Cancer Chemother Pharmacol (2003) 51: 21–28
DOI 10.1007/s00280-002-0534-3

ORIGINAL ARTICLE

Aarati Rai · Anil Gulati

Evidence for the involvement of ET_B receptors in ET-1-induced changes in blood flow to the rat breast tumor

Received: 25 March 2002 / Accepted: 11 September 2002 / Published online: 8 November 2002

Abstract *Purpose*: Structure, growth, and function of the blood vessels in breast tumors are markedly different from those in normal breast tissue due to changes in the production of growth factors such as vascular endothelial growth factor (VEGF), vasoactive substances such as endothelin-1 (ET-1) and cytokines. The role of ET-1 in breast tumor angiogenesis is not adequately understood. Studies have shown that the expression of proET-1, proET-3, and ET_B receptors is increased in breast tumor. However, it is unclear whether there are any changes in ET-1-induced vascular responses in breast tumor. Hence, in the present study we investigated systemic hemodynamics and regional circulatory effects of ET-1 in rats with breast tumors. *Methods: Female Sprague-Dawley rats weighing 180–200 g were divided into the following groups: (1) normal rats treated with saline* ($n=6$), (2) tumor-bearing rats treated with methylnitrosourea (MNU) ($n=6$), (3) normal rats treated with saline plus the specific ET_B receptor antagonist BQ 788 ($n=5$), and (4) tumor-bearing rats treated with MNU plus BQ 788 ($n=5$) Tumor development was monitored by regular palpation and measurement of tumor size. Once tumors had reached approximately 2–4 cm in diameter, the rats were anesthetized with urethane (1.5 g/kg i.p.) and their cardiovascular parameters were measured using a radioactive microsphere technique. Simultaneously, blood perfusion to the breast tissue was also measured using a laser Doppler technique. *Results*: ET-1 produced a significant increase in mean arterial pressure in normal and tumor-bearing rats. Blood flow to the tumor tissue increased significantly in response to ET-1 as compared to breast tissue in normal rats. This response was accompanied by a concomitant decrease in vascular resistance in the tumor tissue. These results were confirmed by laser Doppler flowmetry, which showed a significant increase in blood perfusion to breast tumor compared to normal breast tissue. This increase in blood perfusion was attenuated by pretreatment with BQ 788, suggesting an ET_B receptor-mediated vasodilator action of ET-1 in rat breast tumor. *Conclusions*: The results indicate that ET-1 induced an increase in blood flow to breast tumor tissue mediated through ET_B receptors.

Keywords Breast cancer · Endothelin · BQ 788 (*N-cis*-2,6-Dimethylpiperidinocarbonyl-L-gamma-methylleucyl-D-1-methoxycarbonyltrptophanyl-D-Nle) · Rat · Blood flow

A. Rai · A. Gulati (✉)
Department of Biopharmaceutical Sciences,
The University of Illinois at Chicago,
833 South Wood Street,
Chicago, IL 60612, USA
E-mail: gulati@uic.edu
Tel.: +1-312-9960826
Fax: +1-312-9960098

A. Gulati
Department of Bioengineering,
The University of Illinois at Chicago,
833 South Wood Street,
Chicago, IL 60612, USA

A. Gulati
Department of Neurology and Rehabilitation,
The University of Illinois at Chicago,
833 South Wood Street,
Chicago, IL 60612, USA

Introduction

The development of breast cancer is a complex process involving a combination of factors, such as environmental and genetic factors. One of the extensively studied breast tumor models is the chemically induced rat mammary carcinogenesis model [9, 18, 19, 39, 54]. Chemically induced mammary tumorigenesis in rats may not be an accurate representation of the human mammary tumor growth process but this model seems to most closely resemble human cancer [41].

Chemically induced rat mammary carcinogenesis is commonly achieved by the administration of 7,12-dimethylbenzene(a)anthracene (DMBA) [37] or *N*-methylnitrosourea (MNU) [37]. Tumors induced by DMBA or MNU have different morphological characteristics. Tumors induced by MNU are more localized to the breast and are less likely to metastasize [25]. Therefore, in the present study, MNU was chosen as the chemical agent for the specific induction of breast tumors in rats. These tumors can be benign with fibroadenomas and papillomas or they can be malignant [54]. Rats have six pairs of mammary glands, one in the cervical, two in the thoracic, one in the abdominal and two in the inguinal regions [4, 54]. Virgin rats treated with MNU develop more tumors in the thoracic region than the abdominal region [40].

The development of tumor vasculature has been extensively studied. Tumors that grow beyond the size of a few millimeters require a constant nutrient supply and therefore their own vascular bed and blood flow [10]. Recruitment of new vasculature from preexisting blood vessels is defined as angiogenesis. Without constant nourishment from these developing blood vessels the tumors would become hypoxic and subsequently die. Therefore, the tumor vasculature has been a target of cancer therapy for a long time [10]. Tumor blood vessels develop in a very different way from normal vasculature and have different properties. Single-layered epithelial cells are the first hastily formed tumor blood vessels. It has been suggested that these blood vessels are recruited when the tumor secretes certain growth factors such as vascular endothelial growth factor (VEGF) in response to hypoxic conditions [23]. These newly formed tumor blood vessels do not have a smooth muscle layer or innervation [29, 36, 57]. Tumors also incorporate mature blood vessels that possess all their autoregulatory functions [29]. Normal tissue vascular tone is governed by a host of endogenous factors such as H^+, K^+, Ca^{2+}, pO_2, pCO_2, and nitric oxide (NO), as well as other regulatory substances such as endothelin (ET-1) [24, 46].

ET-1 is a potent vasoconstrictor and contributes significantly to the regulation of vascular tone [11, 61]. In breast cancer tissue ET_B receptors are found on stromal fibroblast cells [5, 34]. ETs have been found to be mitogenic to fibroblasts [53], melanocytes, vascular smooth muscle and endothelium [3, 35, 52]. Alanen et al. have shown an increase in ET-1, ET-3 and ET_B receptor expression in breast carcinoma [1]. It has been shown that both ET-1 and ET-3 cause an increase in VEGF, which is an important angiogenic factor [26, 32, 35]. Thus an increase in ET-1 would promote tumor growth. Increases in ET-1 levels in breast tumors have been shown in several studies [1, 21, 31, 33, 59, 60]. However, this is the first study in which the effects of ET-1 on systemic hemodynamics and blood circulation in the breast tumor tissue have been investigated.

Materials and methods

Animals

Female Sprague-Dawley rats (Harlan Company, Madison, Wis.) weighing 180–200 g were used. All animals were housed, three to a cage, at the Biological Research Laboratories, UIC, under controlled conditions (23 ± 1°C, 50 ± 10% humidity) and under artificial light (0600–1800 h). The animals were given food and water ad libitum. The experiments were done only after the animals had been acclimatized to the environment for at least 4 days. The protocols for the experiment were approved by the Animal Care Committee of the University of Illinois at Chicago, and all animals were used as per the rules and guidelines of the American Association for Accreditation of Laboratory Animal Care (AALAC).

Drugs

MNU was purchased from Ash Stevens, Detroit, Mich. BQ 788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-gamma-methylleucyl-D-1-methoxycarbonyltrptophanyl-D-Nle), a specific ET_B receptor antagonist, and ET-1 were obtained from the American Peptide Company, Sunnyvale, Calif. BQ 788 was dissolved in saline and ET-1 was dissolved in 0.1% albumin.

Experimental protocol

MNU (50 mg/kg i.p.) or saline (1 ml/kg i.p.) was administered to female rats and from the 4th week onwards rats were regularly palpated for identification of breast tumors. Once tumors reached 2–4 cm in diameter the experiments were begun. Tumors of 2–4 cm in diameter took approximately 3 months to develop.

The following groups were studied to evaluate the effect of ET-1 infusion on systemic hemodynamics and blood circulation in the mammary tissue of normal and tumor-bearing rats:

1. Normal (saline-treated) rats ($n = 6$) receiving a 30-min infusion of ET-1 (50 ng/kg per min).
2. Tumor-bearing (MNU-treated) rats ($n = 6$) receiving a 30-min infusion of ET-1 (50 ng/kg per min).

The following groups were studied to evaluate the role of ET_B receptors on systemic hemodynamic and blood flow changes in the mammary tissue induced by ET-1 infusion in normal and tumor-bearing rats:

1. Normal (saline-treated) rats ($n = 5$) receiving a 20-min infusion of BQ 788 (0.5 μmol/kg) min followed by a 30-min infusion of ET-1 (50 ng/kg per min).
2. Tumor-bearing (MNU-treated) rats ($n = 5$) receiving a 20-min infusion of BQ 788 (0.5 μmol/kg) followed by a 30-min infusion of ET-1 (50 ng/kg per min).

Systemic hemodynamic and regional circulation parameters were determined at baseline, and 30, 60 and 120 min after infusion of ET-1 (50 ng/kg per min). Since the ET-1 infusion was carried out for 30 min, the 30-min data show the effect of ET-1 and the 60- and 120-min data indicate the duration of the ET-1 effect.

Surgical preparation

Rats were anesthetized with urethane (1.5 g/kg i.p.) (Sigma Chemicals, St. Louis, Mo.). All surgical areas were shaved and cleaned with alcohol swabs. The left femoral vein was cannulated (PE 50 tubing; Clay Adams, Parsippany, N.J.) for drug administration. The left femoral artery was cannulated (PE 50 tubing)

and was used for withdrawal of reference blood samples in the microsphere studies using a withdrawal pump (Model 22; Harvard Apparatus, South Natick, Mass.). The right femoral artery was cannulated (PE 50 tubing) and connected to a Gould P23 ID pressure transducer for recording the blood pressure on a Grass P7D polygraph (Grass Instrument Company, Quincy, Mass.) through a 7PI preamplifier. The heart rate (HR) was recorded through a 7P4B Grass tachograph (Grass Instrument Company) triggered from blood pressure signals. The right carotid artery was exposed and a PE 50 tube was guided through the common carotid artery into the left ventricle. The presence of the cannula in the left ventricle was confirmed by recording the pressure on the Grass polygraph using a Statham P23 DC pressure transducer (Grass Instrument Company). When the cannula reached the left ventricle, the diastolic pressure dropped to zero. In order to keep the blood pO_2, pCO_2 and pH constant, and to avoid the effect of respiration on blood pressure and HR, animals were kept on constant-rate artificial respiration by inserting an endotracheal cannula connected to a rodent ventilator (Model 683; Harvard Apparatus).

Determination of systemic hemodynamics and regional circulation

Systemic hemodynamics and regional blood circulation were determined using a procedure described previously [12, 13, 47]. At each measurement, a thoroughly mixed suspension of approximately 100,000 microspheres (15 ± 1 μm diameter) labeled with ^{46}Sc (scandium), ^{113}Sn (tin), ^{141}Ce (cerium), or ^{95}Nb (niobium) (New England Nuclear Corporation, Boston, Mass.) in 0.2 ml saline was injected into the left ventricle and flushed with 0.3 ml saline over a 15-s period. In order to calculate the blood flow, arterial blood was withdrawn at a rate of 0.5 ml/min through the right femoral artery. Blood was withdrawn for 90 s starting about 5–10 s before the microsphere injection. At the end of the experiment the animals were killed with an overdose of pentobarbital sodium. All the tissues and organs were dissected out, weighed and placed in vials. The radioactivity in the standards, the blood samples and the tissue samples were counted in a Packard Minaxi Auto-Gamma 5000 series gamma counter (Packard Instruments, Downers Grove, Ill.) with preset windows discriminating the isotope energies. The following parameters were calculated: (1) cardiac output (CO) [(radioactivity injected×withdrawal rate of arterial blood)/radioactivity in sampled arterial blood], (2) stroke volume (SV, CO/HR), (3) total peripheral resistance (TPR, mean arterial pressure (MAP)/CO), (4) regional blood flow [(radioactivity in tissue×withdrawal rate of arterial blood)/radioactivity in sampled arterial blood], and (5) regional vascular resistance (MAP/regional blood flow). The data were calculated using computer programs described previously [45].

Breast blood perfusion measurement by laser Doppler flowmetry

The blood perfusion to the mammary gland of the rats was measured using laser Doppler flowmetry (LDF) as described previously [50, 51]. The animals were shaved around the nipples. The skin surrounding the mammary glands was dissected out as a lambeau about 6 cm wide and 4 cm long. A standard model fiber-optic probe was applied to the surface of the lambeau and secured to the tissue by double-stick tape. It was placed in a metal holder and taped down to prevent movement. It was then connected to a Periflux PF2b 4000 laser Doppler flowmeter (Perimed, Stockholm, Sweden). The time constant was set to 1.5 s and the bandwidth was set to 4 kHz.

Statistical analysis

All data are presented as means ± SEM. Data were analyzed using analysis of variance followed by Duncan's test. A level of $P < 0.05$ was considered significant.

Results

Effect of ET-1 on systemic hemodynamics in normal and tumor-bearing rats

The baseline systemic hemodynamic parameters in normal (saline-treated) rats were: MAP 111.1 ± 4.8 mmHg, CO 268.6 ± 17.6 ml/min, SV 0.87 ± 0.06 ml, TPR 419.6 ± 24.37 mmHg min ml^{-1}, and HR 312.5 ± 20.2 beats/min. In normal rats a significant increase in MAP was observed at 30 min (14.5%, $P < 0.05$) and a decrease at 120 min (17.8%, $P < 0.05$) following ET-1 administration. TPR was increased at 120 min (49.2%, $P < 0.05$). CO was decreased at 60 and 120 min (22.9% and 42.5%, respectively; $P < 0.05$) following ET-1 administration. SV was decreased at 60 and 120 min (20.9% and 36%, respectively; $P < 0.05$). There was no significant change in HR (Fig. 1).

The baseline systemic hemodynamic parameters in tumor-bearing (MNU-treated) rats were similar to those in normal rats. A significant increase in MAP was observed at 30 and 60 min (19.1% and 15.3%, respectively; $P < 0.05$) following ET-1 administration. TPR was increased at 30, 60 and 120 min (73.9%, 39.7% and 71.4%, respectively; $P < 0.05$) following ET-1 administration. CO was decreased at 30, 60 and 120 min (29.4%, 16.7% and 36.1%, respectively; $P < 0.05$). SV was decreased at 30, 60 and 120 min (31.1%, 17.9% and 32.1%, respectively; $P < 0.05$). There was no change in HR (Fig. 1).

Effect of ET-1 on blood flow and vascular resistance in breast tissue of normal and tumor-bearing rats

No change in blood flow to the breast tissue of normal rats was observed following ET-1 administration, but there was a significant decrease (18.61%, $P < 0.05$) in vascular resistance at 60 min (i.e. 30 min after the end of the ET-1 infusion) in the breast tissue of normal rats (Fig. 2).

There were significant differences between the blood flow and the regional vascular resistance in the breast tissue of tumor-bearing and normal rats. A significant increase (153%, $P < 0.05$) in blood flow to the breast tissue of tumor-bearing rats as compared to the value in normal rats was observed 60 min following ET-1 administration. Vascular resistance in tumor-bearing rats was significantly different at baseline (102%, $P < 0.05$) and at 60 min (147%, $P < 0,05$) following ET-1 administration as compared to the value in normal rats.

Effect of ET-1 on blood perfusion in breast tissue of normal and tumor-bearing rats as measured by LDF

Figure 3 shows the changes in perfusion, concentration of moving blood cells (CMBC) and velocity of red blood

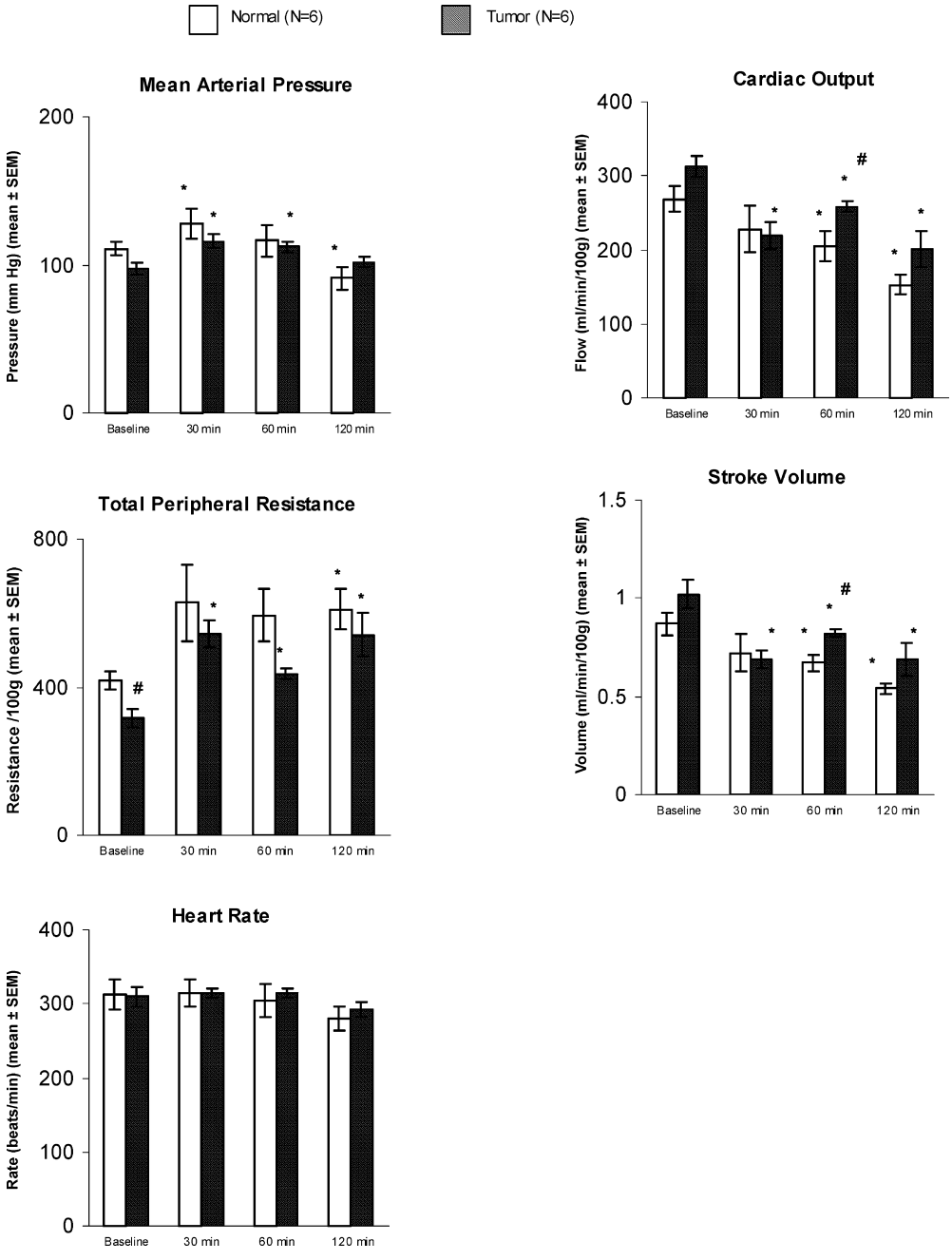

Fig. 1 Effect of ET-1 on systemic hemodynamics of normal and tumor-bearing rats. *$P < 0.05$ compared to baseline, #$P < 0.05$ compared to normal

cells (RBC) in the breast tissue of tumor-bearing and normal rats. Blood perfusion in the breast tissue of normal rats did not change following ET-1 administration. Perfusion in the breast tissue of tumor-bearing rats 30 min following ET-1 administration was increased significantly (176%, $P < 0.05$) compared to the value in normal rats. This increase in perfusion had returned to baseline by 60 and 120 min following ET-1 administration in tumor-bearing rats.

The CMBC in tumor-bearing rats was increased significantly (54%, $P < 0.05$) at 60 min following ET-1 administration as compared to the value in normal rats. CMBC had returned to baseline at 120 min following ET-1 administration. The velocity of RBC had increased significantly (252%, $P < 0.05$) at 30 min following ET-1 administration as compared to the value in normal rats. By 120 min after ET-1 administration the velocity of RBC in tumor-bearing rats had returned to the baseline value.

Effect of BQ 788 on ET-1-induced changes in blood perfusion in breast tissue of normal and tumor-bearing rats as measured by LDF

Figure 4 shows the effect of BQ 788 on changes induced by ET-1 in blood perfusion, CMBC and velocity of RBC in tumor-bearing and normal rats. Blood perfusion in

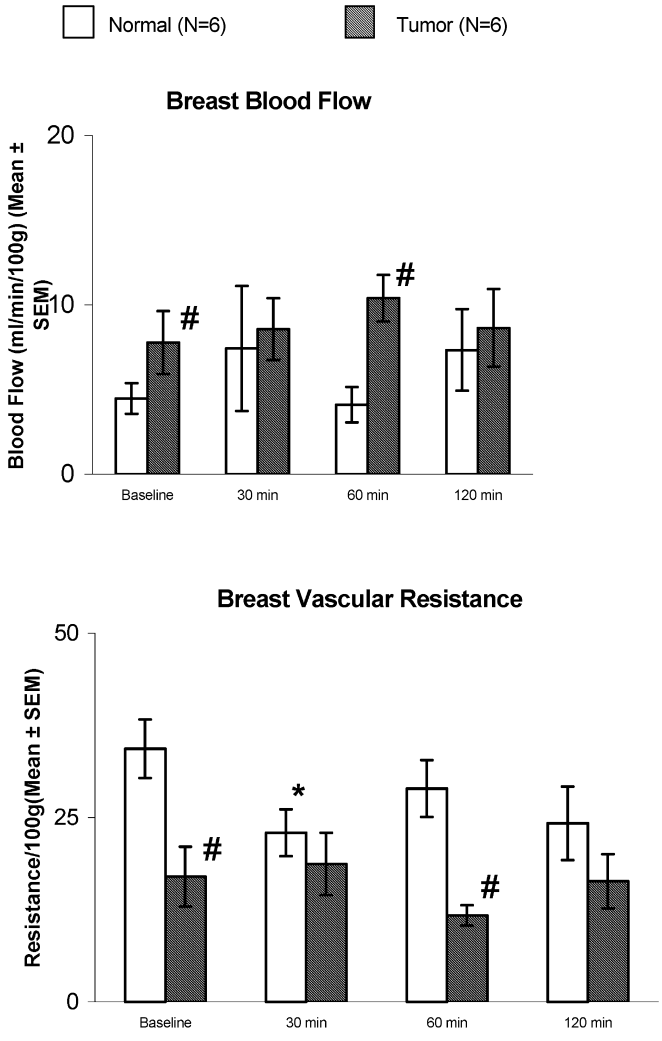

Fig. 2 Effect of ET-1 on blood flow and regional vascular resistance in breast tissue of normal and tumor-bearing rats. $^{*}P < 0.05$ compared to baseline, $^{\#}P < 0.05$ compared to normal

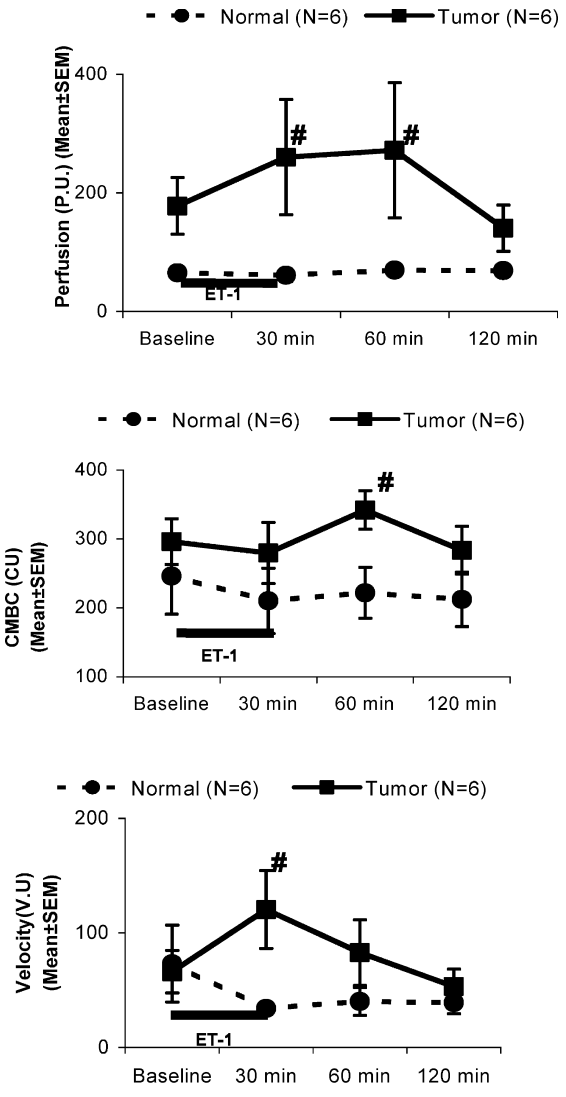

Fig. 3 Effect of ET-1 on perfusion, CMBC and velocity of blood cells in breast tissue of normal and tumor-bearing rats. $^{\#}P < 0.05$ compared to normal

the breast tissue of normal rats did not change significantly following BQ 788 or ET-1 administration. However, perfusion in the breast tumor tissue of tumor-bearing rats was decreased significantly at 30 min ($25.25 \pm 5.7\%$, $P < 0.05$) and 60 min ($25.17 \pm 2.8\%$, $P < 0.05$) following ET-1 administration in BQ 788-pretreated rats. Pretreatment with BQ 788 attenuated the increase in perfusion induced by ET-1 in tumor-bearing rats. No difference between the perfusion in breast tissue of tumor-bearing and normal rats was observed following ET-1 administration in BQ 788-pretreated rats.

The baseline CMBC in tumor-bearing rats was significantly higher than in normal rats (42.4%, $P < 0.05$). However, following BQ 788 administration there was no difference in CMBC between tumor-bearing and normal rats. There was no difference in velocity of RBC between the two groups.

Discussion

In the present study we evaluated the effect of ET-1 on systemic hemodynamics and blood flow to the breast tissue of normal and tumor-bearing rats. ET-1 is a powerful vasoconstrictor [61]. It belongs to a family of peptides approximately 21 amino acids long. At least two forms of ET receptors exist and they are known as ET_A and ET_B. ET_A receptor has a higher affinity for ET-1 but ET_B receptor has equal affinity for both ET-1 and ET-3 [2, 17, 42]. ET-1 has complex cardiovascular effects [27, 48]. When administered to anesthetized and ventilated rats an immediate decrease followed by a sustained increase in blood pressure is observed [22]. It has been found that ET_A receptors are responsible for the vasoconstrictor responses and that ET_B receptors are responsible for the vasodilatory actions of ET-1 [28]. ET-1

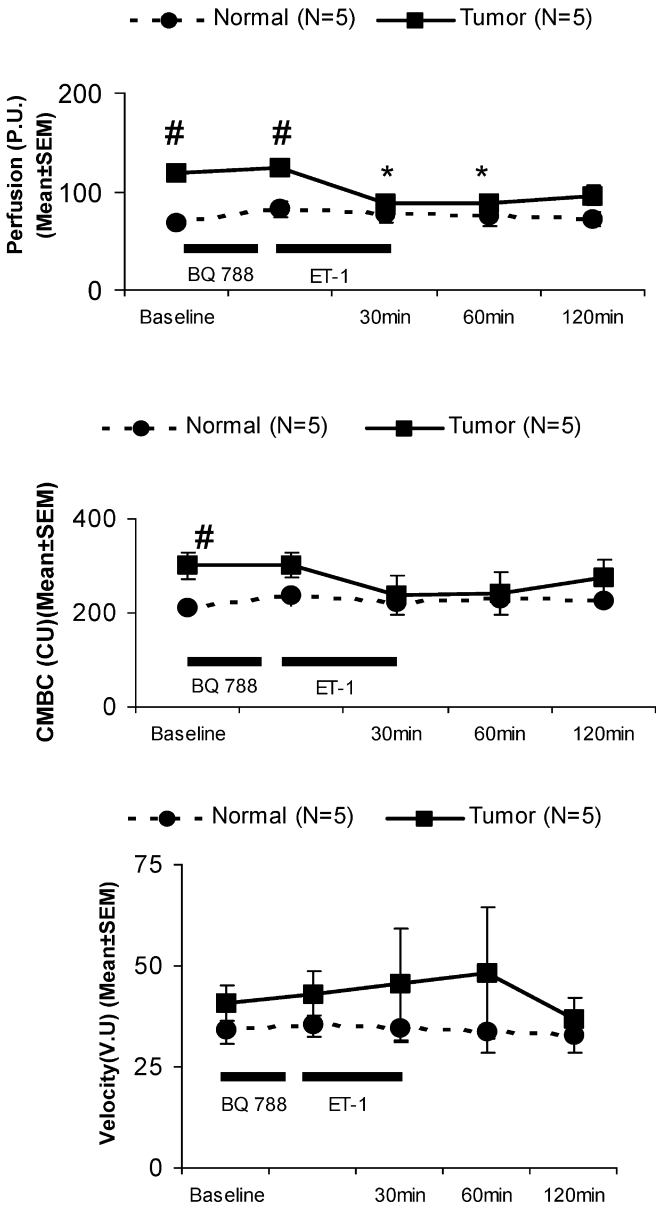

Fig. 4 Effect of BQ 788 on ET-1-induced changes in blood perfusion, CMBC and velocity of blood cells in breast tissue of normal and tumor-bearing rats. $^{*}P < 0.05$ compared to baseline, $^{\#}P < 0.05$ compared to normal

administration results in an increase in blood flow to skin tumors possibly due to the vasodilatory actions of ET_B [6]. If there is an increase in ET-1 and ET_B receptors in breast tumors, we would anticipate similar results in blood flow to breast tumors of rats.

It is known that ET-1 stimulates angiogenesis by promoting production of VEGF [8, 11, 43, 44]. ET-1 is increased in many cancer tissues such as breast carcinoma [59], breast phyllode tumor [60], prostate carcinoma [31], liver carcinoma [21] and some meningiomas [33]. ET receptor expression varies between different cancers [31]. This is the first study to determine changes in ET-1-induced vascular responses in breast tumor. The method used in this study is a well-established radioactive microsphere technique to study systemic hemodynamics and regional blood circulation [13, 14, 15, 16].

Infusion of 50 ng/kg per min of ET-1 caused a biphasic response in blood pressure: an immediate but short-lasting decrease followed by a sustained increase. These results are in accordance with those of previous studies [20, 30, 38, 56]. ET-1 produced a marked pressor response in both normal and tumor-bearing rats, which was accompanied by a significant decrease in SV and CO. TPR significantly increased in both normal and tumor-bearing rats and may explain the observed pressor response.

Baseline blood flow to the breast tumor tissue of tumor-bearing rats was higher than blood flow in normal animals. This has been observed in an earlier study and may be due to the recruitment of new blood vessels in the tumor [55]. Blood flow to the breast tumor following ET-1 administration was significantly increased compared to that observed in the breast tissue of normal rats. LDF showed an increase in blood perfusion to the breast tumor, confirming the increase in blood flow observed in the breast tumor tissue following ET-1 administration. The increase in blood perfusion could have been due to an increase in either the velocity of RBC or CMBC or both. At the end of ET-1 infusion an increase in velocity of RBC was observed while 30 min after ET-1 infusion an increase in CMBC was observed.

We also tested the hypothesis that the observed increase in blood flow in response to ET-1 may be due to ET_B-mediated vasodilation. Studies have shown that ET-1 and ET_B receptor expression is augmented in breast cancer tissue [1, 59]. BQ 788 is a specific ET_B receptor antagonist. It inhibits binding to ET_B receptors with an IC_{50} value of 1.2 n*M*. In the present study we used BQ 788 to determine the role of ET_B receptors in ET-1-induced vasodilation in the breast tumor. It was found that pretreatment with BQ 788 significantly attenuated the increase in blood flow induced by ET-1, indicating that ET_B receptors are involved in ET-1-induced vasodilation. Blood flow changes were determined following pretreatment with BQ 788 in normal and tumor-bearing rats. In the doses used there was a small statistically insignificant increase in basal blood perfusion in the tumor-bearing rats. We cannot comment based on the present results on whether BQ 788 affected baseline breast tumor blood flow. Separate studies will be performed to determine the effect of BQ 788 on basal blood flow.

The expression of ET_B receptors is significantly higher in the endothelial cells than in the smooth muscle cells and is regulated by various growth factors and cytokines [49]. Normal breast tissue has a higher level of ET_B than of ET_A receptors [1] and it is possible that during breast cancer ET_B receptors are over-expressed and contribute to maintaining blood flow to the tumor tissue. As tumors grow they recruit new blood vessels to supply nutrients. This could be by incorporation of existing vessels into the tumor or by the creation of new

blood vessels [7]. Studies have shown that new vessels have different physical properties from normal vasculature. Unlike normal vessels, these vessels do not have any smooth muscle layers or any innervation. They consist only of single layers of endothelial cells.

In summary, the present study clearly demonstrates the infusion of ET-1 produced an increase in blood flow and a decrease in vascular resistance of breast tumor tissue and this increase in blood flow could be blocked by the ET_B receptor antagonist, BQ 788. We suggest that the increased blood flow observed in rat breast tumor may be due to increased ET_B receptors. Therefore, blockade of these receptors could reduce blood flow to the tumors. The clinical significance of these findings are that ET_B receptor antagonists could be used to reduce blood supply to breast tumor tissue and thereby help to reduce growth of the tumor, while ET_B receptor agonists could be used to increase blood flow selectively to the breast tumor and increase delivery of anticancer drugs to the tumor tissue.

References

1. Alanen K, Deng DX, Chakrabarti S (2000) Augmented expression of endothelin-1, endothelin-3 and the endothelin-B receptor in breast carcinoma. Histopathology 36:161
2. Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S (1990) Cloning and expression of a cDNA encoding an endothelin receptor. Nature 348:730
3. Asano T, Aoyagi M, Hirakawa K, Ikawa Y (1994) Effect of endothelin-1 as growth factor on a human glioma cell line; its characteristic promotion of DNA synthesis. J Neurooncol 18:1
4. Astwood EB, Geschickter CF, Rausch EO (1937) Development of the mammary gland of the rat. Am J Anat 61:372
5. Baley PA, Resink TJ, Eppenberger U, Hahn AW (1990) Endothelin messenger RNA and receptors are differentially expressed in cultured human breast epithelial and stromal cells. J Clin Invest 85:1320
6. Bell KM, Prise VE, Chaplin DJ, Tozer GM (1995) Effect of endothelin-1 and sarafotoxin S6c on blood flow in a rat tumor. J Cardiovasc Pharmacol 26:S222
7. Chaplin DJ, Hill SA, Bell KM, Tozer GM (1998) Modification of tumor blood flow: current status and future directions. Semin Radiat Oncol 8:151
8. Cruz A, Parnot C, Ribatti D, Corvol P, Gasc JM (2001) Endothelin-1, a regulator of angiogenesis in the chick chorioallantoic membrane. J Vasc Res 38:536
9. Dao TL, Chan PC (1983) Effect of duration of high fat intake on enhancement of mammary carcinogenesis in rats. J Natl Cancer Inst 71:201
10. Folkman J (1986) How is blood vessel growth regulated in normal and neoplastic tissue? G.H.A. Clowes memorial Award Lecture. Cancer Res 46:467
11. Goto K (2001) Basic and therapeutic relevance of endothelin-mediated regulation. Biol Pharm Bull 24:1219
12. Gulati A, Srimal RC, Bhargava HN (1989) Effect of varying concentration of ethanol on systemic hemodynamics and regional circulation. Alcohol 6:9
13. Gulati A, Sharma AC, Burhop KE (1994) Effect of stroma-free hemoglobin and diaspirin cross-linked hemoglobin on the regional circulation and systemic hemodynamics. Life Sci 55:827
14. Gulati A, Singh R, Chung SM, Sen AP (1995) Role of endothelin-converting enzyme in the systemic hemodynamics and regional circulatory effects of proendothelin-1 (1-38) and diaspirin cross-linked hemoglobin in rats. J Lab Clin Med 126:559
15. Gulati A, Sharma AC, Singh G (1996) Role of endothelin in the cardiovascular effects of diaspirin crosslinked and stroma reduced hemoglobin. Crit Care Med 24:137
16. Gulati A, Sen AP, Sharma AC, Singh G (1997) Role of ET and NO in resuscitative effect of diaspirin cross-linked hemoglobin after hemorrhage in rat. Am J Physiol 273:H827
17. Hori S, Komatsu Y, Shigemoto R, Mizuno N, Nakanishi S (1992) Distinct tissue distribution and cellular localization of two messenger ribonucleic acids encoding different subtypes of rat endothelin receptors. Endocrinology 130:1885
18. Huggins C, Yang NC (1962) Introduction and extinction of mammary cancer. Science 137:257
19. Huggins C, Grand LC, Brillantes F (1959) Critical significance of the breast structure in the induction of mammary cancer in the rat. Proc Natl Acad Sci U S A 45:1294
20. Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, Masaki T (1989) The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci U S A 86:2863
21. Kar S, Yousem SA, Carr BI (1995) Endothelin-1 expression by human hepatocellular carcinoma. Biochem Biophys Res Commun 216:514
22. Kuwaki T, Koshiya N, Cao WH, Takahashi H, Terui N, Kumada M (1990) Modulatory effects of endothelin-1 on central cardiovascular control in rats. Jpn J Physiol 40:827
23. Lissoni P, Malugani F, Bonfanti A, Bucovec R, Secondino S, Brivio F, Ferrari-Bravo A, Ferrante R, Vigore L, Rovelli F, Mandala M, Viviani S, Fumagalli L, Gardani GS (2001) Abnormally enhanced blood concentrations of vascular endothelial growth factor (VEGF) in metastatic cancer patients and their relation to circulating dendritic cells, IL-12 and endothelin-1. J Biol Regul Homeost Agents 15:140
24. Luscher TF, Vanhoutte PM (1990) The endothelium: modulator of cardiovascular function. CRC Press, Boca Raton, p 61
25. Macejova D, Brtko J (2001) Chemically induced carcinogenesis: a comparison of 1-methyl-1-nitrosourea, 7,12-dimethylbenzanthracene, diethylnitroso-amine and azoxymethan models (minireview). Endocr Regul 35:53
26. Matsuura T, Yukimura T, Kim S, Miura K, Iwao H (1996) Selective blockade of endothelin receptor subtypes on systemic and renal vascular responses to endothelin-1 and IRL1620, a selective endothelin ETB-receptor agonist, in anesthetized rats. Jpn J Pharmacol 71:213
27. Matsuura T, Miura K, Ebara T, Yukimura T, Yamanaka S, Kim S, Iwao H (1997) Renal vascular effects of the selective endothelin receptor antagonists in anaesthetized rats. Br J Pharmacol 122:81
28. Matsuura A, Yamochi W, Hirata K, Kawashima S, Yokoyama M (1998) Stimulatory interaction between vascular endothelial growth factor and endothelin-1 on each gene expression. Hypertension 32:89
29. Mattsson J, Appelgren L, Hamberger B, Peterson H (1979) Tumor vessel innervation and influence of vasoactive drugs on tumor blood flow. In: Tumor blood circulation: angiogenesis, vascular morphology and blood flow of experimental and human tumors. CRC Press, Boca Raton, p 129
30. Miyauchi T, Ishikawa T, Tomobe Y, Yanagisawa M, Kimura S, Sugishita Y, Ito I, Goto K, Masaki T (1989) Characteristics of pressor response to endothelin in spontaneously hypertensive and Wistar-Kyoto rats. Hypertension 14:427
31. Nelson JB, Chan-Tack K, Hedican SP, Magnuson SR, Opgenorth TJ, Bova GS, Simons JW (1996) Endothelin-1 production and decreased endothelin B receptor expression in advanced prostate cancer. Cancer Res 56:663
32. Okuda Y, Tsurumaru K, Suzuki S, Miyauchi T, Asano M, Hong Y, Sone H, Fujita R, Mizutani M, Kawakami Y, Nakajima T, Soma M, Matsuo K, Suzuki H, Yamashita K (1998) Hypoxia and endothelin-1 induce VEGF production in human vascular smooth muscle cells. Life Sci 63:477
33. Pagotto U, Arzberger T, Hopfner U, Sauer J, Renner U, Newton CJ, Lange M, Uhl E, Weindl A, Stalla GK (1995)

Expression and localization of endothelin-1 and endothelin receptors in human meningiomas. Evidence for a role in tumoral growth. J Clin Invest 96:2017
34. Patel KV, Schrey MP (1996) Endothelins in breast cancer. Cancer Treat Res 83:369
35. Pedram A, Razandi M, Hu RM, Levin ER (1997) Vasoactive peptides modulate vascular endothelial cell growth factor production and endothelial cell proliferation and invasion. J Biol Chem 272:17097
36. Reinhold HS (1979) In vivo observations of tumor blood flow. In: Tumor blood circulation: angiogenesis, vascular morphology and blood flow of experimental and human tumors. CRC Press, Boca Raton, p 115
37. Rogers AE, Lee SY (1986) Chemically induced mammary gland tumors in rats: modulation by dietary fat. Alan R. Liss, New York, p 255
38. Rohmeiss P, Photiadis J, Rohmeiss S, Unger T (1990) Hemodynamic actions of intravenous endothelin in rats: comparison with sodium nitroprusside and methoxamine. Am J Physiol 258:H337
39. Russo IH, Russo J (1978) Developmental stage of the rat mammary gland as determinant of its susceptibility to 7,12-dimethylbenz[a]anthracene. J Natl Cancer Inst 61:1439
40. Russo J, Russo IH (1987) Biological and molecular bases of mammary carcinogenesis. Lab Invest 57:112
41. Russo J, Gusterson BA, Rogers AE, Russo IH, Wellings SR, van Zwieten MJ (1990) Comparative study of human and rat mammary tumorigenesis. Lab Invest 62:244
42. Sakurai T, Yanagisawa M, Takuwa Y, Miyazaki H, Kimura S, Goto K, Masaki T (1990) Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. Nature 348:732
43. Salani D, Di Castro V, Nicotra MR, Rosano L, Tecce R, Venuti A, Natali PG, Bagnato A (2000) Role of endothelin-1 in neovascularization of ovarian carcinoma. Am J Pathol 157:1537
44. Salani D, Taraboletti G, Rosano L, Di Castro V, Borsotti P, Giavazzi R, Bagnato A (2000) Endothelin-1 induces an angiogenic phenotype in cultured endothelial cells and stimulates neovascularization in vivo. Am J Pathol 157:1703
45. Saxena PR, Schamhardt HC, Forsyth RP, Hoeve J (1980) Computer programs for the radioactive microsphere technique. Determination of regional blood flows and other haemodynamic variables in different experimental circumstances. Comput Programs Biomed 12:63
46. Secombe JF, Schaff HV (1994) Vasoactive factors produced by the endothelium. Landes, Austin, p 40
47. Sharma AC, Rebello S, Gulati A (1994) Regional circulatory and systemic hemodynamic effects of diaspirin cross-linked hemoglobin in the rat. Artif Cells Blood Substit Immobil Biotechnol 22:593
48. Singh G, Sharma AC, Thompson EB, Gulati A (1994) Renal endothelin mechanism in altered thyroid states. Life Sci 54:1901
49. Smith PJ, Teichert-Kuliszewska K, Monge JC, Stewart DJ (1998) Regulation of endothelin-B receptor mRNA expression in human endothelial cells by cytokines and growth factors. J Cardiovasc Pharmacol 31:S158
50. Song CW, Chelstrom LM, Levitt SH, Haumschild DJ (1989) Effects of temperature on blood circulation measured with the laser Doppler method. Int J Radiat Oncol Biol Phys 17:1041
51. Song CW, Chelstrom LM, Haumschild DJ (1990) Changes in human skin blood flow by hyperthermia. Int J Radiat Oncol Biol Phys 18:903
52. Takagi Y, Fukase M, Takata S, Yoshimi H, Tokunaga O, Fujita T (1990) Autocrine effect of endothelin on DNA synthesis in human vascular endothelial cells. Biochem Biophys Res Commun 168:537
53. Takuwa N, Takuwa Y, Yanagisawa M, Yamashita K, Masaki T (1989) A novel vasoactive peptide endothelin stimulates mitogenesis through inositol lipid turnover in Swiss 3T3 fibroblasts. J Biol Chem 264:7856
54. van Zwieten MJ (1984) The rat as animal model in breast cancer research. Martinus Nijhoff, Boston, p 206
55. Vaupel PW (1994) Blood flow, oxygenation, tissue pH distribution, and bioenergetic status of tumors (lecture 23). Ernst Schering Research Foundation, Berlin
56. Vierhapper H, Wagner O, Nowotny P, Waldhausl W (1990) Effect of endothelin-1 in man. Circulation 81:1415
57. Warren BA (1979) The vascular morphology of tumors. In: Tumor blood circulation: angiogenesis, vascular morphology and blood flow of experimental and human tumors. CRC Press, Boca Raton, p 26
58. Watanabe T, Pakala R, Katagiri T, Benedict CR (2001) Angiotensin II and serotonin potentiate endothelin-1-induced vascular smooth muscle cell proliferation. J Hypertens 19:731
59. Yamashita J, Ogawa M, Inada K, Yamashita S, Matsuo S, Takano S (1991) A large amount of endothelin-1 is present in human breast cancer tissues. Res Commun Chem Pathol Pharmacol 74:363
60. Yamashita J, Ogawa M, Egami H, Matsuo S, Kiyohara H, Inada K, Yamashita S, Fujita S (1992) Abundant expression of immunoreactive endothelin 1 in mammary phyllodes tumor: possible paracrine role of endothelin 1 in the growth of stromal cells in phyllodes tumor. Cancer Res 52:4046
61. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T (1988) A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332:411