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### **Cimetidine induces IL-18 production through H<sub>2</sub>-agonist activity in monocytes**

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**Abstract:** 159

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**d) Abbreviations:** cyclic adenosine monophosphate;cAMP, dendritic cells;DC, histamine receptor type2;H2R, interleukin;IL, interferon;IFN, nicotinic acetylcholine receptor  $\alpha$ 7 subunit; $\alpha$ 7-nAChR, natural killer;NK, peripheral blood mononuclear cell;PBMC, prostaglandinE2;PGE2, protein kinase A;PKA, tumor necrosis factor;TNF, Z-tyr-Val-Ala-Asp-fluoromethyl ketone;YVAD-FMK

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### **Abstract**

The present study demonstrated possible mechanism for the improvement of gastrointestinal cancer patients' prognosis by a histamine receptor type2 (H2R) antagonist, cimetidine. This agent, but not the H2R antagonists, ranitidine and famotidine, induced the production of an anti-tumor cytokine, interleukin (IL)-18 by human monocytes and dendritic cells (DC). In fact, cimetidine-induced IL-18 production was antagonized by ranitidine and famotidine. 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 cyclic adenosine monophosphate (cAMP), leading to the activation of protein kinase A (PKA). A 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 (PBMC) from wild 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 anti-tumor activity on gastrointestinal cancers.

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Postoperative administration of cimetidine improves survival in gastrointestinal cancer patients (Tonnesen et al., 1988). The mechanisms of this action might 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-cell activity (Hellstrand and Hermodsson, 1986), stimulation of NK-cell activity and increase in IL-2 production in T-cells (Gifford and Tirberg, 1987). It is reported that the increase of histamine release represents the underlying cause for immunosuppression observed at the time of colonic resection, and that such an effect exerted by histamine can be prevented by perioperative cimetidine (Adams and Morris, 1994). However, such beneficial effects using other H<sub>2</sub>R antagonists, 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 advanced cancer patients compared to normal controls (Kubota et al., 2002).

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IL-18, a monocyte-derived cytokine that requires cleavage with caspase-1 for activity (Gu et al., 1997), enhances local anti-tumor immune responses through activating NK-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).

The present study demonstrated that cimetidine behaved as a partial agonist for H2R in inducing IL-18 production in monocytes and DC derived from PBMC.

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## Materials and Methods

### *Reagents and drugs*

Recombinant human IL-18 and Z-tyr-Val-Ala-Asp-fluoromethyl ketone (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 ten volunteers after acquiring IRB approval (Okayama Univ. IRB No.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-CD 14 Ab. DC were prepared from PBMC as previously described (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 or H2R knockout mice as described previously

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(Yokoyama et al., 2004). We abided by the guidelines on animal experimentation of Okayama University Graduate School of Medicine and Dentistry, and all procedures were licensed by the institutional animal experimentation review committee.

#### *Cytokine assays*

IL-18 in cell-free supernatants was measured by ELISA kit (for human and mouse IL-18, MBL) as described previously (Kohka et al., 2000; Takahashi et al., 2003). The detection limit of the ELISA 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.). After 1 h incubation, monocytes were pelleted by centrifuging (1,000x 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.

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*Assay of cAMP*

After 30 min incubation, monocytes at  $2 \times 10^5$  cells/200  $\mu$ l/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 ANOVA followed by Dunnet's test. The results are the means  $\pm$  S.E.M. of triplicate findings from five donors. A probability value less than 0.05 was considered significant.



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## 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  $\mu$ M. 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. 1B and ID). A caspase-1 inhibitor, 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  $\mu$ M was one third of that seen with histamine at 100  $\mu$ M. It is reported that the effect of histamine on IL-18 production is mediated solely by H<sub>2</sub>R stimulation (Kohka et al., 2000). The concentration range of cimetidine has been used for assessing the H<sub>2</sub>R antagonistic activity of cimetidine on different tissue preparations including stomach and atrium. Other H<sub>2</sub>R antagonists, famotidine and ranitidine had no effect on the production of IL-18 (Fig. 1B). Cimetidine at 100  $\mu$ M induced the production of IL-18 in the presence of histamine at 0.01  $\mu$ M, however, the same concentration of cimetidine

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inhibited the production of IL-18 induced by histamine at 1 and 100  $\mu\text{M}$  (Fig. 1C). Therefore, cimetidine may act as a partial agonist in the presence of histamine at 0.01  $\mu\text{M}$ , whereas it may act as an antagonist in the presence of histamine at 1 and 100  $\mu\text{M}$ . The amount of histamine in the conditioned media of monocytes treated with cimetidine was the same as controls 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 H<sub>2</sub>R, 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 H<sub>2</sub>R stimulation.

The H<sub>2</sub>R antagonists, famotidine and ranitidine antagonized the effect of cimetidine on IL-18 production in monocytes (Figs. 2A and 2B). The inhibitory effects of famotidine and ranitidine were both maximally 70 %. H<sub>2</sub>R 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

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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 (Figs. 2E and 2F). Cimetidine and histamine induced the production of IL-18 by the cells from wild mice, but not from H2R knockout mice. Taken all 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 (Alewijse 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 (Alewijse 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 colon cancer patients' prognosis by cimetidine treatment.

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### Footnotes

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### Legends for Figures

**Fig.1** The effect of cimetidine on IL-18 production and caspase-1 activity in human monocytes and DC

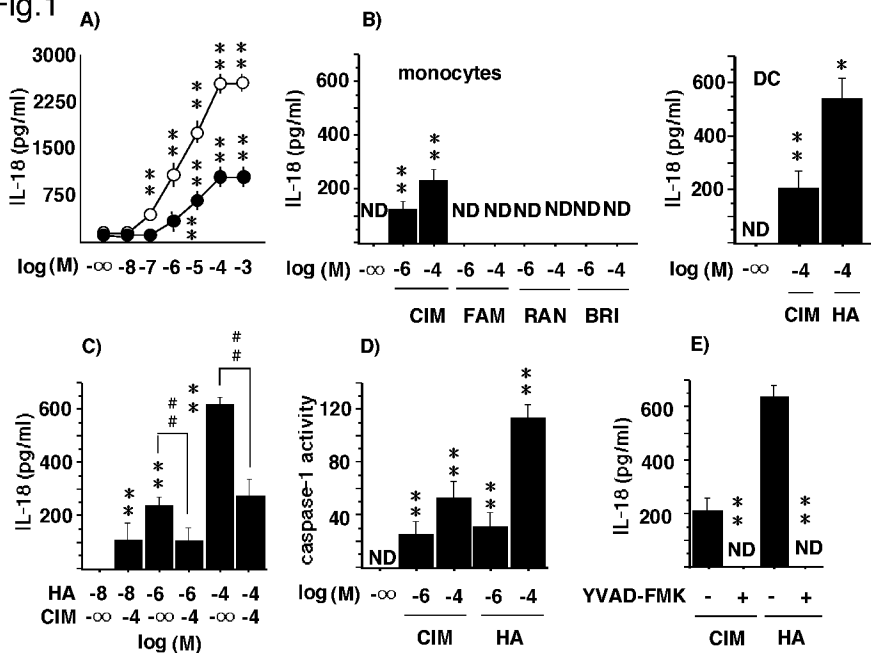
(A) PBMC at  $1 \times 10^6$  cells/ml were treated with histamine (HA) (open circles) or cimetidine (CIM) (filled circles) at concentrations ranging from 10 nM to 1 mM for 24 h. (B) Monocytes at  $1 \times 10^6$  cells/ml were treated with CIM, famotidine (FAM), ranitidine (RAN) or brimamide (BRI) at 1 or 100  $\mu$ M, and DC at  $1 \times 10^6$  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  $1 \times 10^6$  cells/ml were treated with CIM at 100  $\mu$ M in the presence of HA at 0.1, 1 or 100  $\mu$ M 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  $2 \times 10^6$  cells/ml were treated with cimetidine and histamine at 1 or 100  $\mu$ M for 1h. \*\* $P < 0.01$  compared with the corresponding value for medium alone. (E) Monocytes at  $1 \times 10^6$  cells/ml were treated with cimetidine or histamine at 100  $\mu$ M in the presence or absence of YVAD-FMK at 100  $\mu$ M for 24 h. \*\* $P < 0.01$  compared with the corresponding value in the presence of cimetidine or histamine alone.

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**Fig. 2** The involvement of H<sub>2</sub>-receptor in the effect of cimetidine on IL-18 production in monocytes

(A)(B) Human monocytes at  $1 \times 10^6$  cells/ml were treated with famotidine (A) and ranitidine (B) at concentrations ranging from 0.1 to 100  $\mu$ M in the presence of cimetidine at 100  $\mu$ M for 24 h. ##P<0.01 compared with the corresponding value for cimetidine alone. (C) Assay of cAMP. Human monocytes at  $1 \times 10^6$  cells/ml were treated with cimetidine or histamine at 1 and 100  $\mu$ M for 30 min. \*\*P<0.01 compared with the corresponding value in medium alone. (D) Human monocytes at  $1 \times 10^6$  cells/ml were treated with a PKA inhibitor, H89 at concentrations ranging from 0.1 to 100  $\mu$ M in the presence of cimetidine (filled circles) or histamine (filled squares) at 100  $\mu$ M 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. When an error bar was within a symbol, the bar was omitted. (E)(F) Spleen cells or PBMC from wild-type or H<sub>2</sub>-receptor knockout mice at  $1 \times 10^6$  cells/ml were treated with cimetidine (E) or histamine (F) at 1 and 100  $\mu$ M for 24 h. \*\*P<0.01 compared with the corresponding value in medium alone. ND, not detected.

Fig.1



**Fig.2**

