ACCELERATED COMMUNICATION

Cimetidine Induces Interleukin-18 Production through H2-Agonist Activity in Monocytes

Hideo Kohka Takahashi, Takeshi Watanabe, Akira Yokoyama, Hiromi Iwagaki, Tadashi Yoshino, Noriaki Tanaka, and Masahiro Nishibori

Departments of Pharmacology (H.K.T., A.Y., M.N.), Gastroenterological Surgery, Transplant, and Surgical Oncology (H.I., N.T.), and Pathology (T.Y.), Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; and RIKEN Yokohama Institute Yokohama Research Promotion Division, Yokohama, Kanagawa, Japan (T.W.)

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ABSTRACT

The present study demonstrates a possible mechanism for the improvement of gastrointestinal cancer patients' prognosis by the histamine receptor type 2 (H2R) antagonist cimetidine. This agent, but not the H2R antagonists ranitidine and famotidine, induced the production of an antitumor cytokine, interleukin 18 (IL-18), by human monocytes and dendritic cells (DC). In fact, ranitidine and famotidine antagonized cimetidine-induced IL-18 production. Cimetidine induced the activation of caspase-1, which is reported to modify immature IL-18 to mature/active IL-18, and the elevation of intracellular cAMP, leading to the activation of protein kinase A (PKA). The PKA inhibitor H89 abolished the IL-18 production induced by cimetidine. Moreover, the effects of cimetidine on IL-18 production were reproduced in peripheral blood mononuclear cells from wild-type mice, but not in those from H2R knockout mice. In conclusion, cimetidine, a partial agonist for H2R, has a pharmacological profile different from ranitidine and famotidine, possibly contributing to its antitumor activity on gastrointestinal cancers.

Postoperative administration of cimetidine improves survival in patients with gastrointestinal cancer (Tonnesen et al., 1988). This action may be due to a direct inhibitory effect on tumor growth (Adams and Morris, 1994), cell-mediated immunomodulation (Hellstrand and Hermodsson, 1986; Gifford and Tirberg, 1987), or inhibition of cancer cell metastases (Tomita et al., 2003). The cell-mediated immunomodulation includes inhibition of suppressor T cells (Hellstrand and Hermodsson, 1986), stimulation of natural killer cells, and increase in IL-2 production in T cells (Gifford and Tirberg, 1987). The increase of histamine release is reported to represent the underlying cause for immunosuppression observed at the time of colonic resection; such an effect exerted by histamine can be prevented by perioperative cimetidine (Adams and Morris, 1994). However, such beneficial effects using other H2R antagonists (i.e., famotidine and ranitidine), have not been observed in clinical trials (Matsumoto, 1995). Cimetidine treatment inhibits histamine-initiated angiogenesis via reducing vascular endothelial growth factor expression (Gifford and Tirberg, 1987). The activation state of intratumoral DC is a critical factor in the host response to tumors (Furumoto et al., 2004). Cimetidine-induced higher antigen presenting capacity of DC was observed in patients with advanced cancer compared with healthy control subjects (Kubota et al., 2002).

IL-18, a monocyte-derived cytokine that requires cleavage with caspase-1 for activity (Gu et al., 1997), enhances local antitumor immune responses through activating natural killer cells and T cells (Kohno et al., 1997). IL-18 inhibits angiogenesis (Coughlin et al., 1998) and induces apoptosis in tumor cells (Hashimoto et al., 1999). In the mouse colon cancer model, IL-18 inhibits growth of cells (Tamura et al., 2003), and successful prevention of colon cancer establishment is associated with elevation of serum IL-18 level (Goto et al., 2002).

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ABBREVIATIONS: IL, interleukin; H2R, histamine receptor type2; H89, N-[2-(4-bromocinnamylamino)ethyl]-S-isoquinoline; PBMC, peripheral blood mononuclear cell; DC, dendritic cell; Z-YVAD-FMK, N-benzyloxy carbonyl-Tyr-Val-Ala-Asp-fluoromethyl ketone; PKA, protein kinase A.
In the present study, cimetidine behaved as a partial agonist for H2R in inducing IL-18 production in monocytes and DC derived from PBMC.

Materials and Methods
Reagents and Drugs. Recombinant human IL-18 and N-benzyl-
oxy carbonyl-Tyr-Val-Ala-Asp-fluoromethyl ketone (Z-YVAD-FMK) were purchased from MBL (Nagoya, Japan). Histamine dihydrochlo-
ride and cimetidine were purchased from Nakalai Tesque, Inc.
(Kyoto, Japan) and Sigma Chemical Co. (St. Louis, MO). Ranitidine and famotidine were provided by Glaxo Japan (Tokyo, Japan) and Yamanouchi Pharmaceutical Co. Ltd. (Tokyo, Japan). H89 was pur-
chased from Calbiochem (Darmstadt, Germany).
Preparation of Human and Murine Cells. Normal human
PBMC were obtained from peripheral blood of 10 volunteers after
acquiring institutional review board approval (Okayama University;
IRB 279) as described previously (Kohka et al., 2000). Separation of
monocytes from PBMC was carried out as described previously (Ta-
kahashi et al., 2003). The purity of monocytes was 85% as deter-
mined by flow cytometry with anti-CD14 antibody. DC were pre-
pared from PBMC as described previously (Kubo et al., 2004). The
resultant DC showed CD1a(+/CD14(−)/HLA-DR(+)/CD83(+)) pheno-
type, consistent with the previous report (Kubo et al., 2004). PBMC
and spleen cells were obtained from five wild-type or H2R knockout
mice as described previously (Yokoyama et al., 2004). We abided by
the guidelines on animal experimentation of Okayama University
Graduate School of Medicine and Dentistry, and the institutional
animal experimentation review committee licensed all procedures.
Cytokine Assays. IL-18 in cell-free supernatants was measured by
enzyme-linked immunosorbent assay kit (for human and mouse
IL-18; MBL) as described previously (Kohka et al., 2000; Takahashi
et al., 2003). The detection limit of the enzyme-linked immunosor-
bitant assay was 10 pg/ml.
Activity of Caspase-1. The activity of caspase-1 was determined
in a colorimetric assay with a substrate (WEHD-pNA) specific for
this enzyme (R&D Systems, Inc., Minneapolis, MN). After 1-h incu-
bation, monocytes were pelleted by centrifuging (1000
× g, 5 min),
the supernatant was aspirated, and the cells were lysed in accor-
dance with the manufacturer’s instructions. Cell lystate and the
initial supernatant were analyzed for the activity of cell-bound and
released caspase-1, respectively.

As shown in Fig. 1A, the effects of histamine and cimeti-
dine at concentrations ranging from 10 nM to 1 mM on IL-18
production were determined in human PBMC. Histamine
concentration-dependently induced the IL-18 production,
and the effect of histamine was maximal at the concentration
of 100 μM. Using the same preparation, cimetidine concen-
tration-dependently induced the IL-18 production exhibiting
35% agonist activity compared with histamine. Cimetidine
also induced the production of IL-18 in monocytes and DC as
well as caspase-1 activation in monocytes (Fig. 1, B and D). A
caspase-1 inhibitor, Z-YVAD-FMK, prevented this cimetidine-
initiated IL-18 production (Fig. 1E), suggesting that
caspase-1 activation might be involved in the effect of cimeti-
dine. The level of IL-18 production in monocytes and DC
induced by cimetidine at 100 μM was one third of that seen
with histamine at 100 μM. The effect of histamine on IL-18
production was reported to be mediated solely by H2R stim-
ulation (Kohka et al., 2000). The concentration range of cimetidine
has been used for assessing the H2R antagonistic activity of cimetidine on different tissue preparations including
stomach and atrium. Other H2R antagonists (i.e., famo-
tidine and ranitidine) had no effect on the production of IL-18
(Fig. 1B). Cimetidine at 100 μM induced the production of IL-18 in the presence of histamine at 0.01 μM; however, the
same concentration of cimetidine inhibited the production of
IL-18 induced by histamine at 1 and 100 μM (Fig. 1C).
Therefore, cimetidine may act as a partial agonist in the

Results and Discussion
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Fig. 1. The effect of cimetidine on IL-18 production and
caspase-1 activity in human monocytes and DC. A, PBMC at 1 × 106 cells/ml were treated with histamine
(HA; ○) or cimetidine (CIM; □) at concentrations rang-
ing from 10 nM to 1 mM for 24 h. B, monocytes at 1 × 106 cells/ml were treated with CIM, famotidine (FAM),
ranitidine (RAN), or brimamide (BRI) at 1 or 100 μM,
and DC at 1 × 106 cells/ml were treated with CIM or HA
for 24 h. ND, not detected. **, P < 0.01 compared with
the corresponding value for medium alone. C, monocytes
at 1 × 106 cells/ml were treated with CIM at 100 μM in
the presence of HA at 0.01, 1 or 100 μM for 24 h. **, P <
0.01 compared with the corresponding value for medium
alone. **, P < 0.01 compared with the corresponding
value for histamine. D, activity of caspase-1. Monocytes
at 2 × 105 cells/ml were treated with cimetidine and
histamine at 1 or 100 μM for 1 h. **, P < 0.01 compared
with the corresponding value for medium alone. E, monocytes
at 1 × 106 cells/ml were treated with cimetidine or
histamine at 100 μM in the presence or absence of Z-
YVAD-FMK at 100 μM for 24 h. **, P < 0.01 compared
with the corresponding value in the presence of histamine or cimetidine alone.
When an error bar was within a symbol, the bar was omitted.

Burimamide is reported to be a partial H2R agonist. In the present study, we tested whether cimetidine exerted its effect by acting as an agonist for H2R stimulation.

The H2R antagonists famotidine and ranitidine antagonized the effect of cimetidine on IL-18 production in monocytes (Fig. 2, A and B). The maximal inhibitory effect of both famotidine and ranitidine was 70%. H2R stimulation is known to induce intracellular activation of cAMP/PKA pathway (Shayo et al., 1997; van der Pouw Kraan et al., 1998). Cimetidine as well as histamine induced the elevation of cAMP (Fig. 2C); however, famotidine and ranitidine had no effect (data not shown). The maximal effect of cimetidine on cAMP elevation was one third of that obtained by histamine.

As shown in Fig. 2D, the PKA inhibitor H89 partially inhibited the cimetidine- and histamine-induced IL-18 production by 56% and 58%, respectively. These results suggested that the cAMP/PKA pathway is partially involved in the action of cimetidine.

In addition, we examined the effect of cimetidine and histamine on the production of IL-18 by spleen cells and PBMC from H2R knockout mice (Fig. 2, E and F). Cimetidine and histamine induced the production of IL-18 by the cells from wild-type mice but not from H2R knockout mice. Taken together, the present findings indicate that cimetidine stimulated H2R as a partial agonist. Burimamide is reported to be a partial H2R agonist at the recombinant human H2R (Alewijnse et al., 1998); however, burimamide had no effect on the production of IL-18 by human monocytes (Fig. 1B). On the other hand, cimetidine was classified as an inverse agonist using the recombinant human H2R transfected into Chinese hamster ovary cells (Alewijnse et al., 1998). Thus, the pharmacological profile of H2R antagonists may differ depending on the receptor expression cells.

In conclusion, cimetidine induces the production of IL-18 in monocytes via H2R, and this may provide insights into mechanisms underlying the improvement of the prognoses of patients with colon cancer as a result of cimetidine treatment.

References


Address correspondence to: Dr. Masahiro Nishibori, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8558, Japan. E-mail: mbori@md.okayama-u.ac.jp