1974; 33: 103-110.
33) Hilmas, D.E., and Gillette, E.L.: Microvasculature of C3H/Bi mouse mammary tumors after X-irradiation. Rad. Res., 1975; 61: 128-143.
34) Kallman, R.F., Dewardo, G.L., and Stasch, H.J.: Blood flow in irradiated mouse sarcoma as determined by the clearance of Xamen-133. Cancer research. 1972; 32: 483-490.
35) Jolles, B., and Harrison, R.G.: Enzymic processes and vascular changes in the skin radiation reaction. Br. J. Radiol., 1966; 39: 12-18.
36) Song, C.W., and Levitt, S.H.: Vascular change in X-irradiated Walker carcinoma 256 of rats. Radiology. 1971; 100: 394-407.
37) Song, C.W., and Levitt, S.H.: Effect of X-irradiation on vascularity of normal tissues and experimental tumor. Radiol., 1970; 94: 445-447.
38) McAlister, W.H., and Margulis, A.R.: Angiography of malignant tumor in mice following irradiation. Radiology 1963; 81: 664-674.
39) Hall, E.J.,: Radiobiology for the Radiologist, Lippincott. 1987; 12: 241.

Conclusion

The aim of this study is to evaluate the microvascular alterations in salivary gland carcinoma after irradiations. Salivary gland carcinoma was induced in rats by inoculation of several amount of 7,12-dimethylbenzan thracene powder 2.5 *mg*, 5.0 *mg* and 7.5 *mg* respectively into rat submandibular gland. Microangiography was performed by taking soft x-ray with barium infusions, and by indian ink perfusion technique.

The tumors were given a single dose of 20 Gy(to obtain comparatively low grade irradiation dose for isoeffect of dry desquamation of skin to enable the observation of the vascular changes of the tumor[39]) using LINAC 4 *MeV* Mitsubishi unit with field size of 3×3 *cm* at 80 SSD. The dose rate was 2.5 Gy per minute.

The microangiography was performed prior to irradiation and at one, two, and four weeks after irradiation.

The results are as follows.

1. The carcinoma was produced in all rats (100%) between 7 to 11 weeks, the amount of carcinogen was not always in proportion to the development of carcinogenesis, and the most appropriate group for the experiment was 5.0 *mg* inoculated one.

2. The course of the experimental carcinogensis was initiated by ductal proliferation and squamous metaplasia of ductal epithelium which was later transformed into keratocyst, and finally turned into squamous cell carcinoma.

3. Before irradiation, the basic vasculature consisted of peripheral vascular pattern with central penetrating vessels. The peripheral vascular pattern was always richer than that of the center. Irregular and tortuous vessels stretched from the periphery into the center of the tumor mass.

4. In an early stage following irradiation, an increase in the number of smaller, tortuous vessels and decreased intervascular distances were observed in the central portions of tumor nest mass.

5. Later changes of microvasculature after irradiation are increase in tortuousity, irregularity, narrowing, abrupt tapering, fragmentation, and extravasation These findings progressed after a lapse of time.

6. The change of the vascular structure after irradiation such as vasculitis, endothelial swelling and thrombosis on histologic section were coincided with the microangiographic changes.

7. It is suggestive that the mechanisms of the post-irradiation changes of vasculature in the center or periphery of the tumor mass is the destruction of tumor cells and capillaries caused by direct radiation effect, and the progressive degenerative and destructive changes are due to usual residual tissue damage by irradiation.

8. The vascular alterations by irradiation can cause the disruption of blood flow and it can be considered as one of the most effective suppressive factor of tumor growth.

9. The post-irradiative hyalinzation of the connectvie tissue surrounding the tumor tissue was one of the inhibitory factor of the tumor dissemination and the cause of decrease of vascularity.

10. The granulation tissue formation outward the hyalinized zone surrounding the tumor tissue was the reactive feature against the post irradiative central tumor necrosis.

Key Words : Post-irradiation Vasculature, Experimentally Induced Carcinoma, Rat Salivary Gland