Cyclooxygenase-2-Mediated Angiogenesis in Carrageenin-Induced Granulation Tissue in Rats†

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ABSTRACT

The possible participation of cyclooxygenase (COX)-2 in angiogenesis in granulation tissue was analyzed using an air pouch-type carrageenin-induced inflammation model in rats. Injection of carrageenin solution into an air pouch induced gradual increases in the pouch fluid volume and granulation tissue weight as well as angiogenesis in granulation tissue. NS-398 (10–100 μg) inhibited all of these parameters in a dose-dependent manner. NS-398 (100 μg), indomethacin (100 μg), and dexamethasone (10 μg) markedly reduced prostaglandin (PG) E2 levels in the pouch fluid at day 6. NS-398 and indomethacin did not affect protein levels of COX-1 and COX-2 but dexamethasone significantly reduced the level of COX-2 in granulation tissue at day 6. Protein levels of vascular endothelial growth factor (VEGF) in granulation tissue and in the pouch fluid were higher at day 6 than at day 3, and the levels were decreased by treatment with NS-398 (10–100 μg) in a dose-dependent manner. The inhibitory effects of NS-398 (100 μg) were almost the same as those of indomethacin (100 μg). Dexamethasone (10 μg) also reduced VEGF protein levels in granulation tissue at day 6. To clarify the role of PGE2 in VEGF production, minced granulation tissue obtained 3 days after carrageenin injection from the indomethacin-treated rats was incubated in the presence of various concentrations of PGE2. It was shown that VEGF mRNA and protein levels in the minced granulation tissue were increased by PGE2 in a concentration-dependent manner. These findings suggest that COX-2-derived PGE2 plays a significant role in angiogenesis in the carrageenin-induced granulation tissue through VEGF formation.

Angiogenesis is the formation of new capillaries from pre-existing ones (Jackson et al., 1997). It plays a critical role in a variety of physiological and pathological events, including chronic inflammation such as rheumatoid arthritis (Colville-Nash and Scott, 1992), atherosclerosis (Sueishi et al., 1997), diabetic retinopathy (Ishibashi et al., 1999), psoriasis (Li and Li, 1996), wound healing (Suzuki et al., 1998), and chronic airway inflammation (Thurston et al., 1998), in addition to solid tumor growth (Folkman, 1995). The association of angiogenesis with chronic inflammation promotes the formation of granulation tissue as in pannus formation in rheumatoid arthritis (Colville-Nash and Scott, 1992), and skin in psoriatic diseases (Li and Li, 1996). Angiogenesis in a chronic inflammatory state facilitates migration of inflammatory cells to the inflammatory site and supplies nutrients and oxygen to granulation tissue (Jackson et al., 1997). Therefore, the suppression of angiogenesis in granulation tissue is important to suppress the development of chronic granulation tissue.

Cyclooxygenase (COX), which converts arachidonic acid to prostaglandin (PG) G2, has two isoforms, COX-1 and COX-2 (Herschman, 1994). COX-2 is induced at the inflammatory site in an experimental inflammation model (Masferrer et al., 1994; Seibert et al., 1994; Appleton et al., 1995) and in patients with rheumatoid arthritis (Crofford et al., 1994). The injection of carrageenin into an s.c. air pouch of rats increased the protein levels of COX-2 in the cells lining the inner layer of the pouch and in macrophages infiltrating the pouch fluid (Masferrer et al., 1994). In the recurrence of allergic inflammation model in rats, Niki et al. (1997) reported that COX-2 protein is induced in granulation tissue by antigen challenge and COX-2-derived PGE2 participates in the vascular formation and the development of granulation tissue.

It is reported that E-type PGs, such as PGE1 and PGE2, enhance angiogenesis in rabbit corneas (Ziche et al., 1982) and chorioallantoic membrane of 8-day-old chicken embryos (Form and Auerbach, 1983). In addition, the COX-1/COX-2 nonspecific inhibitor indomethacin inhibits angiogenesis in rabbit corneas (Frucht and Zauberman, 1984). COX-2 modulates production of angiogenic factors by colon cancer cells (Tsujii et al., 1998). However, the role of COX-2 in angiogen-

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ABBREVIATIONS: COX, cyclooxygenase; PG, prostaglandin; VEGF, vascular endothelial growth factor; EMEM, Eagle’s minimal essential medium; RT-PCR, reverse transcription-polymerase chain reaction; bp, base pair; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
nosis has not been fully investigated. Therefore, in this study, by using an air pouch-type carrageenin-induced inflammation model in rats, we analyzed the role of COX-2 in angiogenesis in granulation tissue focusing on the formation of vascular endothelial growth factor (VEGF).

Materials and Methods

Induction of Air Pouch-Type Inflammation by Carrageenin in Rats. Air pouch-type inflammation was induced by carrageenin according to the procedure described by Tsurufuji et al. (1978). Male Sprague-Dawley rats, specific pathogen-free, weighing 160 to 170 g (Charles River Japan, Inc., Kanagawa, Japan), were lightly anesthetized with diethyl ether and 8 ml of air was injected s.c. on the back to make an air pouch oval in shape. Twenty-four hours later, 4 ml of a 2% (w/v) solution of carrageenin (Seakem 202; Marine Colloids Inc., Springfield, NJ) in saline was injected into the air pouch under light diethyl ether anesthesia. The carrageenin solution had been sterilized by autoclaving at 121°C for 15 min and supplemented with antibiotics [0.1 mg of penicillin G potassium (Meiji Seika, Tokyo, Japan) and 0.1 mg of dihydrostreptomycin sulfate (Meiji Seika) per milliliter of the solution] after cooling to 40–45°C. The rats were treated in accordance with procedures approved by the Animal Ethics Committee of the Graduate School of Pharmaceutical Sciences, Tohoku University, Japan.

Drug Treatment. NS-398 ([N-2-cyclohexyloxy-4-nitrophenyl] methanesulfonamide; Funakoshi Co., Tokyo, Japan) was dissolved in dimethyl sulfoxide (Wako Pure Chemical Ind., Osaka, Japan). Indomethacin (Sigma Chemical Co., St. Louis, MO) and dexamethasone (Sigma Chemical Co.) were dissolved in 99.5% ethanol (Wako Pure Chemical Ind.). Stock solutions were diluted with saline and 0.5 ml of the diluted solution containing the indicated amount of drugs was injected into the pouch of each rat under light diethyl ether anesthesia just after and 2 and 4 days after carrageenin injection. Final concentrations of dimethyl sulfoxide and ethanol in the saline solution were adjusted to 2% (v/v). Control rats received the same amount of saline solution containing each vehicle at 2% (v/v).

Determination of Pouch Fluid Volume, Leukocyte Infiltration, and Granulation Tissue Weight. One, 3, and 6 days after the injection of carrageenin solution, the rats were sacrificed by cutting the carotid artery under diethyl ether anesthesia. Total pouch fluid was collected and its volume measured. The pouch fluid was diluted 2-fold with saline and the leukocytes in the fluid were enumerated using a hemocytometer. Three and 6 days after carrageenin injection, granulation tissue that formed was dissected and weighed.

Determination of Angiogenesis in Granulation Tissue. Measurement and visualization of angiogenesis in granulation tissue were carried out using carmine dye (Natural Red 4; Sigma Chemical Co.) according to the methods described by Kimura et al. (1986) and Colville-Nash et al. (1995) with slight modifications. One, 3, and 6 days after the injection of carrageenin solution, 3 ml of 5% (w/v) carmine dye in 5% (w/v) gelatin (Sankoh Jun-yaku, Tokyo, Japan) in saline at 37°C was injected into the tail vein of each rat under light diethyl ether anesthesia. The carcasses were chilled on ice for 3 h, and the entire granulation tissue was dissected and weighed. After
being washed with PBS (pH 7.4), granulation tissue was homogenized in 2 volumes of 0.5 mM sodium hydroxide using a Vir-Tis 45 homogenizer (The Virtis Company, Gardiner, NY) for 4 min at scale 40 on an ice bed. The tissue homogenate was centrifuged at 10,000g and 4°C for 30 min. Five hundred microliters of the supernatant was diluted 2-fold with 0.5 mM sodium hydroxide and centrifuged again at 14,000g and 4°C for 30 min. The dye content in 200 μl of the supernatant was determined spectrophotometrically by measuring absorbance at 490 nm. For the standard curve, known amounts of carmine dye were added to the final supernatant of granulation tissue of control rats that were injected with 3 ml of a 10% (w/v) gelatin solution in saline without carmine dye, and the absorbance determined. The amount of carmine dye in the whole granulation tissue was then calculated.

For visualization, granulation tissues were fixed in 10% (v/v) formalin in PBS for 48 h at 4°C. The samples were dehydrated by continuous immersion in 70% (v/v) ethanol for 48 h, 90% (v/v) ethanol for 48 h, and pure ethanol for 48 h. After dehydration, the samples were cleared by immersion in cedarwood oil (Sigma Chemical Co.) for 14 days. Retention of carmine dye within the vascular bed was observed with a light microscope (40× magnification).

**Western Blot Analysis for COX-1, COX-2, and VEGF Proteins.** The supernatant fractions of granulation tissue and the pouch fluid were obtained as described above. The protein content in the supernatant fraction was determined according to the method described by Bradford (1976). Proteins, each at 18.4-μg aliquot of granulation tissue for COX and 4.6-μg aliquot of granulation tissue and 1.4-μg aliquot of the pouch fluid for VEGF were separated by electrophoresis on 8% (w/v) and 12% (w/v) sodium dodecylsulfate-polyacrylamide gel, respectively, and transferred onto nitrocellulose membranes (Schleicher and Schuell Inc., Dassel, Germany). For the detection of COX-1 and COX-2 proteins, the membranes were incubated with goat polyclonal anti-COX-1 and anti-COX-2 (Santa Cruz Biotechnology, Santa Cruz, CA), respectively. The membranes were then incubated with biotinylated anti-goat IgG (Vector Laboratories Inc., Burlingame, CA) and avidin-biotin-peroxidase complex (Vector Laboratories Inc.). For the detection of VEGF proteins, the membrane was incubated with mouse monoclonal anti-VEGF (Santa Cruz Biotechnology) and then with biotinylated anti-mouse IgG (dilution; Vector Laboratories Inc.). The reaction product was visualized with an ECL kit (ECL system; Amersham, Arlington Heights, IL).

**Determination of VEGF Protein Levels in the Conditioned Medium of Granulation Tissue.** Three days after injection of the

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**Fig. 2.** Small vascular network formation in carrageenin-induced granulation tissue. Four milliliters of a 2% (w/v) carrageenin solution in saline was injected into the air pouch. Three (A) and 6 days (B) after carrageenin injection, 3 ml of 5% (w/v) carmine dye with 5% (w/v) gelatin in saline was injected i.v. into anesthetized and prewarmed rat. Granulation tissue was dissected and cleared in cedarwood oil. Retention of carmine dye within the vascular bed was observed with a light microscope (40× magnification).

**Fig. 3.** Effects of NS-398, indomethacin, and dexamethasone on pouch fluid volume, total number of leukocytes in the pouch fluid, and granulation tissue weight 6 days after carrageenin injection. Four milliliters of a 2% (w/v) carrageenin solution in saline was injected into the air pouch. NS-398 (10, 30, and 100 μg), indomethacin (IM, 100 μg), or dexamethasone (DEX, 10 μg) dissolved in 500 μl of saline was injected into the pouch 0, 2, and 4 days after carrageenin injection. The pouch fluid volume (A), the total number of leukocytes in the pouch fluid (B), and granulation tissue weight (C) were determined 6 days after carrageenin injection. Quantities along the x-axis represent micrograms per pouch. Values are the mean with S.E. shown by vertical bars from 10 rats. Statistical significance: *P < 0.05, **P < 0.01, and ***P < .001 versus control. ND, not detected.
carrageenin solution, the rats were sacrificed by cutting the carotid artery under diethyl ether anesthesia and granulation tissue was excised and minced into 1- to 2-mm pieces with a pair of small scissors. The minced granulation tissue was washed twice with 5 volumes of ice-cold PBS. Eight hundred milligrams of the minced tissue was placed in a 60 mm × 15-mm polystyrene sterile tissue culture dish (Corning Costar, Cambridge, MA) and incubated in 4 ml of Eagle’s minimal essential medium (EMEM) (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 10% (v/v) calf serum (Dainippon Pharmaceutical, Osaka, Japan), penicillin G potassium (18 μg/ml), and streptomycin sulfate (50 μg/ml) for 3 h at 37°C under an atmosphere of 95% air, 5% CO₂. The tissues were then washed three times with calf serum-free medium and incubated in 4 ml of Eagle’s minimal essential medium (EMEM) (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) with a thermal cycler (GeneAmp PCR system 2400; Perkin Elmer/Cetus, Norwalk, CT). PCR was performed at 94°C for 3 min, 57°C for 1 min, and 72°C for 3 min in the first round; 94°C for 1 min, 57°C for 1 min, and 72°C for 3 min in the next 38 rounds; and 94°C for 1 min, 57°C for 1 min, and 72°C for 10 min in the last round. For GAPDH mRNA, PCR amplification was performed for 27 cycles at 30-s denaturation at 94°C, 1 min annealing at 57°C, and 2-min extension at 72°C. The other conditions were the same as those used for VEGF mRNA. After PCR, 10 μl of the PCR reaction mixture was loaded onto a 2% (w/v) agarose minigel and the PCR products were visualized by ethidium bromide staining after electrophoresis at 100 V for 40 min.

**Statistical Analysis.** The statistical significance of the results was analyzed by Dunnett’s test for multiple comparisons and Student’s t-test for unpaired observations.

**Results**

**Time Changes of Pouch Fluid Volume, Number of Infiltrating Leukocytes, Granulation Tissue Weight, and Angiogenesis in Granulation Tissue.** After injection of the carrageenin solution into the air pouch, the pouch fluid volume increased gradually for 3 days and then markedly for the next 3 days (Fig. 1A). In contrast, the total number of infiltrating leukocytes in the pouch fluid was highest at day 1, and then declined time dependently (Fig. 1B). One day after carrageenin injection, a very thin granulation tissue was formed in the s.c. tissue that was difficult to dissect quantitatively. Granulation tissue was dissectable at day 3 and the wet weight was further increased at day 6 (Fig. 1C). Total dye content in granulation tissue as an index of angiogenesis was markedly increased from day 3 to 6 (Fig. 1D). The increase in angiogenesis was more prominent than that in granulation tissue formation (Fig. 1C). The vascular network formation was markedly increased from day 3 (Fig. 2A) to 6 (Fig. 2B) after carrageenin injection.

**Effects of NS-398, Indomethacin, and Dexamethasone on Pouch Fluid Volume, Number of Infiltrating Leukocytes, and Granulation Tissue Weight.** The selective COX-2 inhibitor NS-398 decreased the pouch fluid accumulation (Fig. 3A), the leukocyte infiltration into the pouch fluid (Fig. 3B), and granulation tissue formation (Fig. 3C) during 6 days after the carrageenin injection in a dose-dependent manner. The inhibitory effect of NS-398 (100 μg) was almost the same as that of the COX-1/COX-2 nonspecific inhibitor indomethacin (100 μg). By treatment with the anti-inflammatory steroid dexamethasone (10 μg), pouch fluid was predominantly absorbed during 6 days (Fig. 3A). The pouch fluid at day 6 in dexamethasone-treated rats was so viscous that the counting of infiltrating leukocytes could not be carried out. Granulation tissue formation was also strongly inhibited by dexamethasone (Fig. 3C).

**Effects of NS-398, Indomethacin, and Dexamethasone on Angiogenesis in Granulation Tissue.** NS-398 decreased carmine dye content in the whole granulation tis-
sue in a dose-dependent manner (Fig. 4) and the inhibition by NS-398 (100 µg) was almost the same as that by indomethacin (100 µg) (Fig. 4). Dexamethasone (10 µg) decreased the dye content most potently (Fig. 4). Vascular network formation as examined by microscope was also inhibited by NS-398 (100 µg) as shown in Fig. 5. Indomethacin (100 µg) and dexamethasone (10 µg) also inhibited the vascular network formation (data not shown).

Effects of NS-398, Indomethacin, and Dexamethasone on PGE_2 Contents in the Pouch Fluid and Protein Levels of COX-1 and COX-2 in Granulation Tissue. PGE_2 levels in the pouch fluid were decreased by treatment with NS-398 (100 µg) and indomethacin (100 µg) with almost the same potency (Fig. 6). Dexamethasone (10 µg) decreased PGE_2 levels most potently (Fig. 6). The COX-1 protein level in granulation tissue at day 3 was almost the same as at day 6, and was unaffected by treatment with NS-398 (100 µg), indomethacin (100 µg), and dexamethasone (10 µg) at day 6 (data not shown). In contrast, the COX-2 protein level at day 3 was higher than that at day 6, and treatment with NS-398 and indomethacin did not affect the level at day 6, but treatment with dexamethasone (10 µg) significantly reduced it (Fig. 7).

Fig. 5. Effects of NS-398 (100 µg) on angiogenesis in granulation tissue 6 days after carrageenin injection. Four milliliters of a 2% (w/v) carrageenin solution in saline was injected into the air pouch. NS-398 (100 µg) dissolved in 500 µl of saline was injected into the pouch 0, 2, and 4 days after carrageenin injection. Granulation tissues at day 3 and 6 were dissected, homogenized, and centrifuged as described under Materials and Methods. COX-2 protein level in the supernatant fraction of the homogenate of granulation tissue was immunoblotted and analyzed densitometrically. The immunoblots of COX-2 protein in granulation tissue from two rats in each group are shown on the top. Values are the mean with S.E. shown by vertical bars from nine rats. Statistical significance: *P < .05 versus none (control), and **P < .01 versus day 6 none (control).

Detection of VEGF Protein in Granulation Tissue and in the Pouch Fluid, and Effects of NS-398, Indomethacin, and Dexamethasone. The amounts of VEGF protein in granulation tissue (Fig. 8A) and in the pouch fluid (Fig. 8B) were higher at day 6 than at day 3. Treatment with NS-398 lowered the VEGF protein levels at day 6 in granulation tissue (Fig. 9A) and in the pouch fluid (Fig. 9B) in a dose-dependent manner. Treatment with indomethacin (100 µg) also lowered the amount of VEGF protein in granulation tissue and in the pouch fluid and the effect was almost the same as that of NS-398 (100 µg) (Fig. 9, A and B). Dexamethasone (10 µg) also lowered the VEGF protein content of granulation tissue (Fig. 9A). Because the pouch fluid was absorbed by treatment with dexamethasone (10 µg), the level of VEGF protein in the pouch fluid could not be determined.

Induction of VEGF Protein by PGE_2 in Culture of Minced Granulation Tissue. To clarify whether PGE_2 is...
involved in VEGF production, granulation tissues from the indomethacin-treated rats were excised, minced, and incubated in medium containing various concentrations of PGE2. As shown in Figs. 10B and 11A, PGE2 increased VEGF protein levels in the conditioned medium in a time- and concentration-dependent manner. VEGF mRNA levels for the three isoforms of VEGF in granulation tissue were also increased by PGE2 with a maximum at 1 h (Fig. 10C), and in a concentration-dependent manner (Fig. 11B). The amount of VEGF protein in the minced granulation tissue attained a maximum at 3 h and then declined (Fig. 10A).

**Discussion**

Using an air pouch-type carrageenin-induced inflammation model in rats, we demonstrated that COX-2-derived PGE2 plays a role in angiogenesis in the developing chronic granulation tissue. The quantitative analysis of angiogenesis in granulation tissue was carried out using carmine dye according to the method described by Kimura et al. (1986) with slight modification. This method was used because of its high sensitivity. The vascular cast formed by carmine dye in gelatin within the vasculature was unaffected by variations in plasma exudation, and angiogenesis in granulation tissue could be visualized by using cedarwood oil (Colville-Nash et al. 1995). The increase in dye content in granulation tissue seemed to correlate with the increase in the capillary density involved in VEGF production, granulation tissues from the indomethacin-treated rats were excised, minced, and incubated in medium containing various concentrations of PGE2. As shown in Figs. 10B and 11A, PGE2 increased VEGF protein levels in the conditioned medium in a time- and concentration-dependent manner. VEGF mRNA levels for the three isoforms of VEGF in granulation tissue were also increased by PGE2 with a maximum at 1 h (Fig. 10C), and in a concentration-dependent manner (Fig. 11B). The amount of VEGF protein in the minced granulation tissue attained a maximum at 3 h and then declined (Fig. 10A).

**Fig. 8.** VEGF protein levels in granulation tissue and the pouch fluid 3 and 6 days after carrageenin injection. Four milliliters of a 2% (w/v) carrageenin solution in saline was injected into the air pouch. Granulation tissues 3 and 6 days after carrageenin injection were dissected, homogenized, and centrifuged as described under Materials and Methods. VEGF protein levels in the supernatant fraction of the homogenate of granulation tissue (A) and the pouch fluid (B) were immunoblotted and analyzed densitometrically. The immunoblots of VEGF proteins in granulation tissue and the pouch fluid from two rats in each group are shown on the top. Values are the mean with S.E. shown by vertical bars from six rats. The mean density at day 6 is set to 100%. Statistical significance: ***P < .001 versus the value at day 6.

**Fig. 9.** Effects of NS-398, indomethacin, and dexamethasone on VEGF protein levels in granulation tissue and the pouch fluid 6 days after carrageenin injection. Four milliliters of a 2% (w/v) carrageenin solution in saline was injected into the air pouch. NS-398 (10, 30, and 100 μg), indomethacin (IM, 100 μg), or dexamethasone (DEX, 10 μg) dissolved in 500 μl of saline was injected into the pouch 0, 2, and 4 days after carrageenin injection. Granulation tissue 6 days after carrageenin injection was dissected, homogenized, and centrifuged as described under Materials and Methods. VEGF protein levels in the supernatant fraction of the homogenate of granulation tissue (A) and the pouch fluid (B) were immunoblotted and analyzed densitometrically. The immunoblots of VEGF proteins in granulation tissue and the pouch fluid from two rats in each group are shown on the top. Quantities along the x-axis represent micrograms per pouch. Values are the mean with S.E. shown by vertical bars from six rats. The mean density in the control group is set to 100%. Statistical significance: *P < .05, and *** P < .001 versus control.

(Figs. 1 and 2). From the determination of dye content in granulation tissue and histological analysis, it was concluded that the COX-2 inhibitor NS-398 inhibits angiogenesis in granulation tissue as well as inflammatory response (Fig. 3) as the COX-1/COX-2 nonspecific inhibitor indomethacin does (Figs. 4 and 5).

It is reported that E-type PGs, such as PGE1 and PGE2, enhance angiogenesis in rabbit corneas (Ziche et al., 1982) and chorioallantoic membrane of 8-day-old chicken embryos (Form and Auerbach, 1983). In addition, indomethacin inhibits angiogenesis in rabbit corneas (Frucht and Zauberman, 1984). Moreover, VEGF, a potent angiogenic factor, is expressed by PGE2 and PGE1 in osteoblasts (Harada et al., 1994; Ben-Av et al., 1995). In granulation tissue in the air pouch-type carrageenin-induced inflammation model in rats, COX-2-derived PGE2 was suggested to be involved in VEGF production because NS-398 as well as indomethacin significantly reduced VEGF contents in granulation tissue and in the pouch fluid (Fig. 9). To clarify whether PGE2 is involved in VEGF production, granulation tissue from the indomethacin-treated rats in which the effect of endogenous PGE2 on the VEGF production might be minimized, was excised, minced, and incubated in the presence and absence of PGE2. It was demonstrated that PGE2 increased both the VEGF mRNA and protein levels (Figs. 10 and 11). These findings
suggested that NS-398 reduced angiogenesis by inhibiting COX-2-dependent PGE2 production, resulting in the reduction of VEGF production.

VEGF is a secreted protein (Houck et al., 1991) and there are several isoforms of VEGF translated from alternatively spliced mRNAs (Tischer et al., 1991). In rats, it is reported that there are three isoforms of VEGF proteins, VEGF188, VEGF164, and VEGF120, of which the mRNA are 711, 636, and 504 bp, respectively (Bacic et al., 1995). In granulation tissue of rats, all three isoforms were detected (Fig. 11). Among the three, 636- and 504-bp mRNA were detected with higher density, indicating that VEGF164 and VEGF120 may play a significant role in angiogenesis consistent with the observations in ovarian carcinomas (Sowter et al., 1997).

In addition to the observation that COX-2 was detected in granulation tissue 3 and 6 days after carrageenin injection (Fig. 7), NS-398 markedly reduced PGE2 levels in the pouch fluid (Fig. 6) as the nonselective COX-1/COX-2 inhibitor indomethacin. These findings indicated that COX-2 plays an exclusive role in PGE2 production. NS-398 and indomethacin inhibited angiogenesis in granulation tissue induced by growth factor such as basic fibroblast growth factor and...
epidermal growth factor (Majima et al., 1997). In addition, the production of angiogenic factors by colon cancer cell was stimulated by COX-2 (Tsujii et al., 1998). These results suggested that COX-2 stimulates both the production of VEGF and action of angiogenic factors.

Dexamethasone, strongly inhibited the accumulation of pough fluid, leukocyte infiltration into pough fluid, and formation of granulation tissue (Fig. 3). Angiogenesis was also inhibited by dexamethasone as assessed by the determination of total carmine dye (Fig. 4). Although dexamethasone reduced granulation tissue weight more potently than NS-398 and indomethacin, the reductions of angiogenesis and VEGF production by dexamethasone were almost the same as those by NS-398 and indomethacin (Figs. 4 and 9). These findings indicate that dexamethasone inhibits granulation tissue formation via other mechanisms in addition to the inhibition of angiogenesis.

NS-398 and indomethacn at 100 μg inhibited PGE2 production almost completely (Fig. 6) but VEGF production and angiogenesis were inhibited only about 50% (Figs. 4 and 9). Thus, in the carrageenan-induced inflammation model in rats, COX-2-derived PGE2 partially participates in angiogenesis in granulation tissue possibly by stimulating VEGF production.

References


