The Effect of Cimetidine on Survival of Mice Infected with Herpes Simplex Virus Type 2, Murine Encephalomyelitis Virus and Vesicular Stomatitis Virus Infections

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Summary: The effect of cimetidine on survival was investigated in mice infected with herpes simplex virus type 2 (HSV-2), murine encephalomyelitis virus (GD-VII), and vesicular stomatitis virus (VSV). BALB/c mice, 5 weeks of age, were injected intraperitoneally (i.p.) with \(5.5 \times 10^5\) plaque-forming units (PFU) of virus/0.5ml, and cimetidine (1mg/0.5ml) was administered simultaneously. The survival rates of 80% and 85% in the cimetidine groups were significantly greater than the 10% and 23% for the control groups. The GD-VII- and VSV-infected control mice were dead at 3 days after virus inoculation. However, more cimetidine-treated mice survived than control mice. When anti-mouse T-cell serum or cyclosporine, which is a helper T-cell suppressor, was administered to BALB/c mice; the effect of cimetidine against the HSV-2 infection could be observed. When injected with anti-asialo GM1, BALB/c mice or beige mice with low natural killer (NK) cell activity were not affected by cimetidine. Lastly, cimetidine was shown to activate the cytotoxic action on NK cells. The above results indicate that the antiviral effects of cimetidine depend on NK cell activation.

Key words: cimetidine — NK cell activity — beige mouse — herpes simplex virus — encephalomyelitis virus — vesicular stomatitis virus

Introduction

Cimetidine (Tagamet) is a potent H2-receptor antagonist known for its efficient inhibition of gastric acid secretion from the parietal cells of the stomach. It is very useful for the treatment of peptic ulcerations. Many investigators have mentioned that the antiviral and anti-tumor effects of cimetidine depend on inhibition of the activity of suppressor T-cells (Osbond et al. 1981; Mavlight and Talpaz, 1984). However, in the present experiments, ranitidine, famotidine and roxatidine acetate which belong to the same group of histamine H2-receptor antagonists did not show any antiviral effects (Kabuta and Shingu, 1987). NK cells are defined phenotypically as large granular lymphocytes that express both the antigen CD16 (Leu-11) and NKH-1 (Leu-19). NK cells may be defined functionally as cells that mediate non-histocompatibility-restricted cytotoxicity against a variety of cell targets, including tumor and virus infected cells. NK cell activities in tumor-bearing mice or HSV-2 infected mice were decreased in comparison with healthy mice (Fitzgerald et al. 1985; Kikuchi et al. 1985). This paper describes the activation of NK cells by cimetidine.
Materials and Methods

Mice

Five week-old BALB/c and beige mice bred in the animal center of Kurume University were used.

Cells

African green monkey kidney (GMK) cells were used for virus growth and titration. YAC-1 cells, Moloney virus-induced lymphoma cells in A/Sn mice, were used as target cells for NK activity. Effector cells were isolated from the spleens of healthy mice. NK cells were separated by lymphoprep density sedimentation, and adherent cells were removed.

Virus

HSV-2 (strain UW-268), GD-VI and VSV were used. 5.5 x 10^5 PFU/0.5 ml of HSV-2 were inoculated, i.p., into the mice.

Drugs

Cimetidine (Tagamet) was obtained from Smith Kline & Fujisawa Co. Ltd., Tokyo. Cyclosporine was obtained from Sandoz Pharmaceuticals Corp.

Antiserum

Anti-asialo GM1 serum was purchased from Wako Pure Chemical Industries Ltd., and anti-mouse T-cell serum from Cedarlane Lab. Ltd. Five week-old BALB/c mice were injected intravenously (i.v.) with 0.1 ml of the anti-serum (0.2 mg proteins), twice with an interval of one day, starting on the same day as virus inoculation.

Cytotoxic assay

The target YAC-1 cells were labeled with ^{51}Cr. The cells were washed twice, and 10^4 target cells in 0.1 ml RPMI 1640 plus 0.1 ml of effector cells were added to the wells with varying ratios of effector to target cells. The cells were incubated at 37°C in a 5% CO_2 incubator for 4 hr.

NK cell activity is expressed as follows:

\[
\% \text{ } ^{51}\text{Cr release} = \frac{\text{mean cpm experimental} - \text{mean cpm spontaneous}}{\text{mean cpm maximal} - \text{mean cpm spontaneous}}
\]

where mean cpm spontaneous is ^{51}Cr release from each of the three wells by YAC-1 in the absence of NK cells, and mean cpm maximal is ^{51}Cr released by YAC-1 in the presence of 5% Triton X detergent.

Results

Fig. 1 indicates the survival ratios in the cimetidine and control groups. Five week-old BALB/c mice were injected, intraperitoneally, with 5.5 x 10^5 PFU/0.5 ml of HSV-2 or GD-VI or VSV and 0.5 mg cimetidine/0.5 ml, simultaneously. It was confirmed that cimetidine had no direct effect upon virus inactivation and growth (Kabuta and Shingu, 1987). Survival ratios of 80% and 85% in the cimetidine groups were significantly greater than the ratios of 10% and 23% observed in the control groups that received a placebo of phosphate buffered saline (PBS). With HSV-2, GD-VI or VSV, the injected mice

![Fig. 1](image-url)
CIMETIDINE AND VIRUS INFECTION

Fig. 2. The action of cimetidine against HSV-2 infections in BALB/c mice treated with anti-mouse T-cell serum or anti-asialo GM\(_1\) serum

Fig. 3. The effect of cimetidine against HSV-2 infections in BALB/c mice treated with cyclosporine

as well as the control mice died, 3 days after virus inoculation. However, the groups that received cimetidine survived longer than the control groups. This rapid effect of cimetidine against viral infection seems to involve the NK cells.

Fig. 2 shows the effect of cimetidine against the HSV-2 infection in BALB/c mice that were injected with anti-mouse T-serum or anti-asialo GM\(_1\) serum. It was quickly recognized that anti-asialo GM\(_1\) inhibits NK cell activity. The survival duration in mice injected with anti-asialo GM\(_1\) and cimetidine was shorter than in the control group. The survival duration in mice injected with anti-mouse T-cell serum was slightly longer in comparison with the control group. Survival ratios in mice injected with anti-mouse T-cell serum and cimetidine were higher in contrast with the anti-asialo GM\(_1\) group or the control group. These results indicate that a suppression of NK cells by anti-asialo GM\(_1\) is not affected by cimetidine, but a suppression of T-cells by anti-mouse T-cell serum is affected by cimetidine.
Fig. 4. The action of cimetidine against HSV-2 infections in 5 week-old beige mice after cyclosporine and cimetidine resembled that of the cimetidine group. A change of sensitivity due to the effect of cyclosporine as a T-cell suppressor was not observed.

These results could be explained if NK cells are activated by cimetidine. The next experiments were performed using beige mice with low NK cell activity. The action of cimetidine against HSV-2 infected beige mice is shown in Fig. 4. In beige mice, cimetidine conferred only a small amount of protection against the virus infection. Therefore, cytotoxic activation of NK cells by cimetidine was further investigated.

Fig. 5 shows that activation of NK cells in 5 week-old BALB/c mice was enhanced by cimetidine. The $^{51}$Cr release in control mice was 9.4%. The per cent lysis at 3 days after cimetidine injection was 43.5%. NK cell activity at 5 and 6 days after cimetidine injection reached peaks of 60.1 and 61.1%, respectively. In BALB/c mice injected with HSV-2 and cimetidine, the NK cell activity was increased. NK cell activity in 5 week-old beige mice was low and close to the NK cell activity in 3 week-old BALB/c mice. A detailed paper with respect to NK cell activity is in preparation.

Discussion

NK cells are large granular lymphocytes that spontaneously lyse certain tumor cells, virus-infected cells, and normal undifferentiated cells. NK cells are thought to participate in host defenses against malignancies and viral infections, and may also regulate humoral immunity and/or hematopoiesis. There are several modulators of NK activity. An important stimulator of NK activity is interferon, a compound which activates natural killing both in vitro and in vivo. Interleukin I and IL-2 augment NK activity. On the other hand, prostaglandins, molecular oxygen, and acute-phase proteins in the serum suppress NK activity. NK cells from both humans and mice have been shown to lyse a variety of RNA and DNA virus-infected cells, including among others, HSV, cytomegalovirus, measles virus, vesicular stomatitis virus, Epstein-Barr virus, and varicella zoster virus-infected targets.

The antiviral actions of cimetidine are not well established. The present data (Fig. 1) indicates that the effect of cimetidine appears rapidly. Therefore, a reaction related to antibodies can be excluded. Fig. 2 and 3 indicate that the in-
fluence of T-cells is negligible. In Fig. 4, a small effect of cimetidine seemed to depend on low NK cell activity in beige mice. As shown in Fig. 5, augmentation and activation of NK cells is initiated by cimetidine injection, and after 3 and 5 days NK cell activity rose to 4.6 and 6.4 times that seen in control mice. Therefore, the antiviral effects of cimetidine appear to depend on NK cell activation. These observations strongly indicate that cimetidine could be a new strategy for successful immunotherapy of viral diseases.

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