EFFECT OF UBENIMEX ON THE PRODUCTION OF MURINE INTERFERON

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Ubenimex is a low molecular immunomodifier exhibiting antitumor and antimicrobial activities through activation of host defense mechanisms[1~4]. Previously, we reported that forphenicinol which is also a low molecular weight immunomodifier[5] stimulated inducer-elicited production of cytokines such as tumor necrosis factor (TNF) and interferons (IFN)[6,7]. The effect of ubenimex on immune response in mice is different from that of forphenicinol. As for the effect on cytokine production, ubenimex induces interleukin 1 (IL-1) and enhances the concanavalin A (Con A)-induced production of IL-2[8]. However, the effect of ubenimex on IFN production has not been known. In this paper, we report the results of the studies on the effect of ubenimex on IFN production in mice and discuss the difference in the effects of ubenimex and forphenicinol.

ICR mice were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan) and maintained in a barrier system. Single cell suspension of splenocytes was obtained by mechanically dispersing spleens. Cells were cultured in RPMI 1640 medium supplemented with 10% calf serum at 37°C. Ubenimex was synthesized by Nippon Kayaku Co., Ltd. IFN assay was performed by means of inhibition of nucleic acid synthesis using mouse LY cells and vesicular stomatitis virus (VSV), and IFN activity was expressed as an international unit (IU)[6]. In the case of lipopolysaccharide (LPS, *Escherichia coli* 0111, Difco Lab., Detroit, MI, U.S.A.)-induced IFN production, though LPS might exist in serum samples, IFN activity in sera was assayed without removing LPS from the sera because LPS did not affect the LY cells at a concentration up to 100 μg/ml. Moreover, the sera taken from mice injected with LPS contain TNF as well as IFN. By using TNF-resistant LY cells, the interfering effect of TNF on IFN assay was excluded[7].

To determine whether ubenimex was an IFN inducer or not, ICR mice were orally administered 1 or 10 mg/kg ubenimex and bled 2, 4, or 24 hours after the administration. IFN activity in sera was determined. IFN activity was not detected in any sera taken from these mice (<10 IU/ml). Although ubenimex has a mitogenic activity against T cells[8,9], spleen cells exposed to ubenimex at 1 to 100 μg/ml for 24 or 48 hours did not produce IFN into the culture medium. However, when spleen cells of 1×10^7 cells/ml were cultured in the medium containing 20 μg/ml Con A (type IV, Sigma Chemical Co., St. Louis, MO, U.S.A.) for 24 hours they produced 659 IU/ml of IFN (Table 1). Ubenimex added to the culture enhanced the production at a wide concentration range.

The effect of ubenimex administration on the IFN production in mice injected with IFN inducers was examined. ICR mice (n=3) were sensitized by iv injection with 10^7 BCG cells (Japan BCG Manufacturing Co., Tokyo, Japan) on day 0 and were given ubenimex orally once a day daily for 4 days from day 10 to 13. On day 14, they were injected intravenously with 10^7 BCG cells or 50 μg of LPS. As shown in Table 2, 1 mg/kg of ubenimex increased the LPS-induced IFN production 5.8 times. On the contrary, it suppressed the BCG-induced IFN production at a dose of 10 mg/kg, and a

Table 1. Effect of ubenimex on Con A-induced interferon (IFN) production in spleen cell cultures.

Concentration of ubenimex (μg/ml)	IFN activity in culture fluids (IU/ml)
0	659
1	891
10	1,132
100	1,058

Spleen cells (1×10^7 cells/ml) were exposed to ubenimex in the presence of 20 μg/ml Con A for 24 hours. IFN activity was determined by means of inhibition of nucleic acid synthesis using LY cells and vesicular stomatitis virus.

Table 2. Effect of ubenimex on LPS- or BCG-induced interferon (IFN) production in mice sensitized with BCG.

Inducer	Ubenimex (mg/kg)	IFN activity in sera (IU/ml)
LPS	0	1,570
LPS	1	9,140
LPS	10	2,190
BCG	0	6,630
BCG	1	4,210
BCG	10	850

ICR mice ($n=7$) sensitized intravenously with 1×10^7 BCG cells on day 0 were given ubenimex orally once daily for 4 days from day 10 to 13.

On day 14, they were bled 2 hours after iv injection with 50 μg LPS or 10^7 BCG cells.

Table 3. Effect of ubenimex on poly(IC)- or LPS-induced interferon (IFN) production in mice.

Inducer	IFN activity (IU/ml) in sera from mice given			
	None	Ubenimex, po (mg/kg/day)		
		0.1	1	10
Poly (IC)	3,112	3,380	202	3,370
LPS	119	109	35	91

ICR mice ($n=3$) were given ubenimex once daily for 10 days and injected intravenously 10 μg poly-(IC) or 5 μg LPS 1 day after the last administration. They were bled 2 hours after the challenge.

Table 4. Effect of BCG sensitization on poly (IC)-induced interferon (IFN) production in mice.

Number of BCG cells	IFN activity (IU/ml) in sera		
	Poly (IC) (μg), iv		
	1	10	100
0	<10	6,510	21,180
10^5	<10	6,700	18,490
10^6	<10	10,190	34,890
10^7	<10	182	4,290

ICR mice ($n=3$) were sensitized intravenously with BCG cells 14 days before poly (IC) injection. They were bled 2 hours after the challenge.

dose of 1 mg/kg was ineffective. Effect of ubenimex on the LPS- or poly (IC)-induced IFN production in normal mice was also tested. ICR mice ($n=3$) were given ubenimex once a day daily for 10 days and injected with 5 μg of LPS or 10 μg of poly (IC) intravenously 1 day after the last administration. Mice were bled 2 hours after the challenge. In this case, oral administration of 1 mg/kg/day ubenimex markedly inhibited both of the IFN production induced by LPS and poly (IC) (Table 3), though ubenimex enhances immune responses at a wide dose range including 1 mg/kg/day.

To investigate its suppressive effect on poly-(IC)-induced IFN production, we tested the effect of BCG sensitization on the poly(IC)-induced IFN production apart from the subjected drugs. ICR mice ($n=3$) sensitized intravenously with graded numbers of BCG cells 14 days before were injected with 1, 10, or 100 μg poly(IC). BCG sensitization at 10^6 cells increased the IFN production about 1.5 times, however, IFN activity in sera taken from mice injected with 10^7 BCG cells was only from 0.20 to 0.03 time of the control (Table 4). This result is noteworthy because leukocytes sensitized with BCG, moderately such as 10^5 or 10^6 cells, produce larger amount of IFN by poly (IC) induction, but hypersensitization with BCG, such as 10^8 cells, reduces poly (IC)-induced IFN production. Therefore it is possible that immunomodification by ubenimex might suppress the poly (IC)-induced IFN production.

Ubenimex enhanced Con A-induced IFN production in spleen cell cultures and LPS-induced IFN production in mice sensitized with BCG. As reported previously[6,7], forphenicinol enhances both BCG- and LPS-induced IFN production in mice sensitized with BCG, but does not enhance Con A-induced IFN in spleen cell cultures. It has been shown that both low molecular weight immunomodifiers act on macrophages[5,8], and ubenimex also acts on T cells[8,9] but forphenicinol dose not[5]. This difference would be due to their specificity of binding to cells. The one of mechanisms of action of these low molecular immunomodifiers in modulating immune responses can be elucidated from these effects on cytokine production.

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