Modulation of Enhanced Vascular Permeability in Tumors by a Bradykinin Antagonist, a Cyclooxygenase Inhibitor, and a Nitric Oxide Scavenger

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ABSTRACT

The mechanism of the enhanced vascular permeability and retention (EPR) effect seen in solid tumors was investigated with sarcoma 180 (S-180) in mice by using the bradykinin receptor antagonist D-Arg-[Hyp3,Thi5,D-Tic7,01c8]bradykinin (HOE 140), the nitric oxide (NO) scavenger 2-phenyl-4,4$'$,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO), and the cyclooxygenase (prostaglandin synthase) inhibitor indomethacin. In the S-180 solid tumor model, administration of HOE 140 (0.65 or 1.3 $\mu$g/kg/2 h, four times) plus PTIO (167 $\mu$g/kg/2 h, four times/8 h, i.p.), or indomethacin (5 or 10 mg/kg/day, three times, i.p.), significantly suppressed the EPR effect in the tumor, and the combined administration of these agents achieved a stronger inhibition of the EPR effect than did each compound alone. Indomethacin (10 mg/kg/day, three times) plus PTIO (167 $\mu$g/kg/2 h, four times) given i.p. had the greatest inhibition (70%) on the EPR effect. When HOE 140 was administered s.c. at a dose of 13 $\mu$g/kg/12 h for 2 weeks after tumor inoculation, growth of the solid tumor was also suppressed by 32%, by tumor weight. In the ascitic form of S-180, i.p. administration of HOE 140 at 13 $\mu$g/kg/12 h initiated immediately after tumor inoculation significantly suppressed formation of S-180 tumor ascites; the life span of ascitic S-180 tumor-bearing mice was prolonged at the same dose of HOE 140. The expression of inducible NO synthase was elevated, as evidenced by Northern blotting and reverse transcription-PCR. These results indicate that bradykinin, NO, and prostaglandins play an important role in enhanced vascular permeability in tumor tissue and sustain tumor growth. More importantly, bradykinin antagonists such as HOE 140 may be beneficial for the inhibition of tumor growth.

INTRODUCTION

Although significant progress has been made in chemotherapy for certain leukemias and lymphomas, chemotherapy for many solid tumors remains far from satisfactory. Most anticancer agents, which are usually of low molecular weight ($M_\text{r} < 1000$), are not selectively delivered to cancer tissues but are delivered indiscriminately to both normal and tumorous tissues in a diffusion-dependent manner across the blood vessels. Thus, their severe side effects in vital organs limit their therapeutic value, despite their potent tumorcidal action.

We demonstrated previously that biocompatible macromolecules and lipids accumulate in solid tumor more than the low molecular weight anticancer agents do. This mechanism is now understood as a phenomenon observed in many solid tumors, and we coined the term “enhanced vascular permeability and retention effect” (EPR) effect to describe it (1–3). The EPR effect is attributed to multiple causes unique in tumor, namely, (a) a number of permeability-enhancing factors, which are discussed in this article; (b) high-level angiogenesis/hypervascularization (4, 5); (c) defective vascular architecture (6, 7); and (d) deficient lymphatic drainage from tumor tissue (1, 8). On the basis of this EPR effect, a new concept for selective tumor targeting using macromolecular drugs has been proposed (1, 2); some of these drugs are now used successfully in clinical application. Among them, Smancs [poly(styrene-co-maleic acid half n-butylate)-conjugated neocarzinostatin; Refs. 1–3] was approved for use in 1993 by authorities in Japan; HPMA [N-(2-hydroxypropyl)methacrylamide] copolymer-doxorubicin conjugate (9) is now being tested in a Phase I/II study in the United Kingdom. Others at the preclinical stage are HPMA antibodies (10), gelatin-tumor necrosis factor (11), gelatin-interleukin 1 (12), and polyethylene glycol-interleukin 2 conjugates (13). The logical and important approaches in cancer chemotherapy based on the EPR effect are becoming prominent in anticancer drug development. Therefore, for targeted chemotherapy for solid tumors, it is essential to clarify the mechanism of the EPR effect in tumor biology, because one may be able to facilitate delivery of macromolecular and lipid drugs to tumor tissue with great selectivity.

Vascular endothelial growth factor, also called VPF (14–17), mediates extravasation (enhanced vascular permeability) in tumors, although its involvement in the EPR effect in solid tumors is not fully understood. We have demonstrated that bradykinin, which is a well-known mediator of pain and vascular permeability induced in inflammatory responses, is generated in tumor tissue and cancerous ascitic fluid (18–20). Other inflammatory factors, such as NO and prostaglandins, are also known as mediators of vascular permeability in inflamed tissue (21, 22). However, a better understanding of their roles in enhanced vascular permeability in tumor is critical.

In the present study, we elaborate on the role of bradykinin, NO, and prostaglandins in tumor vascular permeability by using their specific antagonists or inhibitors, such as the bradykinin B2 receptor antagonist HOE 140 (23, 24), the NO scavenger PTIO (25, 26), and COX-prostaglandin synthesis inhibitor indomethacin, in an experimental solid tumor model of S-180 in mice. Furthermore, the effects of HOE 140 on ascites formation and on solid tumor growth were demonstrated in S-180-bearing mice.

MATERIALS AND METHODS

Animals and Tumors

Male ddY mice (SLC, Shizuoka, Japan) 5–6 weeks old were used throughout the experiment. S-180 tumor cells, which were maintained by i.p. passage in mice, were injected s.c. into mice with an inoculum size of 2 $\times$ 10$^6$ cells. Usually, the solid tumor becomes palpable 7 days after injection into the animal. In ascitic tumor models, S-180 tumor cells were inoculated i.p. with either 5 $\times$ 10$^6$ cells/mouse for the early-phase treatment regimen, or 3 $\times$ 10$^6$ cells/mouse for the late-phase treatment regimen, followed by administration of HOE 140. All experiments were carried out according to the guidelines of the Laboratory Protocol of Animal Handling, Kumamoto University School of Medicine.
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Chemicals

HOE 140 (Hoechst AG, Frankfurt, Germany) and indomethacin (Sigma Chemical Co., St. Louis, MO) were each dissolved in physiological saline and sterilized by filtration before the experiment. PTIO (Wako Pure Chemical Industry Ltd., Osaka, Japan) was dissolved at 43 mM in MCT obtained from Nippon Fat Oil Co. (Tokyo, Japan). L-Arginine (a substrate of NOS) was provided by Roussel Morishita Co. (Osaka, Japan), and temocapril [angiotensin-converting enzyme (kininase II) inhibitor, i.e., bradykinin potentiator] was a gift from Sankyo Co., Ltd. (Tokyo, Japan).

Treatment of Tumor-bearing Mice with Various Compounds

Suppression of Prostaglandin, NO, and Bradykinin Production, and the Effect on Solid Tumor Vascular Permeability. S-180 solid tumors obtained from at least six mice per group at 10–14 days after tumor cell inoculation were used for the in vivo vascular permeability experiments. Indomethacin was administered to the tumor-bearing mice once daily for 3 days at a dose of 5 or 10 mg/kg (i.p.). PTIO dissolved in MCT was given i.p. to the tumor-bearing mice at 167 mg/kg four times every 2 h. HOE 140 was administered s.c. at initial doses of 0.26 or 0.52 μg/kg, followed by treatment with supplemental doses of 0.13 or 0.26 μg/kg three times at 2-h intervals, respectively. The total dose for each HOE 140-treated group was 0.65 or 1.3 μg/kg (0.5 or 1.0 nmol/kg) per 8 h. To assess vascular permeability in solid tumors, Evans blue dye was injected i.v. at a dose of 10 mg/kg into the tumor-bearing mice with or without treatment with various compounds. Specifically, for the indomethacin-treated group, Evans blue dye was introduced 2 h after the last injection, followed by a 6-h period to allow extravasation of the dye-albumin complex into tumor tissue. In the PTIO or HOE 140 experiment, the dye extravasated in the solid tumor was quantified 6 or 8 h after the Evans blue dye injection, which was administered to the animals 1 h or 30 min after the first injection of PTIO or HOE 140, respectively. MCT or saline as a control vehicle was administered similarly, with the same injection volume as that of PTIO, HOE 140, or indomethacin.

The dye-albumin complex extravasation in the normal tissue (skin) was evaluated similarly with or without the administration of the above agents.

Potentiation of NO and Bradykinin Production, and the Effect on Solid Tumor Vascular Permeability. To facilitate vascular permeability in solid tumors, L-arginine as a substrate of NOS was given by a bolus i.v. injection via the tail vein at 125 mg/kg five times every 2 h, in 10 h. Temocapril as a bradykinin potentiator was administered p.o. once at a dose of 20 mg/kg. Evans blue dye was injected i.v. 30 min before L-arginine or temocapril, and dye leakage in tumor tissues was quantified 24 h later.

Effect of HOE 140 on Solid and Ascitic Tumor Growth. HOE 140 was administered to mice s.c. for solid tumors and i.p. for ascitic tumors, at a dose of 13 μg/kg every day starting immediately after tumor inoculation for the early-phase treatment regimen and continued for consecutive 14 days (solid
tumor model) or 10 days (ascitic tumor model). For the late-phase treatment regimen, HOE 140 treatment was started at 8 days after i.p. tumor inoculation and was carried out consecutively in a manner similar to that for the early-phase treatment regimen for 8 days. After the treatment periods, tumor-bearing mice were killed by dislocation of cervical vertebrae under anesthesia, and ascitic fluid was collected carefully by means of laparotomy; fluid volume and tumor cell number in ascites were measured. The solid tumor tissues were excised and weighed at 14 days after tumor inoculation.

### Quantification of Tumor Vascular Permeability

Evans blue dye binds to albumin noncovalently in vivo after i.v. injection, and the albumin-bound dye (Mr 70,000) extravasates in the tumor tissue due to the enhanced vascular permeability. Thus, extravasation of Evans blue dye is considered to be a marker for the EPR effect of macromolecules in solid tumor. At various time points after Evans blue dye injection, mice were killed and perfused with Krebs-Ringer solution to deplete the dye-albumin complex in the vascular lumen, so only the dye in the solid tumor interstitium was quantitated. The dye-albumin complex in the solid tumor tissue was measured after extraction of the dye with formamide at 620 nm, as described previously (1). Data for two different weights of tumors obtained from each mouse, i.e., 100–200 mg and 200–500 mg, are presented separately.

### Expression of mRNA for iNOS and COX-2 in S-180 Tumor Tissue

Expression of iNOS mRNA in S-180 solid tumor tissue and normal tissue (muscle) was examined by Northern blot analysis. Total RNA was extracted from the tissue by using the guanidine thiocyanate lysis method with Trizol Reagent (Life Technologies, Inc., Gaithersburg, MD) according to the manufacturer's protocol. Northern blot analysis was performed as described (27); 30 µg of total RNA were electrophoresed on agarose gel and transferred to the Hybond-N+ nylon membrane, followed by hybridization of a DNA probe for iNOS mRNA. For COX-2 expression, RT-PCR and Southern blot analyses were performed as described previously (28). The probe used for iNOS was a mouse iNOS cDNA fragment (538 bp); that for COX-2 was a mouse COX-2 cDNA fragment (29), provided kindly by Prof. S. Yamamoto (Tokushima University, Tokushima, Japan); and that for glyceraldehyde-3-phosphate dehydrogenase was of rat glyceraldehyde-3-phosphate dehydrogenase cDNA fragment (805 bp). Each probe was radiolabeled by the Megaprime DNA Labeling Systems (Amersham International, Buckinghamshire, UK).

### Statistical Analysis

Data are shown as means ± SE. The values were analyzed by a two-tailed unpaired t test between the drug-treated group and the control vehicle group. A P of < 0.05 was considered statistically significant.

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**Fig. 2.** Effects of HOE 140 (A), indomethacin (A and B), PTIO (B), L-arginine (C), and temocapril (C) on vascular permeability of solid S-180 tumors in mice. Indomethacin was given at 5 or 10 mg/kg i.p. once daily for 3 days; 2 h after the last injection of indomethacin, Evans blue dye was injected into the mice at 10 mg/kg i.v. PTIO dissolved in MCT was given at 167 mg/kg i.p. four times every 2 h, and HOE 140 was administered as a total dose of 0.65 or 1.3 µg/kg s.c. four times every 2 h. Evans blue dye was injected i.v. 1 h and 30 min after the first injection of PTIO or HOE 140, respectively. Thirty min after Evans blue dye injection (i.v.), L-arginine was injected at 125 mg/kg i.v. five times every 2 h in a day, and temocapril was administered p.o. at 20 mg/kg. Animals given only vehicle (saline or MCT) served as controls. At least six mice were in each experimental group. *, P < 0.05; **, P < 0.01 (n = 6–8 for each group). The effect of various agents on vascular extravasation in normal tissue (skin) is shown in the inset (A). Data are means; bars, SE. See text for details.
The uptake of Evans blue dye into the tumor as a result of enhanced vascular permeability was assessed with or without treatment with various compounds. In the group with small tumors (100–200 mg), extravasation was suppressed by 34 and 46% by HOE 140 at doses of 0.65 and 1.3 μg/kg, respectively; in the large-tumor group (200–500 mg), 30 and 36% suppression was obtained by HOE 140 at the same respective doses. Significant inhibition of dye leakage in HOE 140-treated groups was observed compared with that in the control (saline) group ($P < 0.05$; Fig. 2A). Tumor vascular permeability was reduced by 42 and 50% for the small-tumor group, and by 34 and 51% for the large-tumor group, with indomethacin treatment at doses of 5 and 10 mg/kg, respectively. A significant difference was found between the control group and the indomethacin-treated group (Fig. 2A).

Because both bradykinin and prostaglandin appear to be involved in tumor vascular permeability, we examined the effect of coadministration with both HOE 140 and indomethacin. As shown in Fig. 2A, the combination treatment of HOE 140 (1.3 μg/kg) plus indomethacin (5 mg/kg) achieved 45 and 48% suppression of vascular permeability in the small- and large-tumor groups, respectively. At the higher dose of indomethacin (10 mg/kg) together with HOE 140, 66% (small tumors) and 70% (large tumors) reduction of vascular permeability was observed. Inhibition of vascular permeability in the tumor tissue by these combinations was apparently stronger than that of HOE 140 alone.

Inhibition rates of vascular permeability by scavenging NO with PTIO were 39 and 52% in small- and large-tumor groups, respectively (Fig. 2B). The combined treatment of indomethacin and PTIO achieved a stronger suppressive effect than did indomethacin or PTIO alone. PTIO at 167 mg/kg combined with 5 mg/kg indomethacin given i.p. resulted in 49 and 57% of suppression in small- and large-tumor groups, respectively. The same dose of PTIO with a higher dose of indomethacin (10 mg/kg) showed stronger suppression: 71% (small tumors) and 68% (large tumors). Significant differences were seen between the drug-treated groups and the vehicle MCT control group. The extravasation of the dye-albumin complex in the normal tissue (skin) was not affected appreciably by the administration of PTIO (167 mg/kg; 4 times), HOE 140 (1.3 μg/kg), or indomethacin (10 mg/kg; 3 times) (Fig. 2A, inset).

**Effect of L-Arginine and Temocapril on Solid Tumor Vascular Permeability**

Administration of L-arginine (a substrate for NOS) tended to enhance dye leakage of the solid tumor tissues by 22%, although the...
Effect of HOE 140 on Tumor Ascites Formation in Mice

Early-Phase Treatment Regimen. Administration of HOE 140 was started immediately after tumor inoculation and was continued for the following 10 days. Time profiles of body weight of ascitic tumor-bearing mice treated with or without various compounds were compared with those of the tumor-free control group. The increase in body weight of ascitic tumor-bearing mice can usually be attributed to fluid accumulation in the peritoneal cavity, together with tumor cell proliferation. As demonstrated in Fig. 3A, the increment in body weight of the tumor-bearing mice was markedly inhibited by HOE 140 when it was initiated just after tumor inoculation.

Furthermore, after the end of the treatment course of the tumor-bearing mice, tumor cells and volume of ascitic fluid were measured (Fig. 4). A significant difference in the volume of ascites between the HOE 140-treated group and the control (saline) group was found ($P < 0.05$; Fig. 4A). The total number of tumor cells yielded in the HOE 140-treated group tended to be less (by 31%) than that in the saline control group ($0.1 < P < 0.5; n = 5$). Conversely, in the HOE 140-treated group, the tumor cell number appears to be concentrated because of the decreased fluid volume compared with the saline control group (Fig. 4B).

Late-Phase Treatment Regimen. In this experimental protocol, HOE 140 administration started 8 days after tumor inoculation and continued for 8 days. In contrast to the early-phase treatment regimen, HOE 140 given under the late-phase treatment regimen did not have any appreciable effect on the increase in body weight (Fig. 3B); it also did not significantly affect the volume of ascites fluid and the tumor cell number (data not shown).

Difference was not statistically significant ($0.1 < P < 0.5$; Fig. 2C). Similarly, treatment with temocapril as an inhibitor of kininase II, to prevent the degradation of bradykinin and hence prolong the biological action of bradykinin, had a tendency to increase vascular permeability in solid tumor tissues by about 18% ($0.1 < P < 0.5$; Fig. 2C).

Effect of HOE 140 on Tumor Ascites Formation in Mice

Effect of HOE 140 on Survival (Life Span) of Ascitic Tumor-Bearing Mice and on Solid Tumor Growth

HOE 140 treatment, which was initiated just after tumor cell inoculation and continued for the following 14 days (13 $\mu$g/kg/12 h. i.p.), significantly prolonged the life span of mice bearing S-180 ascitic tumor; the mean life spans of the tumor-bearing mice with and without HOE 140 treatment were 18.6 ± 0.7 and 16.8 ± 0.3 days, respectively ($P < 0.05$; Fig. 5). HOE 140 also showed a significant suppressive effect on S-180 solid tumor growth by 32% when it was administered s.c. at the same dose as in the ascitic tumor model ($P < 0.05$, Fig. 6).

Expression of iNOS mRNA and COX-2 mRNA in S-180 Tumor Tissue

To examine the level of expression of iNOS and COX-2 mRNA in S-180 tumor tissue, Northern blot analysis for iNOS and RT-PCR, with Southern blotting for COX-2, was performed. Very high expression of iNOS mRNA was observed in S-180 tumor tissue, whereas a negligible level of iNOS expression was seen in control (muscle) tissue (Fig. 7A). Also, using RT-PCR and Southern blot analyses, an appreciable level of COX-2 mRNA was identified in S-180 tumor tissue (Fig. 7B). These results confirm that iNOS and COX-2 were significantly up-regulated in tumor tissue, and consequently NO and prostaglandins (especially prostaglandin E2) are produced and are likely to contribute to tumor growth.

DISCUSSION

Most solid tumors exhibit vascular characteristics different from those of normal tissues or organs. The hypervasculature/angiogenesis, defective vascular architecture, and EPR effect are unique biological properties of solid tumor tissues as described earlier. Enhanced vascular permeability may be affected by a number of mediators, including bradykinin, NO, VPF, prostaglandin E2, and tumor necrosis factor. The EPR effect in solid tumors can be demonstrated best as Evans blue dye extravasation in solid tumor tissues, as shown in Fig. 1. Small S-180 solid tumors (less than 10 mm in diameter) usually show extensive homogeneous extravasation (Fig. 1A), whereas large tumors (>10 mm in diameter) show more frequent central necrosis and an avascular area, although the periphery of the tumorous tissue, as well
MMDUATION OF TUMOR VASCULAR PERMEABILITY

Fig. 7. Expression of iNOS (A) and COX-2 (B) mRNA in S-180 tumor tissue and muscle of the mouse. iNOS mRNA was analyzed by Northern blotting, and COX-2 mRNA was tested by RT-PCR and Southern blotting analyses. See text for details. G3PDH, glyceraldehyde-3-phosphate dehydrogenase.

as adjacent normal tissue, shows extensive extravasation (Fig. 1, B–F). It is also known that tumor growth occurs at the periphery corresponding to the area of extravasation. In this regard, we have proposed that the EPR effect in the tumor should sustain solid tumor growth.

Mediators of the EPR effect include bradykinin and the bradykinin system, which have been studied extensively in inflammation, in infection (30) and somewhat in cancer (18–20, 26, 31). It is well known that bradykinin mediates pain and increases vascular permeability and even angiogenesis (32). We documented previously that the bradykinin-generating cascade is activated in the tumor compartment (18–20), and that bradykinin may be involved in malignant ascitic and pleural fluid accumulation, in which [hydroxyprolyl]-bradykinin seems to play a major role (18, 19, 26).

Bradykinin is the most potent agonist for the B2 receptor, and HOE 140 is a B2 receptor-specific antagonist in vitro and in vivo; their biological and pharmacological actions have been studied extensively in inflammatory models (23, 24). The function of bradykinin in tumor biology, however, is partly understood. Here, we report that administration of HOE 140 in the mouse solid tumor model suppressed enhanced vascular permeability. HOE 140 also inhibited the formation of ascitic tumor fluid as well as solid tumor growth. This was consistent with our previous observation that soybean trypsin inhibitor (Kunitz type), which is known to inhibit plasma kallikrein and thus result in reduction of bradykinin generation, reduced S-180 ascites formation (19). These results validate an important role for bradykinin in tumor growth. In addition to the suppressive effect on tumor growth, bradykinin antagonists might be useful for treatment of paraneoplastic syndromes, such as pain, hypoalbuminemia, and malignant fluid accumulation in peritoneal or pleural cavities, which are clinical symptoms frequently associated with cachexia.

A significant suppressive effect of HOE 140 on ascitic tumor growth was demonstrated when the drug was administered in the early-phase treatment regimen. Excessive body weight gain due primarily to ascites formation was suppressed (Fig. 3A), and the life span of the tumor-bearing mice was prolonged by HOE 140 treatment (Fig. 5). It appears, however, that HOE 140 given in the late-phase treatment regimen was less effective for inhibition of ascitic tumor and solid S-180 tumor growth (Figs. 3B and 6). This may be explained by inadequate treatment of HOE 140 in this regimen, because by the time HOE 140 is given, the tumor angiogenesis is already highly developed, and many other mediators may be generated to potentiate the angiogenesis and thus tumor growth compared with the early-phase treatment regimen (17), although HOE 140 treatment shows a strong suppressive effect on extravasation in the solid tumor (Fig. 2). Also, considering the fact that no significant suppression of the tumor growth, as assessed by tumor weight measurement, was observed with HOE 140 in a late-phase treatment regimen, the decreased gain in tumor weight obtained in the early-phase treatment regimen seems to mainly reflect the tumor growth retardation induced by HOE 140 but not simply the inhibition of extracellular fluid accumulation in the solid tumor.

It seems that prostaglandin biosynthesis systems (COX-1 and -2), particularly for prostaglandin E2 production, are appreciably elevated in human and experimental tumors (33, 34). It is intriguing that many studies have shown that administration of nonsteroidal anti-inflammatory drugs, which are prostaglandin synthesis inhibitors such as indomethacin and aspirin, brought about a reduced risk of growth of tumor such as colon cancer, and thus that they are beneficial for cancer prevention (35, 36); however, detailed molecular mechanisms remain unclear. Up-regulation of COX-2 in human colorectal cancer tissue has been reported recently, assessed by an immunohistochemical method (37). Results of our present study on COX-2 with RT-PCR and Southern blot analyses are consistent with this finding in that expression of COX-2 mRNA is highly elevated in S-180 solid tumor tissue (Fig. 7). Furthermore, our results show that prostaglandins are involved in enhanced solid tumor vascular permeability, because the COX inhibitor indomethacin significantly suppressed vascular permeability in our solid tumor model (Fig. 2, A and B). Thus, inhibition of expression and activity of COX might be applicable not only to cancer prevention but also to cancer chemotherapy.

The third permeability mediator involved in the EPR effect of solid tumors is NO, for which we examined the effect of the NO scavenger PTIO (25). We demonstrated previously that the NOS inhibitor Nω-nitro-L-arginine methylster decreased vascular permeability in solid tumors (26). In the present study, we confirmed the notion that NO regulates the EPR effect to a great extent in tumor tissues (Fig. 2). Meyer et al. (38) also found that NOS inhibition by Nω-monomethyl-L-arginine irreversibly attenuated blood flow in R3230Ac rat mammary adenocarcinoma, and Tozer et al. (39) selectively reduced tumor blood flow with the NOS inhibitor Nω-nitro-L-arginine in P22 tumor-bearing rats. To confirm the elevated level of NO synthesis by NOS, we examined the expression of mRNA of iNOS by using Northern blot analysis. The result showed that iNOS mRNA expression is highly up-regulated in the solid tumor (Fig. 7). This result is consistent with our previous observation obtained by the same method as that used in the present experiment and an electron spin resonance study with a different tumor model (rat hepatoma AH-130; Ref. 40).

It has been revealed that bradykinin B2 receptor is coupled to G proteins and that binding of bradykinin to this B2 receptor leads to strong signal amplification (41). Accordingly, receptor binding of bradykinin may further stimulate the biosynthesis of other mediators, such as prostanooids (42, 43) and NO (31, 44), which also play an important role in inflammation (21), angiogenesis (45, 46), and the EPR effect (26). Combined treatment of HOE 140 plus indomethacin had a greater suppressive effect on tumor vascular permeability in an additive manner than did HOE 140 or indomethacin alone (Fig. 2). Similarly, simultaneous administration of PTIO and indomethacin markedly reduced the permeability of tumor vessels (Fig. 2). These results indicate that bradykinin, prostaglandins, and NO are operative in tumor vascular permeability.

Taken together, the present data provide several lines of convincing evidence that at least three factors, i.e., bradykinin, NO, and prostaglandins, are involved in enhanced vascular permeability of solid tumors, as determined by suppression of the EPR effect with HOE 140, PTIO, or indomethacin. Development of macromolecular anti-cancer drugs that use the EPR effect for more selective drug targeting...
delivery (1–3, 9) is gaining increasing attention (47). In view of the EPR effect in solid tumors, our present data have important implications for a new strategy for cancer chemotherapy. Tumor growth and metastasis may be modulated by reduction of the various mediators of vascular permeability, such as NO, prostanooids, and bradykinin. Conversely, by enhancing the generation or by potentiating the effects of permeability mediators locally or transiently, such as bradykinin, NO, and prostaglandins, the solid tumor EPR effect may facilitate more selective targeting of macromolecular anticancer agents, such as Smans, to the tumor tissue.

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