日本医放会誌：46（12），1421—1428，1986（昭61）

Changes in Regional Blood Flow of Normal and Tumor Tissues Following Hyperthermia and Combined X-ray Irradiation

Kazuyoshi Suga
Department of Radiology, Yamaguchi University School of Medicine, Ube, Japan
(Director: Prof. Takashi Nakanishi)

Research Cord No. : 407.9

Key words : Tumor blood flow, ^{133}Xe, Hyperthermia, X-ray irradiation, EATC tumor

加温と放射線併用による正常及び腫瘍組織の血流変化

山口大学医学部放射線医学教室
菅　　一　　能

（昭和61年5月16日受付）
（昭和61年7月16日最終原稿受付）

加温と放射線併用による正常組織及び腫瘍組織の血流量の変化を^{133}Xe局所クリアランス法で比較検討した．

実験に用いた腫瘍はICRマウスの右足底部皮下に移植したEhrlich腫瘍で，正常組織は同マウスの足底部を使用した．

正常組織の血流は41℃，30分間の加温で変化せず，43℃，45℃30分間の加温で増加するのに対して，腫瘍組織では加温温度の上昇に伴い血流は減少した．また，43℃，30分間の加温と30Gyの放射線照射併用では，24時間後も正常組織に比し，腫瘍組織では血流の減少の程度が強かった．

腫瘍組織の血流量は腫瘍の大きさにより異なり，比較的大きい腫瘍（＞250mm^3）は，比較的小さい腫瘍（＜200mm^3）に比較し，加温による血流量が低下が強く，正常組織の血流を基準としたBlood flow ratioの検討でも，250mm^{3}より大きい腫瘍の方が，小さい値を示した．さらに比較的小さい腫瘍（＜200mm^3）では，43℃，30分間加温後約3時間で血流の回復傾向が認められたが，比較的大きい腫瘍（＞250mm^3）では認めなかった．

Introduction

The changes of blood perfusion of tumors and normal tissues, during and after heating, will significantly influence not only the rise in temperature of tumors but also the transport of drugs and oxygen to tumors[1]. Consequently, they alter the effectiveness of hyperthermia, either alone or combined with radiation or chemotherapy.

Hence, in the application of hyperthermia to control cancer, the blood flow must be documented in vivo and at elevated temperatures for both the tumor and normal tissue.

Changes in the vascular system of tumors due to heating will exhibit different patterns according to vascularity of the tumor and the size of the tumor.

This research was investigated changes in regional blood flow by using the ^{133}Xe local clearance method following hyperthermia and combined X-ray irradiation.

Materials and Methods

1) Tumors and normal tissues for experiments

Approximately 2×10^6 Ehrilich ascites tumor cells were implanted subcutaneously in the ventrum of the right hind foot of about 7 weeks old male ICR mice. The mice were supplied by Kyudo Center Co., Kumamoto.

Tumors of various sizes were used to measure local blood flow. The mice with no implantation as control mice were used to measure blood flow of normal tissue.

The tumors were measured with a caliper, and the tumor volumes were calculated by the following formula;

$V=\pi/6 \times$ (width) $\times$ (length) $\times$ (height-the average height of the intact foot, 2.8 mm).

2) The methods of hyperthermia and X-ray irradiation

Hyperthermia was administered by immersing the foot for 30 minutes into a circulating water bath. The temperature of the center of various size tumors measured with a 29 guage copper-constantan thermocouple was 0.2—0.6°C lower than the temperature of the water bath. In this study, the temperature of the water bath was used as the heating temperature.

Tumors were irradiated with a single dose using a SHIMAZU THF-250 type X-ray irradiation apparatus, operated at 200 kvp, 20 mA, with a 1 mm aluminum and a 0.5 mm copper filter, HVL of 1.55 mm copper. The focus tumor distance was 50 cm and the dose rate was 96.7 R/min to a field 8×12 cm in size. Irradiation was given just before hyperthermia.

3) The method of measurement of local blood flow

The regional blood flow of normal and tumor tissues was measured by the ^{133}Xe local clearance method based on the Fick's principle[2)3)].

^{133}Xe solution (about 30—50 μCi, 0.05 ml) was injected into the central portion of the tumor and control mouse's foot by using a 27-guage needle. It took about 20 minutes after heating to begin to measure the blood flow. The mice were anesthetized with 50 mg/kg of nembutal intraperitoneally and fixed. Each mouse was then positioned under a NaI crystal scintillation detector, which was 2 inches in diameter, connected to a rate meter and a chart recorder. The detector was positioned 0.5—1 cm above the tumor. At this time, the mean temperature of the center of tumors was 36.8°C ± 0.4°C, and there was no significant difference between the temperature of small tumors and that of large tumors, though the heating temperature was different in each tumors. The temperature of the heated intact foot at this time was the same as the temperature of the heated tumors. The temperature of the control mouse's foot and the unheated tumors was 36.4°C ± 0.2°C. Therefore, in regard to the temperature in measuring the blood flow, there could be no significant difference between the heated and unheated objects.

When the ^{133}Xe disappearance curve was plotted logarithmically against time on the linear scale, one or two component exponential curves were obtained. There was a tendency that the clearance curves exhibited mono-exponentially in the large tumors and double-exponentially in the small tumors and the intact feet (Fig. 1).

The local blood flow was calculated from the following equation;

$$F=\lambda \times (A/B \cdot k_1 + k_2) \times (A/B) + 1)^{-1}=0.69 \cdot \lambda / T_{1/2}$$

Where k_1 and k_2 are corresponding scale constants for the exponential terms. The calculation was done by using a computer[4)]. The factor λ is the partition coefficient between the nodule tissues and blood, and it was determined to be 1.07 by Gump et al[5)].

The coefficient of variations in the 22 intact feet was 8.7% (The average blood flow was 28.8 ml/100 g/min), and the difference between the first measurement and the second measurement in 15 various size tumors was 7.7% on the average.

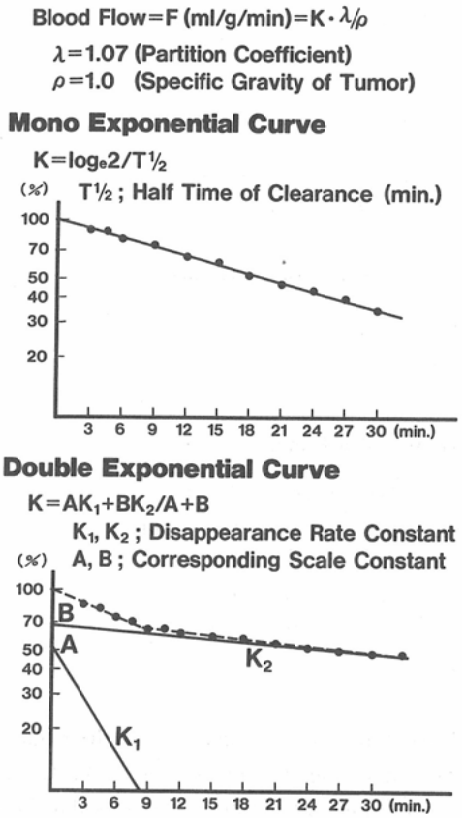

Fig. 1 The ^{133}Xe disappearance curves were plotted logarithmically against time on the linear scale. One or two component exponential curves were obtained. The blood flow was obtained from the disappearance rate constant; k, calculated by computer.

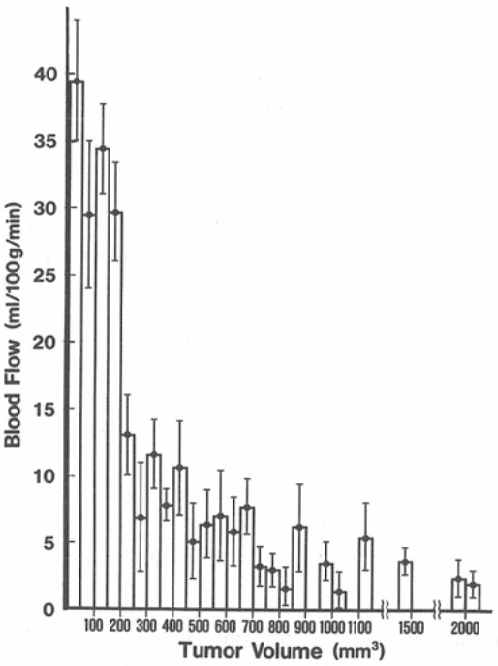

Fig. 2 Blood flow in EATC tumors of the foot of ICR mouse. Columns, average of 6 to 18 tumors Bars, S.D.

4) Statistics

The means and standard errors of the means were calculated for each group. The means of corresponding groups were compared by Student's T-test and P-values less than 0.01 were considered significant.

Results

Fig. 2 shows the relationships between the tumor blood flow and the tumor size. The blood flow value represent the average of 6—18 tumors (means; 14 tumors). It was well known that the tumor blood flow was influenced by anesthetization[6]; however, to compare the values of the blood flow in the same conditions, all of the mice were anesthetized and measured the blood flow.

The blood flow was variable for the size of tumors. The blood flow of the relatively small tumors (<200 mm³) was greater than the blood flow of the intact feet (the average blood flow was 28.8 ml/100 g/min), and the blood flow of the relatively large tumors (>250 mm³) was decreased remarkably. Therefore, tumors were divided into two groups based on tumors volumes, namely the smaller tumor group (<200 mm³) and the larger tumor group (>250 mm³).

The changes in the blood flow after heating for 30 minutes are shown in Table 1. There were 12—16 mice

Table 1 Changes in blood flow in EATC tumors after heating for 30 min..
…Water bath…

	41℃ %(s.d.)	43℃ %(s.d.)	45℃ %(s.d.)
Intact foot	+ 0.69(± 2.9)	+32.32(± 8.1)	+14.21(±12.1)
Tumores<200mm³	−29.28(±12.6)	−55.13(±15.8)	−61.73(±12.8)
Tumores>250mm³	−43.52(±12.5)	−71.80(± 8.8)	−80.69(± 6.3)

The values are average of 14 determinations±S.D..
The value qepresents(the blood flow after heating/the blood flow prior to heating) ×100(%).
+, Blood flow increased compared with that prior to heating.
−, Blood flow decreased compared with that prior to heating.

Table 2 BFR for EATC tumor in the foot of ICR mouse

	Room temp.		41°C, 30 min.		43°C, 30 min.		45°C, 30 min.	
	B.F.	BFR	B.F.	BFR	B.F.	BFR	B.F.	BFR
Intact foot	28.8		29.0		38.1		32.9	
Tumors<200mm³	29.9	1.03	16.1	0.55	11.1	0.29	8.2	0.25
Tumors>250mm³	5.8	0.20	5.6	0.19	1.6	0.04	1.4	0.04

The values are means of more than 18 determinations.

in each group, and the tumors were distributed among each treated group to equalize the distribution of tumor volumes. The blood flow of the control mice's feet were unchanged after heating at 41°C and increased at 43°C to 1.3 fold and at 45°C to 1.1 fold, while the blood flow of the tumors decreased as heating temperature rose ($p<0.01$) (Table 2). Furthermore, the blood flow of the larger tumors decreased more than the smaller tumors at each heating temperature ($p<0.01$).

Observation of the blood flow ratio (BFR; ratio of blood flow in tumor tissue to that in normal tissue) was shown in Table 2. There were 18—22 mice in each group, and the tumors were distributed among each group to equalize the distribution of tumor volumes. BFR values decreased as heating temperture rose, and BFR values of the larger tumor was observed lower than that of the smaller tumors.

The changes of the blood flow of the tumors and normal tissues up to 24 hours after heating and heating combined with X-ray irradiation are shown in Fig. 3, 4, 5. As compared with blood flow of the intact feet, that of the tumors decreased remarkably by heating at 43°C for 30 minutes or with 30 Gy (3000 rad) X-ray irradiation and also in combined use of both treatments. In the smaller tumors, recovery toward normal blood flow began at about 3 hours after heating at 43°C for 30 minutes; however, full recovery was never reached in the larger tumors and a low value of blood flow persisted.

When X-ray irradiation was combined with heating, the tumor blood flow was found to be less than heating alone and X-ray irradiation alone, at 24 hours after treatment ($p<0.01$).

Discussion

Studies of hyperthermia in vivo have indicated that the higher heat sensitivity on tumor cells than that expected on the basis of in vitro cell destruction. And, a markedly rapid cell destruction have been shown in vivo which was not detected generally in vitro studies[7][8][9]. One of the factors which attributed to this phenomenon is considered to be the vascular system, which assumes a critical role in determining the internal environment of both tumor and normal tissue[10].

The ^{133}Xe local clearance method is a simple method for measuring the local blood flow and also can be

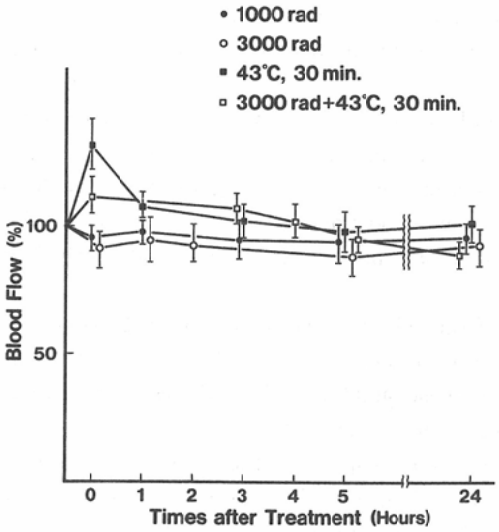

Fig. 3 Changes of the blood flow of the intact foot after treatments. Points, average of 6 measurements. Bars, S.D.

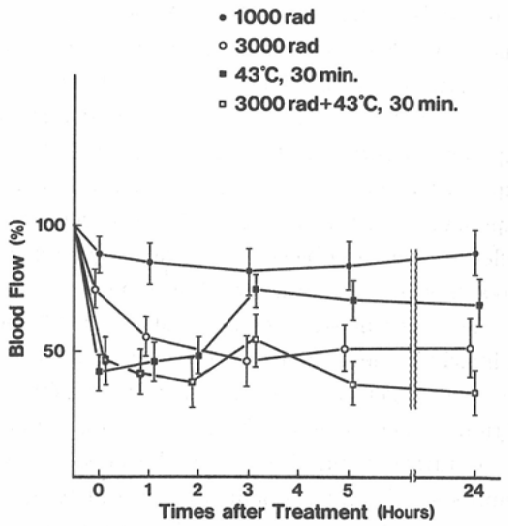

Fig. 4 Changes of the blood flow of the relatively small EATC tumors (<200 mm³) after treatments. Points, average of 6 tumors. Bars, S.D.

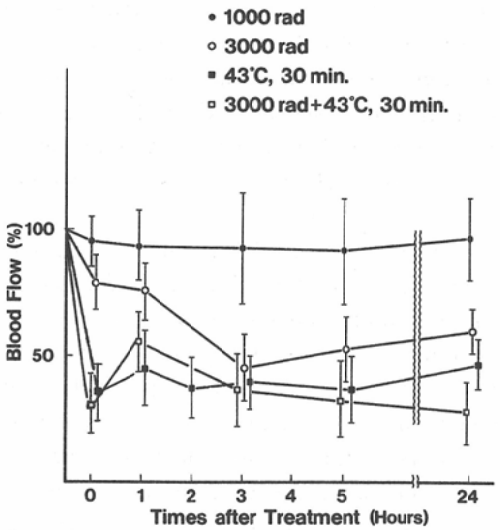

Fig. 5 Changes of the blood flow of the relatively large EATC tumors (>250 mm³) after treatments. Points, average of 6 tumors. Bars, S.D.

applicable for clinical use[11)12)]. In this method, it is possible to measure the functional blood flow of the area where the isotope is distributed, even if the blood flow of the tumor and normal tissue is heterogeneous. However, the mechanical stimulations, such as puncture and compression, cannot be avoided and that these stimulations make some variances in the clearance curve. Kjellmer et al.[12)13)] compared the ^{133}Xe local clearance method with the direct method in which the ^{133}Xe solution was directly injected into the femoral artery, and exhibited that there was no difference in the changing rate of the blood flow between the two methods, although the values of blood flow obtained by the direct method was a little greater than the values

of blood flow obtained by the ^{133}Xe local clearance method. Thus, the local clearance method used in this study is considered useful for comparing the values of the blood flow in the same animal and for determining the changes of the blood flow due to treatments.

The ^{133}Xe clearance curves exhibited mono-exponential curves in the larger tumors and double-exponential curves in the smaller tumors. Because the mono-exponential curves changed to the double-exponential curves when ^{133}Xe solution was injected into the peripheral part of the larger tumors, the double exponential curves were considered to consist of two different blood flow phases: the fast phase which reflects the greater blood flow of the peripheral part of the tumor, and the slow phase which reflects the lower blood flow of the central part of the tumors.

The present study demonstrated that the blood flow of the relatively larger tumors (>250 mm^3) was redmarkably less than the relatively smaller tumors (<200 mm^3) and intact foot (Fig. 2). As the tumor becomes larger, the functional capillary densities gradually decrease, and the total blood flow per unit weight of tumors, particularly in the large tumors, is smaller than that in the normal tissues[1)14)].

Contrary to this observation, the blood flow of the smaller tumors (especially, the mean blood flow of the tumors smaller than 50 mm^3 was 38.8 ml/100 g/min) was greater than that of the normal tissue. It is not a uncommon phenomenon. Some investigators observed a similar phenomenon in the small tumors[15)16)]. The neovascularization to meet the nutritional demand of proliferating tumor cell populations will increase the blood flow of tumor tissue more than normal tissue. Song et al.[10)] reported that the blood flow in the normal tissue adjacent to tumors is greater than that in the normal tissue remote from the tumors. The greater blood flow in small tumors by measuring with the ^{133}Xe clearance method is considered to reflect not only the greater blood flow of tumor tissues itself but also that of the surrounding normal tissues.

In normal tissue, the present study demonstrated that the blood flow of normal tissue was unchanged after heating at 41°C for 30 minutes and increased at 43°C and 45°C for 30 minutes. It is a well known fact that heat induces a prompt increase in blood flow accompanied by dilatation of vessels in normal tissues[6)]. The degree of pathophysiological changes in the vascular system in normal tissue are dependent on temperature and duration of heating.

Following heat treatments, the blood flow of the larger tumor decreased more progressively than of the smaller tumors ($p<0.01$, at each heating temperature) (Fig. 4, 5 and Table 2), and so, it was considered that the heat induced change in the blood flow of tumors was depending on the tumor volume. Song et al.[16)] also observed the different effect by heating depending on the tumor volume in the Walker sarcoma of SD rats.

The response of vascular system to heating varies also depending on tumor types; however, the blood perfusion deteriorates when heated for 30 to 60 minutes at 41°C—43°C in most animal tumors[17)].

In view of the morphological features of tumor vessels, it is not surprising that the response of vessels in tumors to heat is different from that in normal tissues. The rapidly growing vascular system in tumors is made of single layered endotherial cells without an external coat of elastic basement membrane, and tumor blood vessels are usually twisted, sharply bent with coil like features, and extremely dilatated, and abundant sinusoidal opening[18)19)]. Unlikely normal tissue, all functional capillaries in tumors are open and used at near full capacity, even under normothermic condition. The rather fragile capillaries of tumors may not able to cope with the effects by elevated inflow of blood due to heating, and are easily accompanied by leakage of blood[20)21)].

Observation of the blood flow of the tumors up to 24 hours after treatments revealed that there was a gradual tendency toward recovery of the blood flow began 3 hours after heating at 43°C for 30 minutes in the relatively smaller tumors, but the blood flow did not return to the pre-heating level. However, in the larger tumors, there was no recovery tendency of blood flow. Kang et al.[22)] reported that the blood flow was partially recovered by 48 hours after heating at 43.5°C for 30 minutes in SCK tumors of A/J mice. Stewrd and Begg[23)] reported that there was a gradual recovery by 24 hours after heating at 42.8°C for 60 minutes in spontaneous

origin tumors of WHT/CyfBSVS mice. It is considered that the recovery kinetics of tumor blood flow after heating varies depending on tumor volumes and tumor types.

After X-ray irradiation, there were no significant changes in tumor blood flow with dose of 10 Gy (100 rad), although 30 Gy (3000 rad) decreased the tumor blood flow and that low values persisted for 24 hours. Merwin et al.[24] studied the vascular change in C3H mouse in transparent chamber in the skin and observed a narrowing of tumor vessels on the first day after X-ray irradiation with dose of 20 Gy (2000 rad)-50 Gy (5000 rad). These results indicate X-ray alone cause changes in the microcirculation of the tumors.

And also, X-ray irradiation was considered to alter the response of vasculatures to heat, and enhance the heat induced vascular damage. In the present study, lower values of blood flow of the tumors in 24 hours after heating at 43°C for 30 minutes combined with 30 Gy (3000 rad) irradiation were observed, compared with that of heating alone and X-ray irradiation alone ($p<0.01$) (Fig. 4, 5). Eddy[20] reported the same X-ray irradiation with dose of 20 Gy (2000 rad) given 1 hour before heating at 42°C for 30 minutes enhanced the heat induced vascular damage in the cervical carcinoma of hamsters.

The vascular bed in normal tissue; however, appear to be more resistant to the combined use of heat and X-ray irradiation than that in tumors. In the present study, the blood flow of the intact foot was not decreased in 24 hours after combined treatment (Fig. 3). Hume et al.[25] also observed that the vascular beds in normal tissues appeared to be more resistant than that of the tumors in mesenteric blood vessels of mice.

Considering that the decreased pH and nutirition following the lower blood flow enhance well heat killing effect, the less change blood fow in normal tissue and the greater decreasing of blood flow in tumor after combined therapy of hyperthermia and X-ray might result in a good therapeutic gain.

References

1) Bicher, H.I., Hetzel, F.W., Sandhu, T.S., Frinka, S., Vaupel, P., Ohara, M.D. and Obrien, T.: Effect of hyperthermia on normal and tumor microenvironment. Radiology 137: 523—530, 1980
2) Lassen, N.A.: Muscle blood flow in normal man in patients with intermittent claudication evaluated by simultaneous ^{133}Xe and ^{24}Na clearances. j. of Clin. Invest. 43: 1805—1812, 1964
3) Kallman, R.F., de Nardo, G.L. and Stash, M.J.: Blood flow in irradiated mouse sarcoma as determinined by the clearance of Xenon-133. Cancer. Res. 32: 483—486, 1972
4) Wakisaka, G., Sugahara, T., Torizuka, K.: Information management of RI data. Clin. Nuclear Medicine. 89—105, 1981, Nankodo, Tokyo
5) Gump, F.E. and White R.L.: Determination of regional tumor blood flow. Cancer. 21: 871—875, 1968
6) Song, C.W., Kang, M.S., Rhee, J.G. and Levitt, S.H.: Effect of hyperthermia on vascular function in normal and neoplastic tussues. Ann New York Academy of Sciences 335: 35—43, 1980
7) Strom, F.K., Harrison, W.H., Elliott, R.S. and Morton, D.L.: Normal tussues and solid tumor effects of hyperthermia in amimal models and clinical trials. Cancer. Res. 39: 2245—2251, 1979
8) Bichel, P. and Overgaard, J.: Hyperthermic effect on expotential and plateau ascites tumor cells in vitro dependent on environmental pH. Radiat Res. 70: 449—454, 1977
9) Overgaard, K. and Overgaard, J.: Investigations on the possibility of a thermic tumor therapy. Eur J. Cancer 8: 65—78, 1972
10) Song, C.W., Kang, M.S., Rhee, J.G. and Levitt, S.H.: The effect of hyperthermia on vascular function, pH and cell survival. Radiology 137: 795—803, 1980
11) Harada, Y.: Study of the functional tumor blood flow dynamics. Nippon Acta Radiologica 33: 740—755, 1973
12) Tanaka, Y.: Microciruclation of the tumor and radiosensitivity significance in clinical application. Jap. J. Cancer. Clinic. 19: 922—928, 1973
13) Kjellmer, I., Lindbjerg, I. and Tonnesen, H.: The relation between blood flow in an isolated muscle measured with the Xe 133 clearance and a direct recording technique. Acta. Phsiol. Scand. 69: 69—78, 1967
14) Kolstad, P.: The development of the vascular bed in tumors as seen in squamous cell carcinoma of the cervix uteri. Br. J. Radiol 38: 216—233, 1965
15) Dudlar, T.E. and Jain, P.K.: Differential response of normal and tumor microenviroment to hyperthermia. Cancer Res 44: 605—612, 1984

16) Song, C.W.: Effect of local hyperthermia on blood flow and microenvironment. A review. Cancer Res 44: 4721—4730, 1984
17) Tanaka, Y.: Effect of hyperthermia on normal and tumor microcirculation, especially in tumor cell lines. Hyperthermia 101—115, 1984, MAG BROS. INC, Tokyo
18) Eddy, H.A. and Gasarett, G.W.: Development of the vascular system in the hamster malignant neurilemmoma. Microvasc Res 6: 63—82, 1973
19) Hubber, W.R. and Wolf, J.E.: Mealanoma, Tumor angiogenesis and human neoplasia. Cancer 38: 187—192, 1976
20) Eddy, H.A.: Alteration in tumor microvasculature during hyperthermia. Radiology 137: 515—521, 1980
21) Emami, B., Nussbaum, G.H., Tenhaken, P.K. and Huges, W.L.: Pysiological effects of hyperthermia, response of capillary blood flow and structure to local tumor heating. Radiology 137: 805—809, 1980
22) Kang, M.S., Song, C.W. and Levitt, S.H.: Role of vascular function in response of tumors in vivo to hyperthermia. Cancer Res 40: 1130—1135, 1980.
23) Stewart, F. and Begg, A.: Blood flow changes in transplanted mouse tumors and skin after mild hyperthermia. Br. J. Radiol. 56: 477—482, 1983
24) Merwin, R., Olgire, G.H. and Kaplan, H.S.: Transplant chamber observations of the response of a transplantable mouse mammary tumor to local roentgen irradiation. J. Nat. Cancer. Inst. 11: 593—623, 1950
25) Hume, S.P., Marigold J.C.L. and Hirst, D.G.: The effect of hyperthermia on one aspect of the response of mesenteric blood vessels to radiation. Int. J. Radiat. Biol. 39: 321—327, 1981