Roxatidine- and Cimetidine-Induced Angiogenesis Inhibition Suppresses Growth of Colon Cancer Implants in Syngeneic Mice

Kazuyoshi Tomita, Kazuki Izumi, and Susumu Okabe*

Department of Applied Pharmacology, Kyoto Pharmaceutical University, Yamashina, Kyoto 607-8414, Japan

Received June 10, 2003; Accepted September 5, 2003

Abstract. Cimetidine is known to suppress the growth of several tumors, including gastrointestinal cancer, in humans and animals. Nonetheless, whether other histamine H₂-receptor antagonists exert such tumor suppressive effects remains unclear. The effect of roxatidine acetate hydrochloride (roxatidine), an H₂-receptor antagonist, on the growth of colon cancer implanted in mice was examined and compared with that of cimetidine. Drugs were orally delivered for 26 – 29 days beginning before or after implantation of syngeneic colon cancer (Colon 38) in C57BL/6 mice. Tumor volume was determined throughout and histochemical analysis was also performed. Tumor tissue and serum vascular endothelial growth factor (VEGF) levels were measured. In vitro cell growth was assessed by the MTT assay. Both roxatidine and cimetidine significantly suppressed the growth of Colon 38 tumor implants. Histologic analysis revealed that such antagonists markedly increased necrotic areas and decreased the density of microvessels in tumor tissue. Both H₂-receptor antagonists suppressed VEGF levels in tumor tissue and significantly decreased serum VEGF levels in Colon 38-bearing mice. Such drugs, however, failed to suppress in vitro growth of the cell line. In conclusion, both roxatidine and cimetidine were found to exert suppressive effects on the growth of colon cancer implants in mice by inhibiting angiogenesis via reducing VEGF expression.

Keywords: histamine, histamine H₂-receptor antagonist, colon cancer, tumor suppression, angiogenesis

Introduction

Histamine, a biogenic amine, plays a pivotal role in many physiologic and pathophysiologic processes, including inflammation, allergic reactions, gastric acid secretion, and neurochemical transmission (1). Four subtypes of histamine receptors (H₁, H₂, H₃, and H₄) have been identified to date. Among the subtypes, histamine appears to strongly stimulate gastric acid secretion via binding to H₂ receptors in humans and animals. Consequently, histamine H₂-receptor antagonists, such as cimetidine, ranitidine, and famotidine, are clinically used for the treatment of gastritis and gastroduodenal ulcers.

Cimetidine has been demonstrated to improve survival of patients with gastric and colorectal cancer. Tonnesen et al. was the first to report that cimetidine improved survival in gastric cancer patients (2). After such a finding was published, other investigators have reported on the beneficial effects of cimetidine for colorectal cancer (3 – 8). It should be noted, however, that Tonnesen’s report was subsequently questioned after a recent, larger study was performed by The British Stomach Cancer Group (9). There is some evidence suggesting involvement of immunological mechanisms, while other studies link E-selectin-mediated cell adhesion to the tumor-suppressive effect of cimetidine. For example, histamine negatively regulates helper T-cell responses through H₂ receptors (10). Moreover, increased release of histamine has been proposed to represent the underlying cause for immunosuppression observed at the time of colonic resection. It has been demonstrated that such an effect exerted by histamine can be prevented by perioperative cimetidine, thereby improving immune surveillance at the time of surgery (8). Moreover, it has also been reported that cimetidine
treatment was particularly effective in colorectal cancer patients with tumors expressing higher levels of sialyl Lewis-X and sialyl Lewis-A epitopes, which are involved in E-selectin-mediated cell adhesion (6). Nonetheless, the exact mechanisms by which cimetidine affects tumor growth and progression remain poorly understood. Interestingly, other H₂-receptor antagonists, such as ranitidine and famotidine, were found to lack tumor-suppressive effects (11, 12). Such results would appear to suggest that cimetidine possesses the unique pharmacological property of tumor suppression. Roxatidine represents an H₂-receptor antagonist with strong anti-secretory effects that is used for treatment of gastric acid-related diseases in Japan. Nonetheless, no report to date has assessed the effects of roxatidine on tumor growth.

Angiogenesis is involved in many physiologic and pathologic processes, including tumor growth and progression, diseases involving ischemia, and chronic inflammation (13). It has been suggested that angiogenesis represents an important process involved in tumor growth, since new vessels in tumors were found to be absolutely required for solid tumor expansion beyond a size of approximately 1 to 2 mm in diameter. Furthermore, angiogenesis inhibitors were observed to potently suppress solid tumor growth and metastasis (14). Nonetheless, the relationship between angiogenesis and the tumor-suppressive effect of H₂-receptor antagonists remains poorly understood.

Consequently, the present study was performed to determine whether or not roxatidine, as compared with cimetidine, exhibits a tumor-suppressive effect in tumor-bearing mice. In addition, particular attention was paid to the underlying mechanisms, including tumor angiogenesis, that were potentially involved in tumor suppression.

Materials and Methods

Animals

Male C57BL/6 mice (8-week-old, 20 – 22 g) were obtained (Seac Yoshitomi, Ltd., Fukuoka). The animals were kept in an isolated clean room with regulated temperature (approximately 20 – 22°C), humidity (approximately 55%), and light (12-h/12-h light/dark cycle). The animals were allowed free access to food and water. Both animal maintenance and experimental procedures were carried out in accordance with the guidelines of the Ethics Committee of Kyoto Pharmaceutical University. Mice were anesthetized with diethylether and sacrificed by cervical dislocation.

Cell cultures

Colon 38 cell line, a mouse colon adenocarcinoma cell line created in C57BL/6 mice, and Colon 38 tumor fragments were kindly donated (Fujisawa Pharmaceutical Co., Ltd., Osaka). The Colon 38 cell line, which was prepared from Colon 38 tumor fragments resulting from enzyme digestion, was maintained in RPMI 1640 medium (Gibco BRL, Gaithersburg, MD, USA) supplemented with heat-inactivated 10% fetal bovine serum (Gibco BRL), 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.5 μg/ml amphotericin B (Sigma, St. Louis, MO, USA) at 37°C in air with 5% CO₂. The Colon 38 tumor fragments were subcutaneously maintained by serial passage into C57BL/6 mice.

Preparation of Colon 38-bearing mice

Subcutaneous inoculation of a single Colon 38 tumor fragment (8 mm³) in the abdominal region of C57BL/6 mice resulted in production of a single solid tumor in each mouse.

Drugs

Roxatidine (30, 100, and 300 mg/kg per day), which was kindly donated (Teikoku Hormone Mfg. Co., Ltd., Tokyo), and cimetidine (200 mg/kg per day) (Sigma) were dissolved in 0.5% carboxymethyl cellulose sodium (Nacalai Tesque, Kyoto). Test drugs were orally administered twice daily to the mice for either 29 days beginning 3 days before Colon 38 implantation or 26 days beginning concomitantly with Colon 38 implantation. The drugs were administered at a volume of 1 ml/100 g body weight. Control mice with implanted tumor and control mice without tumor received vehicle alone.

Measurement of tumor volume

Colon 38 solid tumor volume (mm³) was calculated every 2 days throughout the experiments with a slide caliper (Mitutoyo, Kanagawa) according to the following formula: \( V = L W^2 \times 0.5236 \), where \( V = \) volume, \( L = \) length, and \( W = \) width (15).

MTT assay

Responses of the Colon 38 cell line to histamine and H₂-receptor antagonists were measured using the MTT assay. Cells were cultured in 48-well culture plates, with the medium mentioned above, until a sub-confluent state was achieved. Cells were then co-incubated in the medium for 18 h with histamine (10⁻³ – 10⁻⁶ M), roxatidine (10⁻⁸ – 10⁻⁴ M), or cimetidine (10⁻⁴ – 10⁻⁴ M). The cells were subsequently incubated in a CO₂ incubator for 3 h with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) (Nacalai Tesque) at a concentration of 0.1%. Thereafter, 300 μl of isopropanol...
containing 0.04 N HCl and 0.3% sodium dodecyl sulfate was added to dissolve the formed formazan crystals. Absorbance was then measured at 595 nm using a THERMOMax Microplate reader (Molecular Devices, Sunnyvale, CA, USA). Viability for each drug-treated cell line was expressed as a percent of the control (vehicle-treated) cell line.

**Determination of mRNA expression**

Colon 38 cell lines were cultured in 6-cm dishes. Total cellular RNA was isolated by means of the acid-guanidinium thiocyanate-phenol-chloroform method using Sepasol RNA-I (Nacalai Tesque). First-strand cDNA was prepared from 5 μg of DNAse I-pretreated total RNA with Moloney murine leukemia virus (M-MLV) reverse transcriptase (RT) (Gibco BRL).

Primers used to amplify specific gene products were as follows: H2-receptor forward primer, 5'-GGA ACA GCA GAA ATG GGA CC-3'; H2-receptor reverse primer, 5'-TGT TCT CTG ATG GTG GCT GC-3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward primer, 5'-TTG TAA CCA ACT GGG ACG ATA TGG-3'; and GAPDH reverse primer, 5'-GAT CTT GAT CTT GGC AGG AGG-3'.

After denaturation at 94°C for 5 min, PCR was performed as follows: 35 cycles of denaturation at 94°C for 45 s, and extension at 72°C for 30 cycles of denaturation at 94°C for 45 s, annealing at 57°C for 45 s, and extension at 72°C for 15 min at 4°C and the supernatants were stored at −80°C until the assay. Mice were next sacrificed and tumor tissue samples were collected; the samples were subsequently minced with scissors in lysis buffer [50 mM Tris-HCl buffer (pH 7.6), 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid, 0.1% Tween 80, 1 mM phenylmethylsulfonyl fluoride, 3 μg/ml leupeptin, and 1 mM dithiothreitol] (10 μl/mg tissue) and homogenized with a Potter-Elvehjem homogenizer in ice. The protein lysates were prepared by centrifugation at 10,000 × g for 15 min at 4°C and the supernatants were stored at −80°C until the assay. Protein concentrations were determined with a Bio-Rad Protein Assay Kit (Nippon Bio-Rad Laboratory, Tokyo), while VEGF concentrations were measured by ELISA (mouse VEGF ELISA kit; R & D Systems, Minneapolis, MN, USA). Tumor tissue VEGF levels were expressed as pg VEGF/mg tissue protein.

**Histologic studies**

Specimens were extracted from each tumor tissue, fixed with 4% paraformaldehyde in 0.1 M phosphate buffer, and embedded in O.C.T. Compound (Tissue-Tek; Sakura Finetecnical, Tokyo). Subsequently, 8-μm cryosections were either prepared and stained with hematoxylin and eosin or examined with immunological assays. Endothelial cells were detected with a rat monoclonal anti-mouse CD31 (Pharmingen, San Diego, CA, USA). The secondary antibody was a rabbit biotinylated anti-rat IgG (Vector Laboratories, Burlingame, CA, USA). CD31-positive cells were visualized with the avidin-biotin-peroxidase complex method, using a Vectastain ABC kit (Vector Laboratories) and a 3',3'-diaminobenzidine tetrahydrochloride preparation (Dojindo Laboratories, Kumamoto). All sections were stained with hematoxylin. Vessels with positive staining for CD31 were quantified in four random microscopic fields per tumor by an independent observer who was blinded to the treatment protocol. Necrotic areas of the exterior region of the tumor tissue were graded as follows: 0, absent or rare (<10%); 1, mild (10 – 25%); 2, moderate (20 – 50%); and 3, severe (>50%).

**Determination of histamine levels**

Histamine concentrations in tumor tissue were also determined by ELISA. The tumor tissue samples were collected and subsequently minced with scissors in 10 mM phosphate buffer (pH 7.4) containing 10⁻⁶ M semicarbazide hydrochloride and homogenized. The homogenate was diluted 1:10 with phosphate buffer and heated at 100°C for 10 min to release bound histamine. The assay samples were then prepared by centrifugation at 3,300 × g for 20 min, and the supernatants were stored at −80°C until the assay. Histamine concentrations were determined with the Histamine ELISA kit (Medical & Biological Laboratories Co., Ltd., Nagoya). Histamine levels in tumor tissue were expressed as nmol/g tissue.

**Statistical analyses**

Data are presented as means ± S.E.M. Statistical
differences in the dose-response studies were evaluated by Dunnett’s multiple comparison test. Student’s t-test was used for comparison between two groups. Mann-Whitney’s U-test was also utilized for comparison of scores. A P value <0.05 was regarded as significant.

Results

Effects of H2-receptor antagonists on growth of Colon 38 tumor implants

On the 26th day after tumor implantation, the tumor volume for control mice was 1244.7 ± 209.5 mm³. Roxatidine, orally administered for 29 days, that is, 3 days before tumor implantation and 26 days after implantation, was found to suppress growth of Colon 38 tumor implants in a dose-related manner after day 26. Cimetidine exhibited similar suppression. Tumor growth suppression was significant at a dose of 300 mg/kg per day for roxatidine (56.6% decrease) and at a dose of 200 mg/kg per day for cimetidine (51.9% decrease) (Fig. 1a). In addition, treatment with roxatidine, at a dose of 300 mg/kg per day, or cimetidine, at a dose of 200 mg/kg per day, beginning on the day of tumor implantation, was also found to suppress growth of Colon 38 tumor implants in mice 26 days after implantation. Tumor growth suppression was significant at a dose of 300 mg/kg per day for roxatidine (61.6% decrease) and at a dose of 200 mg/kg per day for cimetidine (42.2% decrease) (Fig. 1b). Mice treated with H2-receptor antagonists did not demonstrate toxicity or decreased body mass compared with the control group.

Effects of H2-receptor antagonists on in vitro tumor cell growth

To examine whether or not the tumor-suppressive effects of roxatidine and cimetidine were due to a direct effect on the tumor cells, in vitro growth of Colon 38 cell lines was measured with the MTT assay. Histamine, roxatidine, and cimetidine all failed to exert a suppressive effect on in vitro Colon 38 cell growth (Table 1). In addition, RT-PCR analysis revealed that H2-receptor mRNA expression was undetectable in the cell line (Fig. 2).

Histologic changes of Colon 38 tumor tissue samples

Histological analysis was performed on Colon 38 tumor tissue samples obtained from syngeneic mice 26 days after implantation. Roxatidine, delivered at a dose of 300 mg/kg per day; cimetidine, delivered at a dose of 200 mg/kg per day; or vehicle was orally administered twice daily for 26 days. Microscopic observation of Colon 38 tumor tissue samples revealed an increase in necrotic areas in the roxatidine and cimetidine-treated mice compared with control mice (Fig. 3: a, c, e). Significant necrosis was observed at a dose of 300 mg/kg per day for roxatidine and at a dose of 200 mg/kg per day for cimetidine. Median necrosis scores were as follows: control, 0 (n = 7); 300 mg/kg per day of roxatidine, 2 (n = 7; P<0.05); 200 mg/kg per day of cimetidine, 2 (n = 6; P<0.05) (Fig. 4a). In addition, tumor tissue samples were stained for endo-
Roxatidine on Colon Cancer Suppression

The density of tumor microvessels, identified as anti-CD31-positive structures, in Colon 38 tumor tissue samples was decreased by treatment with roxatidine at a dose of 300 mg/kg per day or cimetidine at a dose of 200 mg/kg per day, compared with control mice (Fig. 3: b, d, f). The density of tumor microvessels in tumor tissue samples of control mice was observed to be 351 ± 27 counts/mm². Roxatidine and cimetidine, delivered for 26 days, markedly decreased the density of tumor microvessels in Colon 38 tumor tissue samples, compared with controls, that is, 200 ± 19 (43.2% decrease) and 142 ± 11 (59.5% decrease) counts/mm², respectively (Fig. 4b).

Effects of H₂-receptor antagonists on tumor tissue and serum VEGF levels in Colon 38-bearing mice

To determine the effects of roxatidine and cimetidine on VEGF expression in tumor-bearing mice, VEGF levels in both tumor tissue samples and serum obtained from Colon 38-bearing mice were measured. Roxatidine, delivered at a dose of 300 mg/kg per day; cimetidine, delivered at a dose of 200 mg/kg per day, or vehicle was orally administered twice daily for 26 days. Cimetidine, delivered at a dose of 200 mg/kg per day, significantly suppressed VEGF levels in tumor tissue samples of Colon 38-bearing mice. Similarly, roxatidine, delivered at a dose of 300 mg/kg per day, slightly suppressed VEGF levels in tumor tissue samples of Colon 38-bearing mice (Fig. 5a). In addition, implantation of Colon 38 tumor into mice significantly increased serum VEGF levels 26 days after implantation compared with control mice without tumor. Both roxatidine at 300 mg/kg per day and cimetidine at 200 mg/kg per day significantly suppressed serum VEGF levels in Colon 38-bearing mice compared with tumor-bearing control mice (Fig. 5b).

Effects of H₂-receptor antagonists on histamine levels in tumor tissue of Colon 38-bearing mice

To determine the effects of roxatidine and cimetidine on histamine levels in tumor tissue, histamine levels in tumor tissue samples obtained from Colon 38-bearing mice were measured. Both roxatidine and cimetidine failed to affect histamine levels in tissue samples of Colon 38 tumor implants compared with vehicle-treated control mice. The histamine levels were as follows: control, 11.8 ± 3.0 nmol/g tissue; 300 mg/kg per day of roxatidine, 13.6 ± 1.7 nmol/g tissue; and 200 mg/kg per day of cimetidine, 14.3 ± 3.6 nmol/g tissue (n = 6 or 7).

Table 1. Effects of histamine, roxatidine, and cimetidine on in vitro cell growth of Colon 38 cell lines

<table>
<thead>
<tr>
<th>Reagents (µM)</th>
<th>Vehicle</th>
<th>0.01</th>
<th>0.1</th>
<th>1</th>
<th>10</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>100.0 ± 2.37</td>
<td>101.7 ± 2.74</td>
<td>101.4 ± 2.27</td>
<td>100.6 ± 3.04</td>
<td>102.0 ± 2.90</td>
<td>100.0 ± 2.71</td>
</tr>
<tr>
<td>Roxatidine</td>
<td>100.0 ± 1.31</td>
<td>99.7 ± 1.10</td>
<td>101.3 ± 1.31</td>
<td>99.7 ± 0.64</td>
<td>99.9 ± 1.55</td>
<td>100.9 ± 2.53</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>100.0 ± 1.25</td>
<td>100.0 ± 1.26</td>
<td>99.8 ± 1.25</td>
<td>99.0 ± 1.72</td>
<td>99.6 ± 1.89</td>
<td>103.0 ± 2.64</td>
</tr>
</tbody>
</table>

Cells were pre-incubated with each reagent for 18 h and cell viability was determined by the MTT assay. Data are presented as means ± S.E.M. (percent of the vehicle control) for 8 cultures. Note that histamine, roxatidine, and cimetidine had no effect on the growth of Colon 38 cell lines.
Fig. 3. Immunohistochemical staining of tumor tissue samples obtained from mice treated daily with vehicle (a, b); roxatidine, delivered at a dose of 300 mg/kg (c, d); or cimetidine, delivered at a dose of 200 mg/kg (e, f), for 26 days after tumor implantation. Hematoxylin and eosin-stained (a, c, e) and anti-mouse CD31-stained (b, d, f) sections were evaluated 26 days after Colon 38 implantation. Note that roxatidine, delivered at a dose of 300 mg/kg per day, and cimetidine, delivered at a dose of 200 mg/kg per day, both markedly suppressed the density of tumor microvessels and increased necrotic areas in tumor tissue samples. Arrowheads indicate tumor microvessels. Bars denote 100 μm.
Discussion

The present study demonstrated for the first time that roxatidine was able to significantly suppress growth of colon cancer implanted in mice in a manner similar to cimetidine. In addition, it was found that such agents tended to suppress VEGF protein expression and markedly reduce the density of tumor microvessels in colon cancer tissue samples.

As described in the Introduction, many investigators antagonists, principally cimetidine, for colorectal cancer in clinical studies. In laboratory studies, such tumor-suppressive effects of H2-receptor antagonists were also confirmed in mice bearing several types of colon cancer cell lines with oral cimetidine doses of 25 to 200 mg/kg per day (11, 16). It is even more intriguing that cimetidine has been found to fail to suppress growth of the CT-26 cell line (mouse colon adenocarcinoma) with an subcutaneously dose of 0.12 mg/kg per day in
histidine-decarboxylase knockout mice, that is, mice with undetectable levels of endogenous histamine (17), despite the fact that an identical dose of cimetidine suppressed tumor growth in wild-type mice (18). Based upon the above results, the investigators of the present study reasoned that cimetidine acts as a histamine antagonist at the level of H₂ receptors, ultimately suppressing tumor growth. Accordingly, the present study examined the effects of two different types of H₂-receptor antagonists, ranitidine and cimetidine, on the growth of colon cancer implants. It was first confirmed that cimetidine, delivered at a dose of 200 mg/kg per day, which is a previously established dosage (16), significantly suppressed growth of Colon 38 implants in mice following administration for 26 days after tumor implantation. Moreover, as significant tumor growth suppression was also achieved with ranitidine, delivered at a dose of 300 mg/kg per day, the molar equivalent of a 200 mg/kg per day dosing of cimetidine, it is now apparent that cimetidine does not represent the only H₂-receptor antagonist that suppresses tumor growth. In addition, we also confirmed the tumor-suppressive effects of both ranitidine and cimetidine on the growth of B16BL6 tumor implants, a mouse malignant melanoma model, after 24 days of treatment (unpublished data), implying that the tumor-suppressive effects are not specific for colon cancer implants.

Cimetidine and ranitidine are generally administered at doses of 50 – 300 mg/kg per day and 10 – 60 mg/kg per day, respectively, to prevent ulcer development or enhance ulcer healing in animal experiments. Humans, however, receive doses of cimetidine and ranitidine that are 800 mg per day and 150 mg per day, respectively, so as to achieve similar effects. The cimetidine dose used for this study was selected since it is known that such a dosage maximally inhibits gastric acid secretion in both rats and humans. The cimetidine dose used for rats, however, is approximately 20 times higher than that typically used for patients. Such a difference in dosage might be due to the presence of different pathways for metabolism in rats and humans. In addition, a dose-dependent analysis for growth of colon cancer implants in mice administered ranitidine was performed (Fig. 1a). Despite the fact that mice required delivery of higher doses of ranitidine than is typically utilized for inhibiting gastric acid secretion, no toxicity or decreased body mass was found upon comparison with the control group. In addition, we have already confirmed that ranitidine suppresses in vivo tumor implants in a similar manner to cimetidine and ranitidine (unpublished data). Such results strongly suggest that the H₂-receptor represents the exact site of action for such compounds that suppress tumor growth. The apparent dissociation between the relative potency of H₂-receptor antagonism and tumor suppression for the various agents might be due to pharmacokinetic properties, as the compounds are structurally different. Namely, cimetidine is an imidazole, ranitidine is a furan, and roxatidine is a completely different structural compound from the former two. It remains possible that the ability to act as an effective H₂-receptor antagonist differs depending on the specific tissue type on which the H₂ receptor is expressed.

It has been demonstrated that histamine stimulates cell proliferation in a variety of cell lines, most notably cancer cell lines (19). In addition, the growth-suppressive effects of H₂-receptor antagonists for various cell lines have also been described with in vitro studies (11, 20). To the extent of the authors’ knowledge, no report exists regarding expression of H₂ receptors on colon cancer cells in either humans or mice. Adams et al. reported that histamine increased in vitro proliferation in 2 of 4 different human colon cancer cell lines. Moreover, cimetidine inhibited such histamine-induced proliferation in both cell lines (16). Such results suggest that only certain colon cancer cell lines express H₂ receptors, which appear to contribute to cell proliferation stimulated by histamine. In the present study, histamine, ranitidine, and cimetidine failed to achieve in vitro growth promotive or suppressive effects for the Colon 38 cell line, a cell line that lacks H₂ receptors, although ranitidine and cimetidine did suppress in vivo growth of the tumor tissue implants. Such a finding suggests that the tumor-suppressive effects of H₂-receptor antagonists do not represent the product of direct action on tumor cells.

Histamine also plays an important role in angiogenesis regulation associated with promotion of tumor progression (21, 22). A number of tumor and stromal cells involved in regulation of tumor angiogenesis have been identified, including fibroblasts, smooth muscle cells, and infiltrating immune cells (23, 24). VEGF has been recognized as the most important growth factor involved in angiogenesis induced by numerous cytokines, as well as angiogenesis induced by hypoxic and ischemic stress. VEGF has been found to increase in vivo angiogenesis and vascular permeability, in vitro proliferation and migration of endothelial cells, protease production in endothelial cells, and expression of intercellular adhesion molecules on endothelial cells. In addition, VEGF overexpression has been detected in many human solid tumors (25, 26). Furthermore, inhibition of the VEGF signal pathway was observed to prevent tumor angiogenesis, suppressing solid tumor growth (27, 28). Recent studies have also revealed that histamine directly induces VEGF mRNA expression.
in granulation tissue by binding to H₂ receptors (29). In addition, an in vivo angiogenesis model demonstrated that the adenylate cyclase/protein kinase A signaling pathway, which is involved in H₂-receptor signaling, enhanced angiogenesis via VEGF induction (30). In addition, Kobayashi et al. reported that cimetidine blocked endothelial cell E-selectin expression, a compound that plays an important role in VEGF-induced angiogenesis (12). It is also of interest that Sartippour et al. recently reported that cimetidine failed to exert an effect on in vitro endothelial cell proliferation, suggesting that cimetidine has no effect on angiogenesis (31). Nonetheless, it appears that Sartippour simply demonstrated that cimetidine failed to exert a direct effect on in vitro endothelial cell proliferation. In consideration of the above observations, the present study attempted to examine whether or not the tumor-suppressive effects of H₂-receptor antagonists result from suppression of VEGF expression and tumor angiogenesis. The present study demonstrated that treatment with roxatidine or cimetidine suppressed VEGF protein expression in implanted tumor tissue, implying that angiogenesis was suppressed in the tumor tissue. It is of note that tumor tissue histamine levels were not altered by either H₂-receptor antagonist. Indeed, few microvessels and many necrotic areas were observed in tumor tissue specimens obtained from mice treated with H₂-receptor antagonists. Such findings strongly suggest that the tumor-suppressive effects of H₂-receptor antagonists might be due to angiogenesis attenuation that results from suppression of VEGF expression. In addition, although we confirmed that the Colon 38 cell line does not express H₂ receptors, H₂-receptor mRNA was detected by RT-PCR in Colon 38 tumor tissue implanted in mice (unpublished data). Accordingly, it remains most likely that the tumor cells were stimulated by an as yet unidentified cell expressing H₂ receptors. Such a process could represent the major source of VEGF for the tumor tissue and serum. It should be noted, however, that the VEGF-suppressive effect of roxatidine was weaker than that of cimetidine, although both H₂-receptor antagonists markedly suppressed tumor angiogenesis. Such results suggest that roxatidine might also suppress other angiogenic factors besides VEGF.

In conclusion, both roxatidine and cimetidine were found to suppress the growth of colon cancer implants in mice. Both H₂-receptor antagonists exerted such suppression via reduction of tumor angiogenesis, which appears to involve reduction of VEGF expression.

Acknowledgments

We wish to thank C.J. Hurt, M.D. (St. Vincent’s Manhattan, New York Medical College, USA) for a critical reading of the manuscript; E. Nakamura and K. Matsuno for helpful suggestions; as well as A. Yoshida, T. Ivatsubo, M. Nishimoto, R. Kuramoto, M. Suetomi, and Y. Muneoka for technical assistance.

References

17. Takahashi K, Tanaka S, Furuta K, Ichikawa A. Histamine H₂


