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Comparison of the Effects of Intravenously Bolus-administered Endothelin-1 and Infused Angiotensin II on the Subcutaneous Tumor Blood Flow in Anesthetized Rats

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To evaluate the effects of endothelin-1 (ET-1) on tumor blood flow, the authors measured the mean arterial blood pressure (MABP) of enflurane-anesthetized male Donryu rats and the tissue blood flow of subcutaneously implanted tumor (Yoshida rat ascites hepatoma LY-80) by using a hydrogen clearance method. The tumor blood flow was evaluated in terms of the ratio to the maximum blood flow, which was defined as the largest flow in the same position during successive measurements. After bolus intravenous administration of ET-1 (1.0 nmol/kg), MABP reached approximately 140 mmHg (at 5–30 min), diminishing gradually to the baseline level over 2 h. The tumor blood flow increased from 36.7±20.6 to 59.5±30.2% (n=32, $P<0.001$, at 2 min), returning to the baseline level at 10 min. On the other hand, at 2 min after the beginning of continuous intravenous infusion of [Asp^1, Ile^5]-angiotensin II (AII; the dose was determined by a blood pressure control system for keeping MABP at approximately 150 mmHg, consequently 0.26 μg/kg/min on the average), the tumor blood flow increased from 42.3±21.6 to 76.4±22.6% (n=32, $P < 0.001$), which was significantly larger than the flow after ET-1. The results indicate that hypertension induced by systemic ET-1 injection is less effective than hypertension induced by continuous systemic AII infusion in increasing tumor blood flow; AII is probably a suitable agent as a safe and effective enhancer of tumor blood flow. Moreover, ET-1 appears to constrict arterial vessels in the microcirculation time-dependently, while AII constricts probably only normal peripheral arterioles.

Key words: Hydrogen clearance method — Arterial blood pressure — Microcirculation — Tumor vessel — Rat ascites hepatoma

Tumor blood flow is an important factor influencing the ease of cancer detection and therapy, and many researchers have tried to modify tumor blood flow with vasoactive agents.[1-11] In particular, intravenously infused angiotensin II (AII) can enhance the tumor blood flow, both selectively and passively[7, 12, 13]; and chemotherapy with AII has been employed under the name of "induced hypertension chemotherapy" (IHC) for more than 10 years.[14, 15] On the other hand, endothelins (ETs),[16] which have various biological effects,[17] produce a potent and sustained vasoconstriction *in vitro* and *in vivo* and have often been compared with AII. Since AII enhances tumor blood flow secondarily by constriction of the normal vessels,[12] it is important to determine whether ETs are capable of increasing tumor blood flow effectively and safely. However, little work has been done on the effects of ETs on tumor blood flow.

The purpose of the present study was to evaluate the effects of ET-1 on tumor blood flow in comparison with those of AII. We used a hydrogen clearance method to measure tumor blood flow. This method permits precise measurement of tissue blood flow *in situ*[18] before and after drug administration. A proper interval time was set between the administrations, because ET-1 could possibly affect the arterial pressure and blood flow for more than 1 h.[19] Comparison of the actions of AII and ETs would provide information concerning not only the mechanism of IHC, but also the localization of the vasoconstrictions by AII and ETs in microcirculation systems.

MATERIALS AND METHODS

Animals and tumor Male Donryu rats (Nippon Rat Co., Urawa), each weighing 250–350 g at inoculation, were used. They received a standard pellet diet and tap water *ad libitum*. Solid tumors which grew to 4–5 cm in diameter at 15–25 days after a subcutaneous inoculation into the rat lower back of 2×10^6 cells of Yoshida rat ascites hepatoma LY-80, maintained in our laboratory, were examined. All rats were measured only once.

Chemicals Pentobarbital sodium and enflurane were purchased from Dainippon Pharmaceutical Co., Ltd., Osaka. Endothelin-1 was purchased from Peptide Institute Inc., Osaka. [Asp^1, Ile^5]-angiotensin II (angiotensin II human) was kindly provided by Toa Eiyou Ltd., Tokyo.

Blood pressure measurement The Donryu rats (295–380 g at measurement) with the tumor were anesthetized with pentobarbital (25 mg/kg, subcutaneously injected

and supplemented as required) and enflurane (0.9–1.8% in the inhaled air at 1.0 liter/min), on a heated stage at 35°C. The concentration of enflurane was maintained by an anesthetic machine for small animals which was made in our laboratory.[20] The mean arterial blood pressure (MABP) was measured with a pressure transducer (TNF-R, Spectramed Medical Products, Singapore) connected to a cannula inserted into the right femoral artery.

Measurement of tumor blood flow A detailed description of our method, which was modified from that of Aukland *et al.*,[21] was given in our previous report.[13] In brief, after saturation of the tissue with hydrogen following the inhalation of 9% hydrogen gas in air (at 1.0 liter/min), the blood flow value (in ml/min per 100 g tissue) was calculated from the clearance curve. In the present experiment, the value reflected the average flow for 2 min; two electrodes (UHE-201, Unique Medical Co., Tokyo), 0.08 mm in diameter, were inserted into the tumor at least 2 cm apart. The above two kinds of measurement were performed at the same time, between noon and midnight.

Bolus endothelin-1 injection The method in this study was a modification of that of Mortensen and Fink.[22] Rats were given a bolus injection of 1 nmol/kg ET-1 in 0.3 ml of physiological saline from the tail vein. The injection was given in 60 s (within 15 s in the original report) with an infusion pump (Compact Syringe Pump, Model 975, Harvard Apparatus Co., Inc., Millis, Mass.). The measurement of MABP and the tumor blood flow was performed just before the ET-1 intravenous administration (i.v.) and at 2, 5, 15, 30, 45, 60, 90, and 120 min after the beginning of the transient decline of MABP after the ET-1 i.v.

Continuous angiotensin II infusion AII, in physiological saline at a concentration of 2.5 μg/ml, was continuously administered from the tail vein with an infusion pump (Transfusion Pump, Model 235, ATOM Co., Ltd., Tokyo). In particular, the infusion rate was controlled corresponding to MABP by an experimental prototype of a computer-aided fuzzy logic blood pressure control system developed by Fukui and his coworkers (Tokyo Denki University) and the authors. The details of the system have been described in the report of Fukui and Masuzawa.[23] The measurement was carried out immediately before AII i.v., at 2 min after the beginning of the increase of MABP with AII i.v., and at a constant phase in which MABP was approximately 150 mmHg, on the supposition that clinical IHC would be performed under such conditions.[14]

Experimental protocol In protocol 1, AII infusion was followed by ET-1 i.v. The interval between the stoppage of AII infusion and the start of ET-1 injection was at least 60 min. Eight rats were used. In protocol 2, ET-1 i.v. was followed by AII i.v. with an interval of 120 min. Eight rats were used. At each interval, the rat was kept in the previously described anesthetic condition with measurement of tumor blood flow (protocol 2) and MABP monitoring.

Data analysis The ratio (%) of the tumor blood flow in relation to the maximum blood flow (MBF) at the points measured was calculated. In protocol 1, data were ob-

Table I. Mean Arterial Blood Pressure (MABP) and Tumor Blood Flow after Angiotensin II or Endothelin-1 Administration

	Protocol 1			Protocol 2	
	MABP[a] (n=8)	Tumor blood flow[b] (n=16)		MABP[a] (n=8)	Tumor blood flow[b] (n=16)
Before AII	103.9±2.9	14.3±14.5	Before ET-1	105.2±8.1	15.1±12.2
2 min after AII	143.4±9.1	26.3±23.6	2 min after ET-1	128.0±12.2	21.4±16.9
Constant phase	147.9±1.9	19.3±20.5	5	140.0±5.2	17.8±18.0
Interval of 60 min			10	142.3±6.0	16.7±15.7
Before ET-1	103.8±4.3	13.2±14.3	15	142.6±6.7	16.8±15.7
2 min after ET	130.8±9.4	25.2±30.3	30	141.4±5.3	17.2±15.8
5	139.9±7.2	14.4±22.8	45	138.1±7.4	16.4±14.0
10	139.4±6.3	10.1±16.2	60	132.9±11.3	15.7±13.2
15	139.3±8.0	10.4±13.3	90	124.8±13.0	15.5±12.3
30	137.4±7.8	9.5±10.5	120	119.3±15.4	14.5±11.6
45	135.3±8.2	11.3±11.4	(i.e. before AII)		
60	132.4±10.5	14.0±14.9	2 min after AII	147.0±12.9	28.8±27.3
90	123.4±11.9	15.3±16.0	Constant phase	150.6±3.3	33.1±34.1
120	116.9±14.8	15.7±15.7			

Values are means±SDs.
a) mmHg. *b*) ml/min/100 g tissue.

tained sequentially before AII, at 2 min after the beginning of AII, at a constant phase with AII, before ET-1, at 2 min, at 5 min after ET-1, etc. For example, if the values of the tumor blood flow (ml/min per 100 g tissue) were 8.2, 10.4, 11.9, 8.5, 9.6, 7.1, etc., respectively, then the MBF for that point was recognized to be 11.9 (at the constant phase with AII) and taking that as 100%, the above tumor blood flow percentages were 68.9, 87.4, 100.0, 71.4, 80.7, 59.7, etc.

The significance of differences was evaluated by using Student's t test. Probability levels of less than 5% ($P<0.05$) were considered significant.

RESULTS

Because AII-induced hypertension was maintained until the constant-phase data were obtained, the infusion time of AII was 12.0 ± 5.0 min and the total dose was 3.13 ± 1.70 μg/kg; the average dose per body weight was 0.26 μg/kg/min under these conditions.

Table I presents MABP and the tumor blood flow (2 points per rat) in both protocols. AII was usually capable of making MABP approximately 150 mmHg in 2–5 min, increasing tumor blood flow to twice the baseline level (i.e. the value before the first administration). After an interval of 60 min MABP and tumor blood flow with AII returned to baseline levels (Protocol 1). ET-1 increased MABP after a transient (less than 1 min) decrease, usually to 55–65 mmHg (data not shown). At 2 min after ET-1, tumor blood flow increased slightly and transiently. There was no significant difference between protocol 1 and 2, except the MABP of "before AII."

Figure 1 shows the means and the standard deviations of MABP (n=16) and the previously described tumor blood flow percentage (n=32). Significant differences in MABP were recognized between before and after administration of both agents. AII-induced hypertension significantly increased tumor blood flow above the baseline level and above every point after ET-1, except that no significant difference was observed between the constant phase with AII and the point 2 min after ET-1. In Fig. 1, the numbers of data are double those of Table I because the data of protocols 1 and 2 in Table I were computed together (see Discussion).

In preliminary experiments, the MABP of rats with 2 nmol/kg ET-1 reached 150 mmHg at 5–10 min after the administration, but could not be held there: some rats with 5 nmol/kg ET-1 injected suddenly died during the experiments although no cause could be determined macroscopically or microscopically.

DISCUSSION

No comparison of the effects of ET-1 and AII on tumor blood flow has been published to date, probably due to the variety of problems involved in such a comparison. In the present study, it was difficult to choose a suitable method of measurement of tumor blood flow, as well as the sequence, the interval, the route, the rate, and the dose of administrations, and the site of tumor inoculation.

A hydrogen clearance method permits *in situ* measurement of tissue blood flow.[18] Successive measurements in the same position were necessary in order to study the effects of agents on the tumor blood flow because the tumor blood flow has spatial and temporal heterogeneity even within an individual tumor.[24, 25] If the values of the tumor blood flows were computed in the present study,

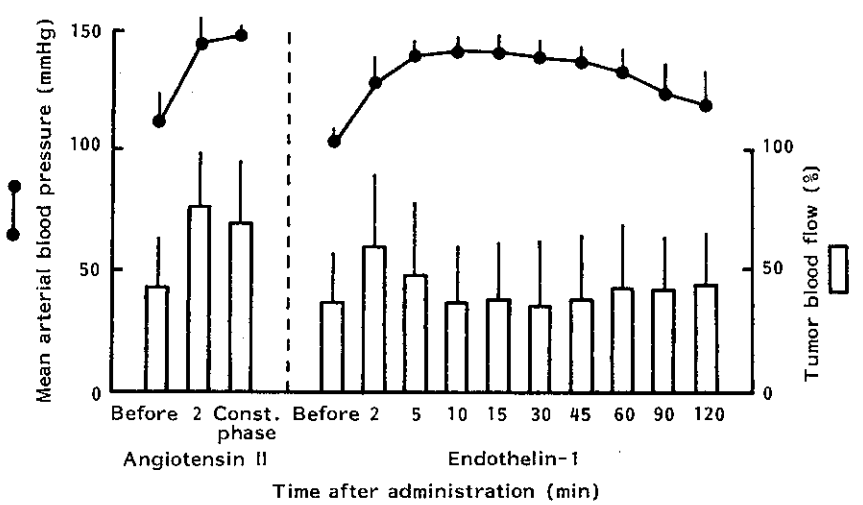

Fig. 1. Mean arterial blood pressure (MABP) and tumor blood flow percentage after angiotensin II or endothelin-1 administration. Means and standard deviations are presented (n=16: MABP, n=32: tumor blood flow percentage).

the standard deviations would be so large (Table I) that statistical analysis would not be possible. Thus, for data analysis, we had to calculate the ratio of blood flow to MBF.

The results of both administration sequences (protocols 1 and 2) suggested that the intervals between the administrations were suitable in terms of tumor blood flow, because the tumor blood flow at the beginning of the second drug did not differ from the baseline level (Table I). Thus, we considered the data of protocols 1 and 2 to be equally valid in terms of tumor blood flow (Fig. 1). Parameters other than tumor blood flow, however, could be influenced by the first agent even after some interval.

In the case of ET-1, bolus systemic injection is a standard method for *in vivo* analysis,[16, 19, 22] and long-term infusion could confuse the results because of the long-term effects of ET-1.[19] In addition, Mortensen and Fink[22] have shown that bolus administration and continuous infusion of ET-1 result in similar hemodynamic effects.

With AII, it has been suggested that administration into the so-called "tumor-feeding artery" would make the tumor blood flow larger than would i.v. administration.[26] Nevertheless, we considered that the elevation of MABP to approximately 150 mmHg with continuous AII i.v. would cause a selective and effective increase of the tumor blood flow.[7, 12, 13] Furthermore, intravenous infusion is performed in most cases of clinical IHC. On the other hand, intraarterial administration induces a high concentration of AII in the tumor microcirculation; the systemic MABP cannot indicate the regional tumor blood flow. Consequently, we could not use MABP as a parameter to select the dose of AII in chemotherapy. Hence, we chose continuous intravenous infusion as the AII administration method in the present study.

The dose (1 nmol/kg) of ET-1 used in the present experiment is comparable to the doses used in previous studies in rats *in vivo*.[16, 19, 22] Mortensen and Fink have said that the dose would be a "maximal" *in vivo* dose.[22] In the present study, moreover, the tumor possibly affected the systemic circulation and the blood coagulation in the rat. Some rats given more than 2 nmol/kg ET-1 developed complications in our preliminary experiments, while the rats given AII survived until death due to the tumor. Careful infusions of AII can control the blood pressure in rats and human patients,[14] because AII has a short biological half-life.[27] On the other hand, ET-1 evokes sustained hypertension even after bolus i.v. ET-1 constricts coronary arteries[16, 28] and can possibly induce lethal ischemia of the heart.[29] These conditions could occur in humans, and would be particularly dangerous for elderly people suffering from cancer. Thus from the viewpoint of safety, ET-1 is less desirable than AII.

The subcutaneously transplanted tumor is a very specific system. In the study field of the tumor blood flow with/without AII, however, this system suggests the actual blood flow in methylcholanthrene-induced primary tumors, transplanted tumors in various organs,[13] lymph node metastasis[30] in rats and human cancers transplanted to nude mice and nude rats.[31] The effects of ET-1 on blood flow in tumor tissue and normal tissue in other organs largely remain to be examined.

Thus, comparing bolus systemic injection of ET-1 and continuous systemic infusion of AII, AII increased tumor blood flow more than did ET-1, at least in our system, and AII is also considered more suitable for cancer therapy and diagnosis from the viewpoint of safety.

Vasoconstriction in microcirculation with AII and ET-1

Concerning the mechanism of the increase of tumor blood flow by AII, we added nothing to the theory previously described,[12] i.e. AII i.v. appears to cause "peripheral" vasoconstriction[32] in the normal tissue, so the blood flow is forced into a tumor vessel in a parallel circuit[3, 8] to a normal vessel. However, intravenously administered adrenergic stimulants cause "central" vasoconstriction,[32] decreasing the blood flow of tumor and normal tissue, as a rule.[33, 34]

In relation to the effects of ET-1 on tumor blood flow, it is necessary to distinguish the early phase (up to 5 min) from the late phase (after 5 min). The early fast flow at 2 min after ET-1, which was often the MBF of a given position, could be a very attractive phenomenon if ET-1 could be utilized for clinical oncology. The observation of Borić *et al.*[35] in the hamster cheek pouch suggested that arterioles of the 4th branching order responded to superfusion with 10 n*M* ET more quickly than arterioles of the 1st or 2nd order; however, the constriction lasted considerably longer in the latter vessels. These results and our results favor the hypothesis that in the early phase ET-1, like AII, may constrict only peripheral arterioles, enriching the tumor blood flow, while in the late phase ET-1, like adrenergic stimulants, may constrict more "central" arterial vessels.

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