ENHANCEMENT OF GRAFT-VERSUS-HOST REACTION AND DELAYED CUTANEOUS HYPERSENSITIVITY IN MICE BY UBENIMEX†

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The effect of ubenimex on graft-versus-host reaction and delayed cutaneous hypersensitivity in mice was studied. Ubenimex enhanced the graft-versus-host reaction in a dose range from 0.005 to 0.5 mg/kg. Ubenimex inhibited the aging-caused decrease of delayed cutaneous hypersensitivity to oxazolone and the efficacy was greater with older mice. Ubenimex also inhibited mitomycin C and L1210-caused decreases of delayed cutaneous hypersensitivity to picryl chloride. However, the excess decrease caused by mitomycin C was not significantly altered by ubenimex.

Ubenimex, a dipeptide isolated from the culture filtrate of *Streptomyces olivoreticuli*[1], has antitumor activities[2~6], low toxicities[7] and aminopeptidase-inhibitory activities[8,9]. Ubenimex also has immunomodulating activities[2,3,10~14]. In an immunological study of ubenimex ISHIZUKA *et al.* found that ubenimex enhanced delayed-type hypersensitivity (DTH) to sheep-red blood cells (SRBC)[15]. In the present study we examined the effect of ubenimex on delayed cutaneous hypersensitivity (DCH) to oxazolone and picryl chloride in mice. We also examined the effect on graft-versus-host reaction (GVHR).

Materials and Methods

Animals

The strains of mice used here were as follows; BALB/c, C57BL/6 and BDF_1. The first strain was purchased from Charles River Japan Inc. and the other two from Shizuoka Laboratory Animal Center. The mice were kept in a clean environment as previously described[4].

Chemicals

Ubenimex, (−)-*N*-[(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutyryl]-L-leucine, was a product of Nippon Kayaku Co., Ltd., dissolved in saline at 5 mg/ml, sterilized by filtration through a Millipore filter and stored frozen at −20°C until use. Mitomycin C was purchased from Kyowa Hakko Co., Ltd. Picibanil (OK432)[16] and Krestin (PSK)[17] were from Chugai Pharmaceutical Co., Ltd. and Sankyo Co., Ltd., respectively.

GVHR

GVHR was induced by the method of SIMONSEN *et al.* and FORD[18,19]. Spleen cells used as the graft were taken from adult C57BL/6 mice and intraperitoneally injected to 7-day-old BDF_1 mice in an inoculum form of 5×10^6 cells. Agents were subcutaneously injected into the back of the BDF_1 mice immediately after the spleen cell inoculation. Seven days later mice were sacrificed and the spleen

† Hereafter, by recommendation of WHO, the name of ubenimex is used for bestatin.

weights were measured to evaluate GVHR.

DCH to Oxazolone

The method of ISHIZUKA *et al.*[2] was applied with slight modifications as described below. BALB/c mice were used. To sensitize the mice, the shaved abdomens were painted with 0.1 ml of 5% oxazolone (4-ethoxymethylene-2-oxazolone-5-one, Aldrich Chemical Co., Ltd.) in absolute EtOH. Three days later the sensitized mice were challenged by painting a hind footpad with 0.1 ml of the same oxazolone solution. DCH was evaluated 24 hours after the challenge by measuring the thickness of the footpad with calipers. Ubenimex was orally administered immediately after the sensitization.

DCH to Picryl Chloride

BDF_1 mice having an age of 8 weeks were similarly sensitized with 0.1 ml of 5% picryl chloride (Nakarai Chemical Co., Ltd.) in absolute EtOH. The sensitized mice were challenged by applying the same volume of 0.1% picryl chloride in olive oil to an earlobe. Twenty-four hours later DCH was evaluated by measuring the thickness of the earlobe with a Dial Thickness Gauge (Ozaki Seisakusho Model G special "Peacock"). Ubenimex was orally and mitomycin C intraperitoneally administered both starting on the day of sensitization.

Results

The effect of ubenimex on GVHR in infant BDF_1 mice was first investigated. Ubenimex enhanced GVHR in a dose range from 0.005 to 0.5 mg/kg as shown in Table 1. In another experiment the effect of ubenimex was compared with those of Krestin and Picibanil. Krestin and Picibanil doses used here were the reportedly maximum ones[16,17]. Ubenimex again enhanced GVHR (Table 2).

Table 1. The effect of various doses of ubenimex on GVHR in mice.

GVHR	Ubenimex (mg/kg)	Number of mouse	Spleen weight[a] (mg/g)	Index
Not induced	0	9	3.67±0.48	1.00
Induced	0	8	12.54±1.16	3.42
	0.0005	8	11.95±1.54	3.26
	0.005	9	15.94±0.84*	4.34
	0.05	9	16.34±2.27*	4.45
	0.5	8	17.85±2.69*	4.86

Spleen cells used as the graft were taken from 10 C57BL/6 mice with an age of 8 weeks. Prior to this experiment the effect of ubenimex on the spleen weight of normal or GVHR-noninduced mice was examined under the same conditions. Ubenimex reduced the spleen weight by about 15% at 0.05 and 0.5 mg/kg.

[a] Mean of the values per body weight ± SD.

* $P<0.001$ by Student's t-test vs. the value for the control group of GVHR-induced mice.

Table 2. The effect of ubenimex, Krestin and Picibanil on GVHR in mice.

GVHR	Agent	Dose (mg/kg)	Number of mouse	Spleen weight[a] (mg/g)	Index
Not induced	None	0	10	3.92±0.52	1.00
Induced	None	0	10	9.27±1.04	2.36
	Ubenimex	0.05	9	15.60±2.33*	3.98
	Krestin	50	10	19.92±2.28*	5.08
	Picibanil	20 KE	10	14.34±2.29*	3.66

Spleen cells used as the graft was prepared from 10 C57BL/6 mice having an age of 10 weeks.

[a] Mean of the values per body weight ± SD.

* $P<0.01$ by Student's t-test vs. the value for the control group of GVHR-induced mice.

Table 3. The effect of ubenimex on DCH to oxazolone in mice with varied ages.

Mouse age (week)	Ubenimex (mg/kg)	Number of mouse	Increase in footpad thickness (mm±SD)	T/C (%)
8	0	5	1.12±0.20	100
	0.5	8	1.03±0.34	92
12	0	7	0.78±0.25	100
	0.05	7	1.11±0.29	142
	0.5	7	1.07±0.18	137
	5.0	7	1.23±0.17*	158
16	0	6	0.69±0.37	100
	0.05	6	0.88±0.31	128
	0.5	6	1.11±0.28	161
	5.0	6	1.17±0.34*	170
40	0	6	0.48±0.19	100
	0.05	6	0.61±0.24	127
	0.5	6	1.07±0.13*	223
	5.0	6	0.81±0.13*	169

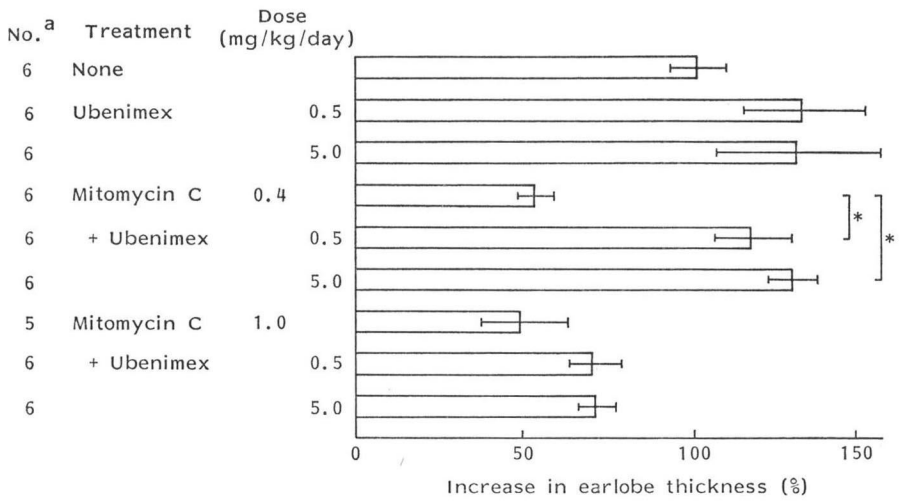
* $P<0.05$ by Student's t-test vs. the value for each control group.

Fig. 1. The effect of ubenimex on DCH to picryl chloride in mice.

Challenge was done 7 days after the sensitization. Ubenimex and mitomycin C were administered once a day on successive 8 days.

[a] Number of mouse.

* $P<0.05$ by Student's t-test.



Krestin and Picibanil also similarly enhanced the reaction.

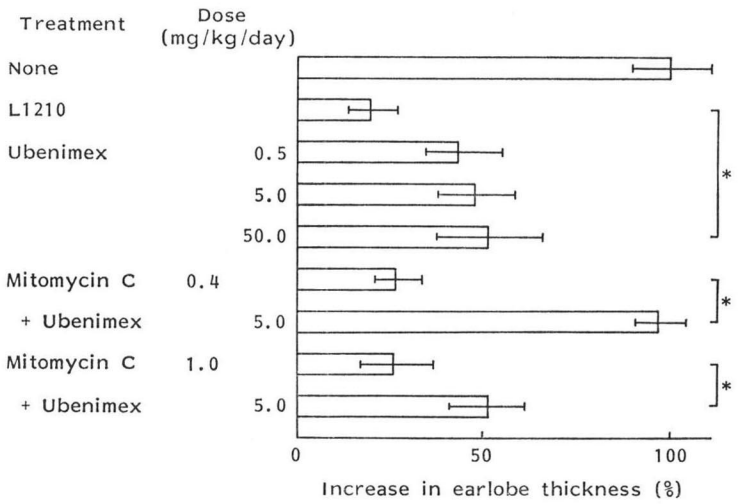
The effect of ubenimex on DCHs in BALB/c mice was next investigated. DCH to oxazolone decreased with age of mouse as shown in Table 3. Ubenimex inhibited the aging-caused decrease and the efficacy was greater with older mice. Ubenimex given at 5 mg/kg completely inhibited the decreases in mice having ages of 12 and 16 weeks. As to the decrease in much older mice having an age of 40 weeks, ubenimex even at a low dose of 0.5 mg/kg was completely inhibitory.

Ubenimex at 0.5 and 5.0 mg/kg had practically no effect on DCH to picryl chloride in BDF_1 mice (Fig. 1). Mitomycin C at 0.4 mg/kg reduced the DCH by about 50%. Ubenimex completely in-

Fig. 2. The effect of ubenimex on DCH to picryl chloride in L1210-transplanted mice.

L1210 (1×10^5 cells) was transplanted in one of the lowest lateral regions of the abdomen one day before the sensitization. Challenge was done 6 days after the sensitization. Ubenimex and mitomycin C were administered once a day on successive 7 days. Each group consisted of 5 mice.

* $P<0.05$ by Student's t-test.



hibited the reduction when administered at 0.5 and 5.0 mg/kg. Mitomycin C at a higher dose of 1.0 mg/kg also reduced the DCH to a similar extent as that at 0.4 mg/kg but ubenimex could not inhibit this reduction at the same doses as above.

DCH to picryl chloride was considerably decreased in BDF_1 mice when 1×10^5 cells of L1210 were transplanted (Fig. 2). Ubenimex did not inhibit the decrease at 0.5 and 5.0 mg/kg. Ubenimex given at as much as 50 mg/kg did inhibit but the inhibition was partial. When mitomycin C was administered to the L1210-transplanted mice at 0.4 mg/kg the DCH level remained decreased. Ubenimex at 5 mg/kg completely inhibited this decrease. This dramatical effect of ubenimex is considered to be exerted due to the aid of the anti-tumor effect of mitomycin C against the transplanted L1210[20]. When dose of mitomycin C was increased to 1.0 mg/kg ubenimex at the same dose had a much less effect. This result may indicate that the excess immunosuppression caused by mitomycin C is difficult to be prevented by ubenimex administration.

Discussion

In the present study the effect of ubenimex on GVHR and DCHs in mice was investigated. Ubenimex enhanced GVHR in a wide dose range as shown above. In the GVHR spleen cells from adult C57BL/6 mice were given to infant BDF_1 mice. When the spleen cells were given after X-ray-irradiated GVHR was not induced (data not shown). In addition spleen cells from adult BDF_1 mice did not induce GVHR in infant mice of the same strain (data not shown). Therefore, the enhancing effect of ubenimex is considered to be chiefly due to the proliferation of the transplanted spleen cells. When the spleen cells from C57BL/6 mice were transplanted after the macrophages were eliminated, the enhancing effect of ubenimex was significantly decreased (data not shown). This indicates that ubenimex requires macrophages for enhancement of GVHR.

T cells have been known to be involved in DCH[21,22]. ISHIZUKA *et al.* found that ubenimex enhanced DTH to SRBC in mice. In the present study ubenimex inhibited the aging-caused decrease

of DCH to oxazolone in mice. Ubenimex also inhibited the mitomycin C and L1210-caused decreases of DCH to picryl chloride. These ubenimex effects on DCH may be exerted *via* a mechanism involving T cells.

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