LETTER TO THE EDITOR

Dear Sir,

Cimetidine: An inhibitor or promoter of tumor growth?

Cimetidine, a histamine type 2 (H2) receptor antagonist, is a commonly prescribed medication for the treatment of gastrointestinal reflux disease (GERD) as well as for gastric and duodenal ulcer disease. In addition, cimetidine has been shown to have immunomodulatory effects. This has led investigators to study the effects of cimetidine in the treatment of various neoplasms.

Chemotherapy for colorectal cancer is based on fluorouracil, which has only minimal activity on high-stage disease. Pharmacologic agents that can improve survival in patients with colorectal cancer will have profound impact in the treatment of this condition. Cimetidine was first reported to have antitumor effects in 1979 (Armitage and Sidner, 1979). This anecdotal case presentation was followed by a report of immunomodulation by cimetidine (Osband et al., 1981). Studies involving cimetidine on xenograft mouse models have been reported (Gifford et al., 1981; Tutton and Steel, 1979; Watson et al., 1993; Adams et al., 1994; Suonio et al., 1994). The majority of these studies (Gifford et al., 1981; Tutton and Steel, 1979; Watson et al., 1993; Adams et al., 1994) describe an antitumor effect of cimetidine. In addition, there have been several clinical trials with equivocal results (Svendsen et al., 1995; Links et al., 1995; Matsumoto, 1995).

The xenograft models that were previously used to test cimetidine involved human tumor cell line implantation in the subcutaneous (s.c.) space (Gifford et al., 1981; Tutton and Steel, 1979; Watson et al., 1993; Adams et al., 1994) and the subrenal capsule (Suonio et al., 1994). We have previously developed a “patient-like” tumor model using surgical orthotopic implantation (SOI) of histologically intact tumor tissue for human colon cancer (Tu et al., 1991) as well as many other tumor types (Hoffman, 1994). Tumor growth and progression after s.c. implantation, where metastasis rarely occurs, differ greatly when compared with orthotopic implantation, which can allow for a high metastatic potential correlating with the clinical course of the disease (Hoffman, 1994). In addition, different drug responses on tumor growth have been reported for the s.c. vs. orthotopic site (Kuo et al., 1993). These significant differences suggest that orthotopic implantation is a more clinically relevant tumor model. This study was designed to examine the efficacy of cimetidine on human colon carcinomas that were implanted by SOI compared with s.c.

TUMOR AND PATIENT PROFILE

Human colon cancers (AC 3445 and AC 3557) were obtained originally from fresh surgical specimens and implanted s.c. to obtain stock tumor tissue. Tumors AC 3445 and AC 3557 are well-differentiated adenocarcinomas invading up to the serosa, with no lymph node involvement (T3N0M0, stage II). Permission to use patient-derived tissue was obtained from the Human Subjects Committee of the University of California at San Diego.

TUMOR IMPLANTATION IN NUDE MICE

Four- to 6-week-old CD-1 nu/nu mice of both sexes were used. All mice were kept in a pathogen-free facility with controlled light/dark cycle, temperature and humidity, under NIH guidelines. Cages, bedding, food and water were autoclaved prior to use. Tumor tissue, harvested from s.c. tumor stock, was cut into small pieces (1 mm3) and mixed thoroughly in RPMI 1640 culture media.

Orthotopic implantation

After an appropriate state of anesthesia was induced with isoflurane inhalation, the nude mice were put in the supine position. A 1 cm lower abdominal incision was made. The cecum and ascending colon were then exteriorized. A small serosal tear was then created in the ascending colon. Ten tumor pieces were then affixed to the subserosa, at the site of the serosal tear, using an 8-0 nylon suture. The cecum and ascending colon were then returned to the peritoneal cavity. The abdominal incision was closed using a 6-0 silk suture.

S.c. implantation

After an appropriate state of anesthesia was induced with isoflurane inhalation, the mice were placed in the lateral position. A small incision was made in the flank. Ten tumor pieces were placed in the s.c. space. The incision was then closed with a 6-0 silk suture. The take rate was 100% for both tumors.

EXPERIMENTAL DESIGN

SOI

Sixteen mice were used for each case of AC 3445 and AC 3557. They were randomly divided to cimetidine-treated and control groups with 8 mice in each group.

S.c. transplantation

Ten mice were used for each case of AC 3445 and AC 3557. They were randomly divided to cimetidine-treated and control groups with 5 mice in each group.

Cimetidine

Cimetidine, obtained from Sigma (St. Louis, MO), was dissolved in autoclaved drinking water at a concentration of 1 mg/ml. This corresponds to a dose of 100 mg/kg/day, which has previously been shown to produce an effective cimetidine level in murine plasma (Gifford et al., 1981; Adams et al., 1993). Cimetidine containing water was prepared fresh twice per week.

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Cimetidine was given on the day of tumor implantation (day 0) until the experiment was terminated on day 60. Mice were sacrificed using CO₂ asphyxiation and then fixed in 10% formalin for subsequent autopsy.

Tumor measurement
Calipers were used to measure tumor size in the two largest dimensions. Tumor volume was then calculated using the formula

\[ \text{Volume} = \frac{1}{2} \times \text{length} \times \text{width}^2 \]

(Eulerst et al., 1996). Tumor measurement of s.c. implanted tumors began 2-3 weeks after implantation, and weekly thereafter. In addition, tumors were weighed after the mice were sacrificed, prior to placing them in formalin. In the orthotopically implanted mice, tumor measurement was performed at the time of sacrifice.

Histopathology
Upon autopsy of orthotopically implanted mice, tissues of the primary tumor (colon), liver, lung and lymph nodes were collected. All tissues were then subsequently processed and stained with standard hematoxylin-eosin (H&E) staining techniques. The slides were examined microscopically, by an independent pathologist.

Statistics
A Mann-Whitney U-test was used for all statistical analysis with \( \alpha = 0.05 \).

RESULTS
Primary tumor growth
Orthotopic group, cimetidine treatment. Tumor volumes (mm³) of both AC 3445 and AC 3557 for the cimetidine-treated animals

<table>
<thead>
<tr>
<th>Tumor Volume (mm³)</th>
<th>AC 3445</th>
<th>AC 3557</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>116.0 ± 37.0</td>
<td>756.1 ± 244.0</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>93.2 ± 30.5</td>
<td>144.5 ± 24.4</td>
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</table>

Mean tumor volume (mm³) ± standard error (SE) are shown for control and cimetidine-treated mice implanted orthotopically with AC 3445 and AC 3557. Cimetidine resulted in increased tumor growth of AC 3445 and AC 3557 by 65% (\( p = 0.016 \)) and 55% (\( p = 0.21 \)), respectively.

Histopathology
Histopathological examination revealed that implanted tumors AC 3445 and AC 3557 were well-differentiated adenocarcinomas, as were the original patient tumors. In the orthotopic group, there was no evidence of tumor growth in the liver, lung or lymph nodes of either cimetidine-treated or control animals.

DISCUSSION
Consistent with previously published reports of cimetidine's antitumor effects (Gifford et al., 1981; Tatum and Steel, 1979; Watson et al., 1993; Adams et al., 1993, 1994), we were able to demonstrate growth inhibition by cimetidine when the human colon tumors were transplanted s.c. In marked contrast, our experiments

were higher when compared with controls. Mean tumor volumes for AC 3445 in control and cimetidine-treated groups were 116.0 and 756.1, respectively (Table I). This corresponds to a 63% increase in tumor growth for AC 3445 in mice given cimetidine compared with controls. This difference was statistically significant at \( p = 0.016 \). For AC 3557, mean tumor volumes (mm³) for the control and cimetidine-treated groups were 93.2 and 144.5, respectively (Table I). Although statistical significance of the difference could not be generated (\( p = 0.21 \)), there was a 55% increase in mean tumor volume for the cimetidine-treated mice.

S.c. group, cimetidine treatment. Tumor volumes (mm³) of AC 3445 and AC 3557 were recorded weekly after the tumors were palpable. For both cases, there was a trend for cimetidine to inhibit growth of the s.c. implanted tumors. This was more pronounced for AC 3445 than for AC 3557. On days 28 and 35, control mean tumor volumes were 531.5 and 1,129.3, respectively. In contrast, on days 28 and 35, cimetidine-treated mean tumor volumes for AC 3445 were 242.0 and 513.5, respectively (Table II, Fig. 1). This resulted in \( p < 0.05 \). Overall, cimetidine resulted in greater than 45% inhibition of growth when compared with controls (Table II). For AC 3557, the degree of inhibition was less (range 4-23%). There were no time periods that reached statistical significance (Table III, Fig. 2).

Cimetidine level
Prior to sacrifice, blood was collected for cimetidine level determination, which was performed by the Poison Lab (San Diego, CA). Three pooled specimens were analyzed and had the following cimetidine levels: 1.5, 2.3 and 1.9 μg/ml. The average was 1.9 μg/ml. This is within the therapeutic range of human plasma level (0.5-2.0 μg/ml) for cimetidine.

TABLE I – COMPARISON OF ORTHOTOPIC TUMOR GROWTH OF AC 3445 AND AC 3557, Cimetidine vs. Control

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TABLE II – COMPARISON OF CIMETIDINE VS. CONTROL, FOR THE S.C. IMPLANTED TUMOR AC 3445

<table>
<thead>
<tr>
<th>Tumor Volume (mm³) ± SE</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 35</th>
<th>Day 42</th>
<th>Day 49</th>
<th>Day 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>264.1 ± 56.4</td>
<td>551.5 ± 116.7</td>
<td>1,129.3 ± 202.6</td>
<td>1,557.8 ± 321.6</td>
<td>2,310.0 ± 484.2</td>
<td>2,848.9 ± 862.2</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>147.3 ± 36.7</td>
<td>242.0 ± 57.0</td>
<td>513.5 ± 149.4</td>
<td>677.1 ± 188.7</td>
<td>1,198.2 ± 900.4</td>
<td>1,461.0 ± 507.7</td>
</tr>
<tr>
<td>( p )</td>
<td>0.17</td>
<td>0.047</td>
<td>0.058</td>
<td>0.076</td>
<td>0.117</td>
<td>0.17</td>
</tr>
<tr>
<td>% Inhibition</td>
<td>45</td>
<td>56</td>
<td>54</td>
<td>56</td>
<td>48</td>
<td>49</td>
</tr>
</tbody>
</table>

Mean tumor volume (mm³) ± standard error (SE) are shown for tumor AC 3445 implanted in the s.c. space. Cimetidine-treated mice compared with controls, \( p < 0.05 \) was obtained on days 28 and 33.

TABLE III – COMPARISON OF CIMETIDINE VS. CONTROL, FOR S.C. IMPLANTED TUMOR AC 3557

<table>
<thead>
<tr>
<th>Tumor Volume (mm³) ± SE</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 35</th>
<th>Day 42</th>
<th>Day 49</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>123.9 ± 12.4</td>
<td>155.0 ± 32.5</td>
<td>304.9 ± 54.0</td>
<td>665.1 ± 206.9</td>
<td>1,387 ± 590.9</td>
<td>2,211.1 ± 1,283.4</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>112.1 ± 12.8</td>
<td>147.2 ± 17.1</td>
<td>292.1 ± 25.7</td>
<td>506.5 ± 63.8</td>
<td>1,165.4 ± 301.4</td>
<td>1,750.0 ± 239.6</td>
</tr>
<tr>
<td>( p )</td>
<td>0.6</td>
<td>0.92</td>
<td>0.6</td>
<td>0.6</td>
<td>0.92</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Mean tumor volume (mm³) ± standard error (SE) are shown for tumor AC 3557 implanted in the s.c. space. There was no statistically significant difference noted in tumor growth.
with orthotopically implanted tumors resulted in increased tumor volume in mice treated with cimetidine when compared with controls. This was statistically significant for tumor AC 3445.

It is clear that there are contradictory data in the literature as to the effects of cimetidine on colon tumor growth. In the references reviewed, the vast majority of animal studies used an s.c. model (Gifford et al., 1981; Tutton and Steel, 1979; Watson et al., 1993; Adams et al., 1994) or the subrenal capsule (Suonio et al., 1994). We have previously reported a new model developed with SOI of histologically intact tissue. This model demonstrates more clinically relevant features of human cancer growth in vivo, including metastasis (Fu et al., 1991), which is a rare event following s.c. implantation (Hoffman, 1994).

It has been proposed that the appropriate microenvironment is crucial for tumor growth and metastasis (Wilman et al., 1992). In addition, Wilman et al. (1992) and Kuo et al. (1993) reported different effects of chemotherapy agents on colon carcinoma in the orthotropic vs. ectopic implantation. The orthotropic microenvironment may explain cimetidine’s stimulation of AC 3445 and AC 3557.

Tutton and Steel (1979) claimed short-term suppression of tumor growth of a colorectal cell line by cimetidine and postulated histamine blockade as one mechanism. The treatment was begun 20 days after s.c. implantation and lasted only 12 days. However, when tumor growth was analyzed, it appeared that after the 5th day of treatment, tumor growth was accelerated. Also, the histamine H2-receptor antagonist Dimpriz had no effect on tumor growth (Tutton and Steel, 1979). Suonio et al. (1994) tested histamine and cimetidine on fresh human colon cancer tissue in the subrenal capsule assay, also an ectopic site, and concluded that histamine resulted in reduction of tumor size. Cimetidine caused reduction in tumor size in 3 of 10 assays. However, when all 10 cases are considered, cimetidine resulted in increased average tumor growth (Suonio et al., 1994).

Further evidence for histamine blockade as the mechanism for tumor suppression was proposed by Adams et al. (1994). They tested the effects of histamine and cimetidine on 4 human colon cancer cell lines (C170, LIM2412, LIM2405 and LoVo) in the s.c. xenograft nude mouse model. They found that histamine, given locally, stimulated growth of colorectal cell line C170. This stimulatory effect of histamine could be suppressed by the administration of high doses of cimetidine (200 mg/kg/day). However, an earlier study by Watson et al. (1993) failed to show this stimulatory effect of histamine on the growth of C170. It is possible that this variation could be due to cell line heterogeneity.

There are several reports that seem to contradict histamine blockade as the mechanism of action for tumor growth suppression. Lawson et al. (1996) compared the effects of 2 histamine receptor (H2) antagonists, cimetidine and ranitidine, on 2 human colon cancer cell lines (C170 and LIM2412). The results show that while cimetidine was able to inhibit growth of C170, ranitidine did not. Interestingly, both cimetidine and ranitidine caused increased tumor growth of cell line LIM2412. Tutton and Bacska (1983) tested 3 H2-receptor antagonists, cimetidine, metiamide and ranitidine, in a s.c. xenograft mouse model (using fresh human colorectal tumors), as well as a carcinogen-induced tumor model in rats. They concluded that all 3 had antitumor effects. However, the effects of ranitidine and metiamide were greater than those of cimetidine. Also, Hahn et al. (1995) tested the immunomodulating effects of cimetidine, ranitidine and famotidine (all are H2-receptor antagonists). They tested mononuclear cells from patients with gastric cancer and found different immunomodulating effects among the 3 agents. They concluded that the antitumor effects of cimetidine were unlikely due to interaction with the H2-receptor. Comparison of the data of others and with each other and with ours is difficult due to differences in tumor models, doses and dosing regimens.

Clinical trials of cimetidine in colorectal cancer were not conclusive (Svensen et al., 1995; Links et al., 1995; Matsumoto, 1995). Of these, only one was a placebo-controlled, randomized study (Svensen et al., 1995). There was no effect of cimetidine on overall survival, although a subset analysis revealed a trend to improved survival in Dukes’ C patients (p = 0.11). However, the study did not mention the number of patients in each group who received chemotherapy (Svensen et al., 1995). This is significant, because Harvey et al. (1994) demonstrated increased plasma concentration of fluorouracil when administered with cimetidine. This could account for the trend in improved survival seen in these clinical trials. Links et al. (1995) failed to show clinically significant differences in survival between cimetidine treated and control.

Contrary to other reports, our results show clearly that cimetidine does not inhibit colon tumor growth in the orthotopic nude mice model, and more importantly, we show that cimetidine can stimulate human colon cancer growth. Data obtained from an orthotopic model should be more reliable, especially in the SOI model, which allows clinical-like replication of the course of the tumor (Hoffman, 1994). It is clear that further research is needed to elucidate the effect and mechanism of cimetidine on tumor growth, which has important ramifications for its safe use. In future experiments, additional patient colon tumors as well as other types will be transplanted into nude mice both orthotopically and s.c. to determine their response to cimetidine.

Yours sincerely,


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HOFFMAN, R.M., Orthotopic is orthodox: why are orthotopic transplant metastatic models different from all other models? J. cell. Biochem., 56, 1–3 (1994).


