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ACCELERATED COMMUNICATION

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

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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 (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]-5-isoquinoline; PBMC, peripheral blood mononuclear cell; DC, dendritic cell; Z-YVAD-FMK, *N*-benzyloxycarbonyl-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*-benzyloxycarbonyl-Tyr-Val-Ala-Asp-fluoromethyl ketone (Z-YVAD-FMK) were purchased from MBL (Nagoya, Japan). Histamine dihydrochloride 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 purchased 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 (Takahashi et al., 2003). The purity of monocytes was 85% as determined by flow cytometry with anti-CD14 antibody. DC were prepared from PBMC as described previously (Kubo et al., 2004). The resultant DC showed CD1a(+)CD14(−)HLA-DR(+)CD83(−) phenotype, 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 immunosorbent 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 incubation, monocytes were pelleted by centrifuging (1000*g*; 4°C, 5 min), the supernatant was aspirated, and the cells were lysed in accordance with the manufacturer's instructions. Cell lysate and the initial supernatant were analyzed for the activity of cell-bound and released caspase-1, respectively.

Assay of cAMP. After a 30-min incubation, monocytes at 2×10^5 cells/200 μl/well were supplemented and assayed for cAMP using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI), consistent with the previous report (Kubo et al., 2004). We performed no acetylation procedures.

Statistical Examination. The statistical significance of differences was evaluated by analysis of variance followed by Dunnett's test. The results are presented as the means ± S.E.M. of triplicate findings from five donors. A probability value (P) less than 0.05 was considered significant.

Results and Discussion

As shown in Fig. 1A, the effects of histamine and cimetidine 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 concentration-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 cimetidine. 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 stimulation (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., famotidine 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

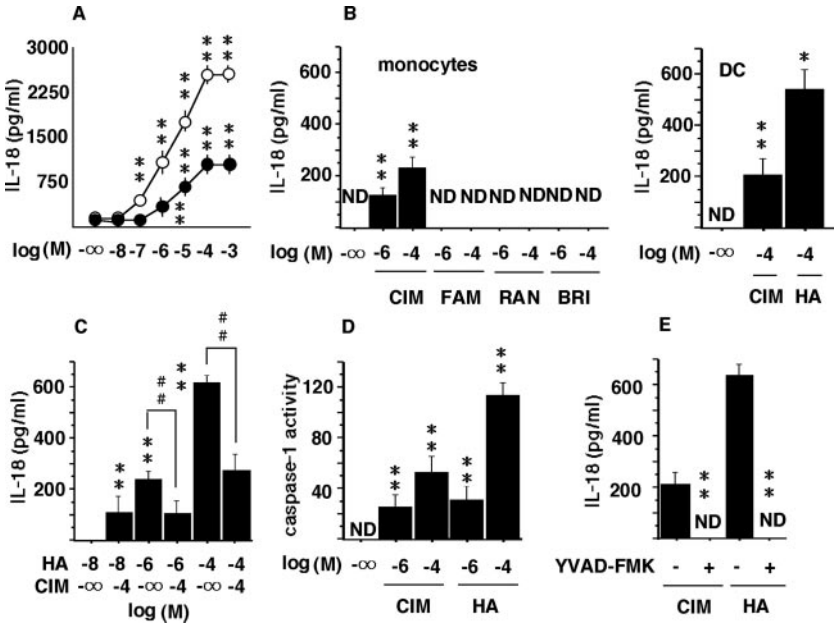

Fig. 1. The effect of cimetidine on IL-18 production and caspase-1 activity in human monocytes and DC. A, PBMC at 1×10^6 cells/ml were treated with histamine (HA; ○) or cimetidine (CIM; ●) at concentrations ranging from 10 nM to 1 mM for 24 h. B, monocytes at 1×10^6 cells/ml were treated with CIM, famotidine (FAM), ranitidine (RAN), or brimamide (BRI) at 1 or 100 μM, and DC at 1×10^6 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 1×10^6 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 2×10^6 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 1×10^6 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 cimetidine or histamine alone.

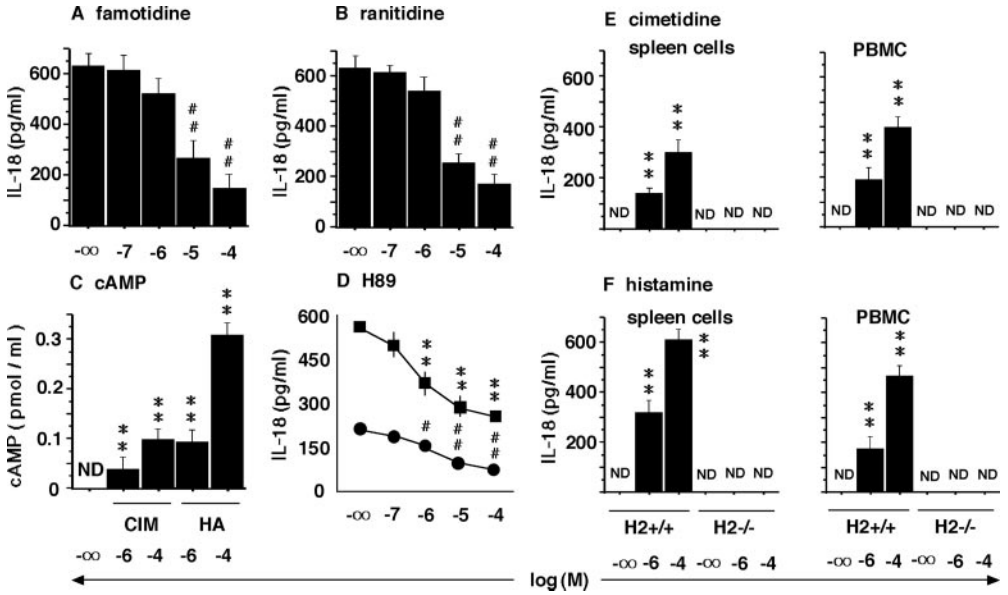

Fig. 2. The involvement of H2-receptor in the effect of cimetidine on IL-18 production in monocytes. A and B, human monocytes at 1×10^6 cells/ml were treated with famotidine (A) and ranitidine (B) at concentrations ranging from 0.1 to 100 μM in the presence of cimetidine at 100 μM for 24 h. ##, $P < 0.01$ compared with the corresponding value for cimetidine alone. C, assay of cAMP. Human monocytes at 1×10^6 cells/ml were treated with cimetidine or histamine at 1 and 100 μM for 30 min. **, $P < 0.01$ compared with the corresponding value in medium alone. D, human monocytes at 1×10^6 cells/ml were treated with the PKA inhibitor H89 at concentrations ranging from 0.1 to 100 μM in the presence of cimetidine (●) or histamine (■) at 100 μM for 24 h. **, $P < 0.01$ compared with the corresponding value for histamine alone. #, $P < 0.05$; ##, $P < 0.01$ compared with the corresponding value for cimetidine alone. E and F, spleen cells or PBMC from wild-type or H2-receptor knockout mice at 1×10^6 cells/ml were treated with cimetidine (E) or histamine (F) at 1 and 100 μM for 24 h. **, $P < 0.01$ compared with the corresponding value in medium alone. ND, not detected. When an error bar was within a symbol, the bar was omitted.

presence of histamine at 0.01 μM, whereas it may act as an antagonist in the presence of histamine at 1 and 100 μM. The amount of histamine in the conditioned media of monocytes treated with cimetidine was the same as control cells without cimetidine. Moreover, histamine at the concentration present in such conditioned media did not have any effect on cytokine production. These results indicate that the effect of cimetidine was not mediated via antagonism of histamine action on H2R and that cimetidine may behave as an agonist when the concentration of histamine is low. This finding prompted us to test 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 mechanism underlying the improvement of the prognoses of patients with colon cancer as a result of cimetidine treatment.

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