Cimetidine, an unexpected anti-tumor agent, and its potential for the treatment of glioblastoma (Review)

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Abstract. Cimetidine (CIM), the prototypical histamine H2 receptor antagonist (H2RA), was brought to market based on its ability to accelerate healing of gastrointestinal ulcers through the inhibition of gastric acid secretion. Cimetidine, the most studied H2RA, has been demonstrated to possess anti-tumor activity against colon, gastric and kidney cancers, and melanomas. This activity involves a number of different mechanisms of action: a) CIM antagonizes tumor cell-mediated interleukin-1-induced activation of selectins in liver sinusoids, inhibiting tumor cell binding on liver sinusoids, thereby reducing the development of liver metastasis; b) histamine acts as a growth factor in various tumor cell types via the activation of H2 receptors; CIM therefore may antagonize this effect; c) CIM acts as an immunomodulator by enhancing the host's immune response to tumor cells. With respect to malignant gliomas, CIM added to temozolomide was superior *in vivo* when compared to temozolomide alone in extending survival of nude mice with human glioblastoma cells orthotopically xenografted into their brain. We review the various mechanisms of action potentially associated with the therapeutic effects of CIM in the case of experimental glioblastomas, observations we hope will encourage clinical investigation of CIM in the management of highly malignant gliomas.

Contents

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Abbreviations: CIM, cimetidine; H2RAs, histamine H2 receptor antagonist

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1. Origin of cimetidine

Cimetidine [N''-cyano-N-methyl-N'-(2(((5-methyl-1H-imidazol-4-yl)methyl)thio)ethyl)guanidine] is a substituted imidazole with a specific antagonistic effect on histamine H2 receptors. Briefly, cimetidine (CIM) is a weak base with a high level of water solubility which can be measured in biological fluids including the cephalo-spinal fluid (1). CIM is metabolized in the liver by oxidative hydroxylation and conjugation. Up to 80% of a single dose of CIM is excreted in the urine (1), with up to 70% in an unchanged form (1). Its principal action is on parietal cell histamine H2 receptors, and by binding to these receptors, inhibits gastric acid secretion stimulated by histamine, pentagastrin, acetylcholine, insulin, food and other secretagogues (2).

2. Initial therapeutic indications of cimetidine

CIM was the first registered histamine H2RA, its wide acceptance was based on its clinical effectiveness in the healing of gastrointestinal ulcers through inhibition of gastric acid secretion (1-3). CIM was one of the most widely used H2RA during the 1980s (3). At the time of its introduction in the late 1970s, CIM was rarely considered an agent with clinical utility other than its primary indication (3). A primary concern was if by virtue of their acid-inhibitory activity, H2RAs increased the risk of developing gastrointestinal malignancies (3); tiotidine, one of the earliest H2RAs developed, was abandoned when preclinical toxicity tests demonstrated an increased incidence of gastric tumors in rats (4). CIM inhibits several isozymes of the cytochrome P450 enzyme system, including CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. This inhibition forms the basis of the numerous drug interactions. While CIM proved to be a safe medication, its use in peptic ulcer disease was supplanted by the development of longer-acting H2RAs with reduced adverse effects and the introduction of highly specific proton pump inhibitors (2).

Table I. Description of the various clinical trials using cimetidine in oncology.

Oncological indication	Type of trial	Cimetidine dose	No. of pts. enrolled	Results (patient survival)	Authors/Refs.
Gastric cancer	Randomized	Post-operative 800 mg/d	181	Significant increase	Tonnesen *et al* (6)
Gastric cancer	Randomized	1-1.2 g/d	65	Significant increase	Burtin *et al* (5)
Colorectal cancer	Randomized	5 d pre-/2 d post-operative 800 mg/d	34	Significant increase	Adams and Morris (7)
Colorectal cancer	Randomized	5FU+/-post-operative 800 mg/d, 1 y	64	Significant increase	Matsumoto (8)
Colorectal cancer	Randomized	Post-operative 400 mg twice/d, 2 y	45 (Dukes C)	Significant increase	Svendsen *et al* (9)
Colorectal cancer	Randomized	Pre-operative, 7 d	42	3-y survival benefit	Adams and Morris (10)
Colorectal cancer	Randomized	Pre-operative 800 mg twice/d, 5 d	125	Survival benefit	Kelly *et al* (11)
Colorectal cancer	Non-randomized	5FU+/-post-operative 800 mg/d, 1 y	64	10-y survival benefit	Matsumoto *et al* (13)
Advanced melanoma	Phase II	300 mg, 4 x/d	19	1 CR, 2 PR	Morton *et al* (16)
Advanced melanoma	Phase II	INF + 1.2 g/d	35	7 PR	Creagan *et al* (15)
Metatastatic RCC	Non-randomized	Coumarin + 300 mg, 4x/d upd	42	3 CR, 11 PR	Marshall *et al* (17)
Metatastatic RCC	Phase II	Coumarin + 300 mg 4x/d	50	4 PR	Dexeus *et al* (18)
Metatastatic RCC	Non-randomized	600 mg/d upd	42	2 CR	Inhorn *et al* (19)
Metatastatic RCC	Phase III	INF +/- (coumarin + 400 mg 3x/d)	148	No significant increase	Sagaster *et al* (20)

upd, until progression of disease; d, day; y, year; CR, complete response; PR, partial response; INF, interferon; RCC, renal cell carcinoma.

3. Cimetidine as an anti-tumor drug

The first reports suggesting CIM exhibited a clinical oncologic effect appeared in 1988 in the context of gastric cancer (5,6). In a randomized study including 65 patients selected because their condition contraindicated all other forms of treatment, Burtin *et al* (5) found that a course of CIM (1-1.2 g/day) or ranitidine (450-900 mg/day) significantly improved the patients' survival rates. These patients survived six times longer than others receiving palliative treatment with analgesics (5). Another multicenter, randomized, double-blind, placebo-controlled study carried out by Tonnesen *et al* (6) on 181 patients showed that a post-operative course of CIM at a normal therapeutic dosage (800 mg/day) significantly prolonged the survival of gastric cancer patients.

In colorectal cancer patients, Adams and Morris (7) were the first to demonstrate the beneficial effect of a short-course perioperative treatment with CIM on surgically-induced immunosuppression. Their randomized study involving 34 patients showed a strong trend towards enhanced survival in the patients treated with CIM (800 mg/day) when compared to controls, a finding correlated with an increase of lymphocyte infiltration into the tumors (7).

Matsumoto (8) performed a multicenter randomized controlled study in 64 colorectal cancer patients receiving postoperative 5-fluorouracil. Post-operative treatment with CIM (800 mg/day) and 5-fluorouracil (150 mg/day) for about a year was efficacious, increasing the disease-free period and survival when compared to the treatment with 5-fluorouracil alone (8).

Several subsequent studies, summarized in Table I, have been published showing considerably enhanced survival rates in gastric and colorectal cancer patients treated with CIM (9-13).

The use of CIM also has intriguing implications in the management of advanced malignant melanomas (14-16) and metastatic renal cell carcinomas (17-20) (Table I).

Our group (21) has demonstrated that CIM complements the cytotoxic agent temozolomide in experimental glioblastomas, a point detailed in the section entitled Cimetidine and malignant gliomas.

4. Mechanisms of action of cimetidine in oncology

Studies of the anti-tumor effects of CIM indicate multiple potential mechanisms of action, characterized by three overall characteristics: a) a direct inhibitory effect on tumor growth by blocking the cell growth-promoting activity of histamine (22-24) (Fig. 1) and an indirect effect by inhibiting tumor-associated angiogenesis (Fig. 2) (25); b) a cell-mediated immunomodulation by enhancing the host's immune response to tumor cells (Fig. 1) (26-28); c) an inhibition of cancer cell migration (21) and adhesion to endothelial cells (29) and therefore an inhibition of tumor neo-angiogenesis (25) (Fig. 2) and metastasis development (29) (Fig. 3).

Inhibitory effects on tumor growth. While the mechanisms involved are incompletely understood, CIM is known to inhibit the growth of several types of tumors, including gastrointestinal cancers, both *in vitro* and *in vivo* in animal models (23,24). An active role is strongly suggested for histamine of autocrine or paracrine origins in malignant cell proliferation (Fig. 1) (12).

Histamine is a receptor-dependent growth factor in some, but not all, human colon cancer cell lines, as well as in some gastric, breast and melanoma cell lines (23,24,30,31). In a culture study of four different colorectal tumor cell lines

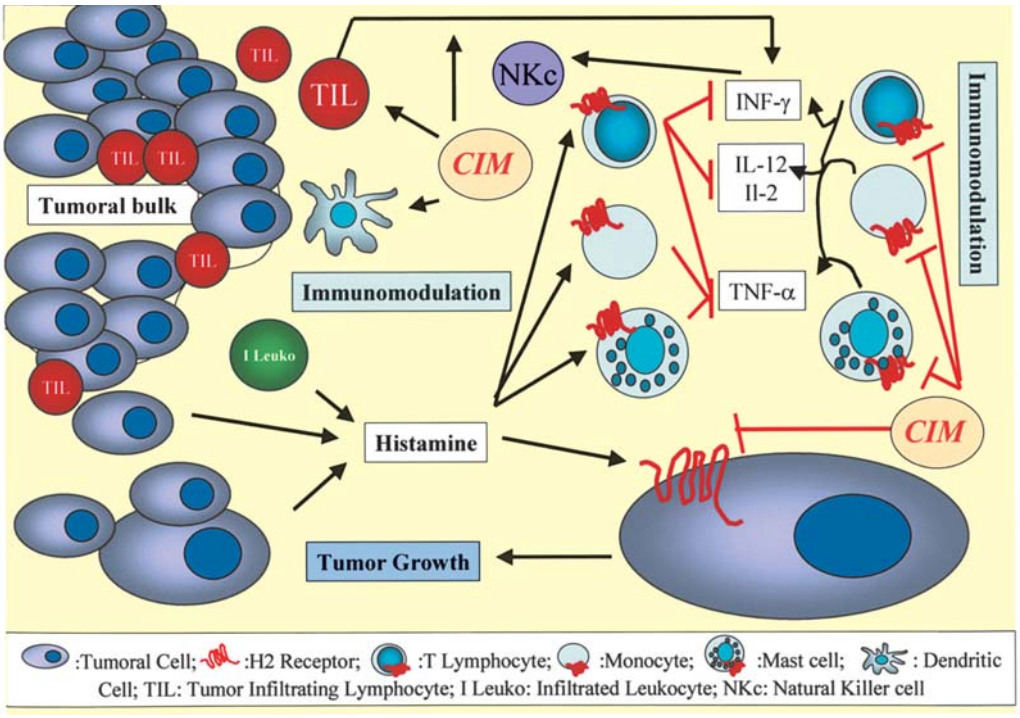

Figure 1. CIM inhibitory effect on tumor growth and CIM-mediated immunomodulation. CIM blocks the cell growth-promoting activity of histamine. The mechanisms proposed for the cell-mediated immunomodulation of CIM include the inhibition of suppressor T lymphocyte activity, the stimulation of natural killer cell (NKc) activity, an increase in interleukin-2 (IL-2) and interleukin-12 (IL-12) production in helper T lymphocytes, an increase in tumor inhibitory cytokines and the enhancement of the host's anti-tumor cell-mediated immunity.

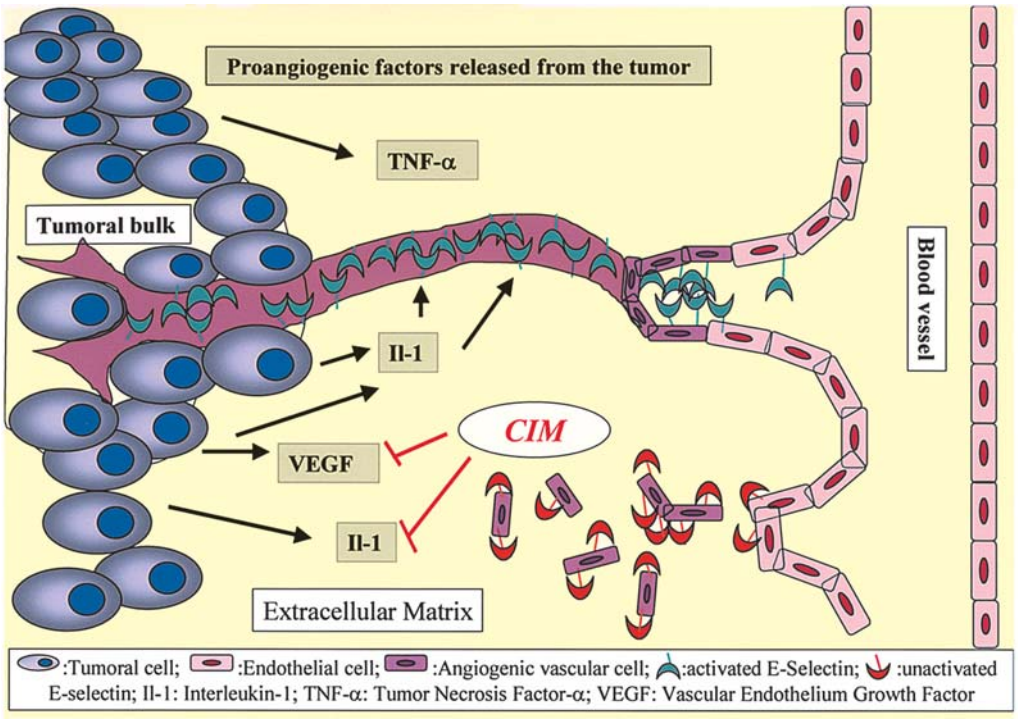

Figure 2. CIM-mediated neo-angiogenesis inhibition. CIM induces a significant decrease in VEGF expression levels and the vascular-like tube formation by endothelial cells is significantly impaired.

(C170, Lovo, LIM2412 and LIM2405) histamine was found to stimulate cell proliferation in two of them (C170 and LIM2412) in a dose-dependent manner (23). This effect was reversed by CIM in the presence of histamine, but not in its absence (23). When the C170 cell line was grown in nude mice as a subcutaneous xenograft, CIM had a significant dose-dependent growth-inhibitoring effect leveling out at a dose of 50 mg/kg/day (23).

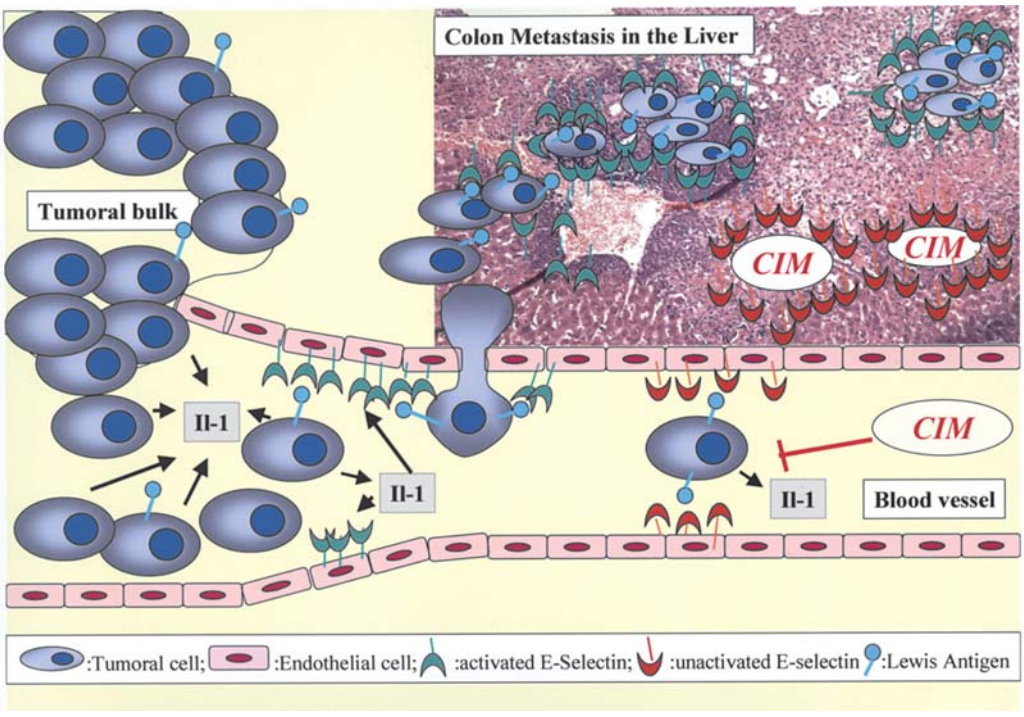

Figure 3. CIM-mediated inhibition of cancer cell migration and the development of liver metastasis. Epithelial cells detaching themselves from primary epithelial tumors (tumoral bulk) and migrating through the lymphatic or the blood vessels eventually colonize the liver because epithelial cancer cells exhibiting Lewis antigens on their surfaces are able to adhere to endothelial cells in liver sinusoids due to the presence of selectins (the ligands for Lewis antigens) in these endothelial liver cells. CIM prevents liver metastasis of colon cancer cells by blocking E-selectin activation by means of the inhibition of interleukin-1 (IL-1) secretion by the tumor cells.

Rajendra *et al* (32) demonstrated that CIM at 10 μM inhibited the *in vitro* proliferation of the Caco-2 colorectal cancer cell line in the presence of histamine by causing apoptotic cell death. In the human gastric tumor cell lines MKN45 and MKN45G, CIM (10 μM) reversed the histamine-stimulated proliferation (30). CIM also inhibited the proliferation of MKN45 subcutaneous xenografts in nude mice (100 mg/kg/day, given in the drinking water) (30). In another *in vitro* study, histamine significantly stimulated cells proliferating in a dose-dependent manner on the gastric cancer cell lines KATO-III and AGS, with the maximum effect again occurring around a 10 μM concentration (31). CIM reversed the histamine-stimulated cell proliferation, with the maximum effect at concentrations above 10 μM (31). Ranitidine and famotidine did not show such an effect (31). Histamine significantly stimulated growth in two of four human melanoma cell lines, and this effect was inhibited by CIM in a dose-dependent manner, and also by ranitidine and famotidine (24). CIM also inhibited tumor growth of human pancreatic cancer xenografts in immunodeficient mice (33).

Adams *et al* (23) suggested a role for H2 receptors located either on the tumor cells themselves, on immunocompetent cells in the host, or both. Using L-histidine decarboxylase (HDC)-deficient mice with undetectable levels of endogenous histamine, Takahashi *et al* (34,35) have shown that the daily administration of CIM (0.12 mg/kg/day) failed to suppress the growth of a syngeneic colon adenocarcinoma despite the fact that an identical dose of CIM suppressed tumor growth in wild-type mice, as the result of the inhibition of the H2-mediated actions of endogeneous histamine. Curiously, ranitine did not seem to exert most of the *in vitro* and *in vivo* effects mentioned, an observation which would argue against H2 receptors playing a role in the effects of CIM, since ranitine is marginally more potent as an H2 receptor antagonist (36). In fact, in a prospective randomized controlled study, the use of ranitidine in patients with gastric cancer did not show any significant increase in their survival rates (37). In contrast, roxatidine significantly decreased the *in vivo* growth of colon 38 implants in mice (38). In their study, Tomita *et al* (38) showed that *in vitro*, histamine, roxatidine, and CIM failed to achieve any growth-promotive or suppressive effects in the case of the colon 38 cell line, a cell line that lacks H2 receptors, although roxatidine and CIM suppressed the *in vivo* growth of the tumor tissue implants. Such a finding suggests that in this case, the tumor-suppressive effects of H2 receptor antagonists do not constitute the product of any direct action on tumor cells. Szincsak *et al* (39) have shown that *in vivo* tumor proliferation in immunodeficient mice xenotransplanted with a human melanoma cell line was diminished by CIM (50 mg/kg/day), if combined with a tamoxifen derivate acting on cytochrome P450 molecules. This suggests again that the effect of CIM cannot be restricted to an H2 receptor blocker alone. The anticancer actions of CIM might not be mediated via histamine antagonist only. Therefore, the mechanisms of action by which CIM prolongs the survival of patients with various forms of cancer remain to be clarified and are probably multifactorial. The inhibitory effect of CIM on tumor-associated angiogenesis (25,38) is developed below.

Cell-mediated immunomodulation. Many tumors, and particularly colorectal and breast cancer, secrete histamine, a process that results in high histamine levels within the tumors (13,40). Moreover, histamine is also frequently secreted in response to the surgical resection of colorectal cancers (40). All these factors working together create an immunosuppressive environment both in the area of tumor growth and in the whole body, and in so doing they facilitate tumor growth. A number of clinical studies have shown that the administration of CIM may help in reducing the immunosuppression due to increased histamine levels in a tumor's environment (11,41). Adams and Morris (7) first desribed that pre-operative treatment with CIM (800 mg/day) significantly increased the proportion of colorectal cancers that elicited a lymphocyte response, and that the presence of tumor-infiltrating lymphocytes was associated with a survival advantage. In a pilot study, they showed that CIM enhanced the lymphocyte infiltration of human colorectal carcinomas (10). Forty-two patients scheduled for the elective resection of colorectal carcinomas were randomized either to receive CIM for one week preoperatively, or to act as control (10). A positive lymphocyte response was observed in 10 of 18 CIM-treated carcinoma patients compared with only 5 of the 24 control patients ($p=0.03$) (10). Moreover, the presence of a lymphocyte response correlated with improved survival (10). Gastric cancer patients also have higher levels of suppressor lymphocyte activity when compared to normal controls, and these levels are restored to normal with CIM treatment (42). In a controlled randomized clinical trial, Lin *et al* (43) recently showed that pre-operative CIM administration at the dose of 400 mg/day promoted peripheral blood lymphocytes and tumor infiltrating lymphocytes in patients with gastrointestinal cancer.

The mechanisms proposed for the cell-mediated immunomodulation of CIM (Fig. 1) include the inhibition of suppressor T lymphocyte activity (26), stimulation of natural killer (NK) cell activity (27), an increase in interleukin-2 (IL-2) production in helper T lymphocytes (28), an increase in tumor inhibitory cytokines (35) and the enhancement of the host's anti-tumor cell-mediated immunity by improving the suppressed dendritic cell function in advanced cancer patients (44).

Takahashi *et al* (35) have demonstrated that: a) a daily injection of CIM suppressed tumor progression in mice after the syngeneic transplantation of CT-26 cells (a colon adenocarcinoma cell line); and b) decreased expression of TNF-α and INF-γ associated with the tumor development was restored following treatment with CIM. CIM dramatically increased IFN-γ production by human lymphocytes (Fig. 1) via a possibly histamine-independent (non-histamine receptor mediated) pathway, most likely through cytochrome P450 moieties (45). High concentrations of INF-γ resulted in the inhibition of cell proliferation by the direct stimulation of natural killer cells (Fig. 1) (45). The use of CIM also retarded the growth of human melanomas in a nude mouse model and prolonged the survival of the tumor-bearing mice by directly inhibiting the proliferation of tumor cells and indirectly promoting the infiltration of activated macrophages into the tumor site (39). It is also reported that H2RAs such as CIM can reverse the inhibition of the secretion of human interleukin-12 (IL-12) induced by histamine via H2 receptors expressed on monocytes (the precursors of dendritic cells) (Fig. 1) (46). While it remains unclear whether or not H2 receptors are expressed on dendritic cells, the effect of CIM on the antigen presenting ability of dendritic cells appears to increase because of CIM-specific actions (Fig. 1) (44). It also remains unclear whether or not the modulating effects of CIM on the dendritic cell function observed *in vitro* by Kubota and colleagues (44) have any clinically substantial meaning: the clinical effectiveness of CIM against gastrointestinal malignancies is considered to be due to the combined total of immunological and non-immunological actions.

CIM has been reported as having better cell-mediated immunomodulation than other H2RAs such as famotidine and ranitidine, and the differences between CIM and other H2RAs might be due to their structures and/or affinities to H2 receptors (22,36).

Immunologically based therapies for various types of cancers are improved by adjuvant CIM therapy (47). Interestingly enough, one study has reported that a small number of patients with metastatic renal cell carcinomas (5%) responded with long-term remission to CIM monotherapy (19). But, immunologically based therapies for renal cell carcinomas or disseminated malignant melanomas have usually been combined with CIM and the contributions of CIM have not been adequately controlled (17,20,48,49).

Inhibition of cancer cell migration and the development of liver metastasis. *In vitro* studies have demonstrated that CIM inhibits the adhesion of some breast (50) and colon (29) cancer cells to human umbilical cord cells, a process that is a crucial biological step in tumor neo-angiogenesis and, consequently, in tumor progression and metastasis. Tomita *et al* (38) have shown that CIM-induced angiogenesis inhibition suppresses the growth of colon cancer implants in syngeneic mice and is associated with a significant decrease in VEGF expression levels in tumor tissue and the serum of colon 38-bearing mice (Fig. 2). In the syngeneic murine colon cancer CMT93 model, CIM also significantly reduced the growth of the subcutaneously grafted tumor and neovascularization in the tumor (25). CIM at this dose had no effect on the *in vitro* proliferation of this cell line (25). The cancer cells' production of the vascular endothelial growth factor was not affected by CIM, whereas the vascular-like tube formation by endothelial cells *in vitro* was significantly impaired in the presence of CIM (Fig. 2) (25). Their findings suggest that CIM suppresses tumor growth, at least in part by inhibiting tumor-associated angiogenesis. One of the major classes of adhesion molecules present on the surface of endothelial cells includes selectins (51). The direct implication of P-selectin in endothelial cell migration has been reported previously (52) and we recently suggested a direct implication of E-selectin in human endothelial cell migration during tubulogenesis (53). Both E- and P-selectins are induced in endothelial cells by proangiogenic cytokines such as the tumor necrosis factor (TNF)-α or IL-1ß (51). Since Kobayashi *et al* (29) have shown that CIM prevented liver metastasis of colon cancer cells in nude mice by blocking the E-selectin expression on the endothelial cells, the anti-angiogenic effect of CIM could also be related to the decrease in E-selectin expression on endothelial cells and therefore to its anti-metastatic effect against carcinoma cells invading the liver (Figs. 2 and 3).

Kobayashi *et al* (29) have also shown that CIM (daily doses of 200 mg/kg) prevented liver metastasis of colon cancer cells in nude mice by blocking E-selectin expression on the endothelial cells, a ligand for sialyl Lewis antigens on tumor cells (Fig. 3). Epithelial cells detaching themselves from primary epithelial tumors (carcinomas) and migrating through the lymphatic or the blood vessels (Fig. 3) eventually colonize the liver due to the fact that epithelial cancer cells exhibiting Lewis antigens [involving CD15 with fucose moieties, i.e. fucosyl-N-acetyl-lactosamine (fucosyl-LacNAc)] on their surface are able to adhere to endothelial cells in liver sinusoids because of the presence of selectins (the ligands for Lewis antigens) in these endothelial liver cells (Fig. 3) (13,54-56). Kaji *et al* (54) and Khatib *et al* (55) showed that upon entry into the hepatic circulation, epithelial tumor cells can rapidly trigger a molecular cascade (involving interleukin-1 secretion by tumor cells) leading to the induction of E-selectin expression on the sinusoidal endothelium (Fig. 3). Khatib *et al* (55) thus suggested that E-selectin induction in liver sinusoids by carcinoma cells contributes to the liver-colonizing potential of carcinoma cells (Fig. 3). Again, these actions of CIM probably do not occur via the blocking of the histamine receptor because famotidine and ranitidine did not show any similar effect. CIM 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 with endothelial cells (13).

5. Cimetidine and malignant gliomas

Malignant gliomas are the most frequently encountered primary brain tumors in adults and children (57,58); these malignant gliomas include neoplasms of astrocytic (anaplastic astrocytomas and glioblastomas) and oligodendroglial (anaplastic oligodendrogliomas) lineages (59). The standard treatment for these malignant gliomas is typically surgery, followed by radiotherapy and chemotherapy (58,60-63). However, only those malignant gliomas that exhibit a loss of heterozygosity (LOH) of chromosomes 1p and 19q are chemoresponsive (64,65). Unfortunately, gliomas exhibiting 1p/19q LOH are mainly malignant oligodendrogliomas, i.e. a minor proportion of malignant gliomas (59,66). In other words, most malignant gliomas are of astrocytic origin, without 1p/19q LOH, and are therefore weakly sensitive to any type of chemotherapy if at all (58). Malignant gliomas are biologically heterogeneous and include sub-populations of proliferating and migrating cells (58,67,68). While certain intracellular signaling pathways specifically control cell proliferation and/or apoptosis, other intracellular signaling pathways control cell migration (58,68-71). For example, the CAS/Crk assembly serves as a 'molecular switch' for the induction of cell migration and appears to contribute to the invasive property of tumors (70). Moreover, accumulating evidence suggests invasive glioma cells associated with high levels of migration display a decreased proliferation rate and a relative resistance to apoptosis (57,58,68,70,71), a feature that may contribute to chemotherapy and radiotherapy resistance (71). It is these migrating glioma cells that renders dismal the prognosis associated with high-grade malignant gliomas (58,68). Because experimentally decreasing migration in apoptosis-resistant migrating tumor astrocytes restores sensitivity to apoptosis (58,68) and thus to pro-apoptotic drugs, it would be interesting to elaborate new therapeutic strategies targeting migrating glioma cells. Cell migration includes very complex cellular and molecular processes in which at least three independent but highly coordinated biological steps are involved, i.e.: a) cell adhesion to specific components of the extracellular matrix (ECM) (72-74); b) modifications to the organization of the actin cytoskeleton (75-77); and c) the secretion of proteases (78). Gene-expression profiling has implicated numerous genes involved in glioma cell migration, and many of these genes relate to cell adhesion molecules that directly interact with specific ECM components (79-84). Gladson has detailed the molecular nature of ECM in gliomas (85), the crucial roles of which have been emphasized for the first time by Rutka and colleagues (86,87) with respect to gliomas. Apart from integrins (85,88,89), galectins (75,90-92) also play a number of crucial roles in glioma cell migration. While integrins employ protein-protein interactions with ECM components, galectins use protein-carbohydrate interactions between themselves and ECM glycoproteins, with the core of carbohydrate ligands for the galectins being represented by LacNAc moieties, i.e. Lewis antigens without fucosylation (58). We have shown that the interactions between the oligosaccharide moieties present in the glioma ECM and cell adhesion molecules present on the surface of glioma cells play a number of major roles in glioma cell migration (75,90-93). Among these oligosaccharide moieties that play a number of major roles in glioma cell migration are fucose and lactose (75,85,90-92,94).

One major target in the fight against glioma cell migration is connected with the successful decrease in protease expression by glioma cells (78). Another major target involves adhesion molecules and their ligand in the extracellular matrix. By example, tenascin, an integrin ligand, is overexpressed in the extracellular matrix of malignant gliomas when compared to low-grade gliomas and normal brain parenchyma (85), and clinical applications serve to specifically combat this particular feature of glioma cell migration (95).

Complementary to conventional chemotherapy, CIM has been used successfully to inhibit cancer cell migration of epithelial origins (carcinomas) towards the liver (13,29). It should be remembered that metastatic implantation of epithelial cancers in the liver involves cancer cell-mediated oligosaccharide moiety (the fucose moiety present on Lewis antigens) interactions with cell adhesion molecules (selectins) present in liver microvasculatures (13,29,56). In view of the fact that levels of expression of fucose binding activities in malignant gliomas differ in relation to the levels of malignancy (91) and that these receptor types could influence the levels of proliferation of human glioma cells (93), we postulated that addition of CIM to temozolomide treatment would improve survival of human glioblastoma orthotopic xenograft-bearing immunodeficient mice when compared to temozolomide therapy alone. We chose the human U373 model because it is of astrocytic origin, devoid of 1p/19q LOH and weakly sensitive to temozolomide (96), and the rat 9L sarcoma model because of its diffuse invasive abilities with respect to the brain parenchyma (97). We observed that combining CIM

with temozolomide improved survival of the U373 orthotopic xenograft-bearing nude mice (21). However, human glioblastoma U373 cells do not express H2 receptors (98), an observation which again argues against the possibility of H2 receptors on tumor cells playing a role in the CIM-induced effects.

In vitro colorimetric MTT-based assay have revealed that cimetidine significantly decreased growth of both human U373 glioblastoma and rat 9L gliosarcoma cells at concentrations ≥100 μM (21). Van der Ven and colleagues (99) and Finn and colleagues (100) had previously tested the growth-modulating effects of CIM on glioma cultures derived from human brain tumors. They observed that high dose (1 mM) CIM induced inhibition of *in vitro* proliferation of gliomas, while lower concentrations (1 μM) were less effective (99,100). We observed that *in vitro* 0.1-1 μM CIM significantly decreased migration of both U373 and 9L brain tumor cells (21). We also demonstrated that 30 daily intraperitoneal injections of 30 mg/kg CIM markedly decreased the percentage of 9L tumor cells exhibiting endogenous receptors for fucose moieties and the concentration of endogenous receptors for fucose moieties in 9L tumor cells (21). This CIM-mediated decrease in endogenous receptors for fucose moieties could partly explain the cimetidine-induced decrease in 9L (and also U373) tumor cell migration and, in turn, the *in vivo* benefit of adding cimetidine to temozolomide.

Fucose-containing glycans with potential clinical applications are hypothesized to combat the development of malignant gliomas. Indeed, it has long been known that under normal circumstances, the astrocyte number is kept constant in the mammalian central nervous system during adulthood and old age, as a result of the balance of division promoters and division inhibitors (101). Moreover, Nieto-Sampedro (102) identified the mitogen inhibitors as immunologically related to blood group oligosaccharides (i.e. Lewis antigen-related structures) and to glycan epitopes of the epidermal growth factor receptor. On the basis of these data, Aguilera *et al* (103) synthesized a family of oligosaccharides with a common Lewis-X-type structure, i.e. fucosyl-LacNAc-related structures, and these compounds are the source of a significant level of antiproliferative activity against malignant glioblastoma cells (104). Our recent study also revealed that CIM significantly decreased the expression of endogenous receptors for LacNAc moieties (21), knowing that such endogenous ligands involve, for example, different types of galectins whose levels of expression can be modulated by anti-inflammatory compounds (105-107). We defined the role played by galectin-1 on glioma cell migration features (75,90). Thus, this CIM-induced decrease in endogenous ligands for LacNAc (and maybe galectin-1) can act synergistically with the CIM-induced decrease in endogenous receptors for fucose on both 9L and U373 tumor cell migration levels and on the benefit *in vivo* of adding CIM to temozolomide.

6. Conclusions

Cimetidine is a histamine receptor-type H2 blocker whose clinical usefulness was clearly demonstrated several decades ago in the treatment of peptic ulcer disease. More recently, cimetidine has been proven to be a useful adjunct in colon cancer chemotherapy because it delays the formation of liver metastasis. Cimetidine also displays anti-tumor effects in gastric and renal carcinomas, and in melanomas. Cimetidine can also act as an immunomodulator by enhancing the host's immune response to tumor cells. We have recently shown that combining CIM with temozolomide improved survival when compared to temozolomide alone in human glioblastoma orthotopic xenograft-bearing nude mice. As reviewed in the present report, various mechanisms of action can be associated with the beneficial therapeutic effects contributed by cimetidine in the case of experimental glioblastomas, a fact that should encourage clinical investigators to enter highly malignant gliomas to cimetidine-related clinical trials.

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