THE EFFECT OF UBENIMEX† ON *N*-METHYL-*N'*-NITRO-*N*-NITROSO-GUANIDINE-INDUCED STOMACH TUMOR IN RATS

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The effect of ubenimex on the progression of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)-induced stomach tumor in rats was studied. Tumor induction was performed by giving MNNG *via* drinking water for 34 weeks. Ubenimex was ip administered twice a week at 0.5 mg/kg for 84 weeks in one group and 49 weeks in another starting from the first and 36th weeks after initiation of MNNG administration, respectively. The stomachs were endoscopically examined 2 times. At the 64th week after initiation of MNNG administration tumorous lesions were observed with about 70% of the rats in both ubenimex administration groups. In the ubenimex non-administration group nearly 90% of the rats had the lesions. After completion of ubenimex administration almost all the rats had the lesions in the three groups but the sizes were much smaller with the two ubenimex administration groups. Almost all of these lesions were histopathologically identified as tumorous. The tumor volume per rat in the two ubenimex administration groups from the 1 and 36th weeks was 21.0 and 19.2% of the volume in the control group, respectively. Tumor number per rat was similar among the three groups.

The natural killer activity of rats was also examined after completion of the above experiment. The activity markedly increased when ubenimex was administered from the 36th week after initiation of MNNG administration. When ubenimex was administered from the first week, the activity did not increase demonstratively. From all the results described above we conclude that ubenimex exerts an inhibitory action against the progression of MNNG-induced stomach tumor in rats. Contribution of the increase of natural killer activity to ubenimex antitumor action may be dependent on schedule of ubenimex administration.

Ubenimex, a dipeptide produced by *Streptomyces orivoleticuli*[1], inhibited aminopeptidase activities[2,3] and enhanced the functions of immunocompetent cells[4~9]. In addition ubenimex had activity against a large variety of transplantable tumors such as IMC carcinoma[6], Gardner lymphoma[6], colon 26 adenocarcinoma[10], C1498 leukemia[10], human stomach cancer in nude mice[11] *etc.*[12,13]. These results on the antitumor activity were thus far obtained only with mice. In the present study the effect of ubenimex administration on *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine(MNNG)-induced stomach tumor was studied using rats. The natural killer activity of the spleen cells was also examined after completion of ubenimex administration.

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

Materials and Methods

Male Wistar rats (SPF) were purchased from Shizuoka Laboratory Animal Center (Hamamatsu), kept in a conventional animal room and fed with a solid chow CE-2 (CLEA Japan Inc.). Ubenimex was synthesized by the previously reported method[14]. Ubenimex was dissolved in physiological saline and sterilized by using a Millipore filter.

MNNG, Tween 60, sodium pentobarbital and $Na_2{}^{51}CrO_4$ were purchased from Aldrich Chemical Inc., Milwaukee, Wis., Wako Pure Chemicals Co., Tokyo, Abbott Labs., North Chicago, Ill. and New England Nuclear Co., Boston, Mass., respectively.

Stomach tumors were induced in male Wistar rats with a drinking water containing MNNG and Tween 60 at 50 μg/ml and 0.4%, respectively[15,16]. This drinking water was given for the first 34 weeks and thereafter a normal drinking water for 50 weeks. Ubenimex was administered ip under the following two schedules. Under the first schedule ubenimex was administered twice a week principally on Monday and Thursday at 0.5 mg/kg for 84 weeks starting with the initiation of MNNG administration. Under the other schedule ubenimex was similarly administered for 49 weeks from the 36th to the final 84th week, that is, starting one week after completion of MNNG administration. In addition a ubenimex non-administration group was included. The first, second and control groups were composed of 30, 25 and 30 rats, respectively.

Stomachs were endoscopically examined at the 64th week after initiation of MNNG administration and the 85th week, that is, after completion of ubenimex administration using a human bronchofiberscope (type 3C4, Olympus Optical Co., Tokyo). Prior to insertion of the fiberscope, the rats were anesthetized with sodium pentobarbital, the stomach cavities were washed 3 times with H_2O and filled with 5 ml of air using a catheter syringe. For histopathological examination, the excised stomachs were fixed with 10% formalin solution and embedded in paraffin. The paraffin sections were stained with hematoxylin and eosin.

The natural killer activity was determined on spleen cells using the method of KIESSLING *et al.*[17]. YAC-1 and K562 cells were used for the target cells. The target cells were disrupted by freezing and thawing after being fed with ^{51}Cr and the resultant ^{51}Cr was considered as the maximal ^{51}Cr level to be released from the cells at the incubation. Radioactivity was determined with a Beckman model Gamma 800 gamma spectrometer.

Results

The effect of ubenimex on the progression of MNNG-induced stomach lesion in rats was first examined endoscopically. From the size of the lesion the rats were classified into 5 groups from those having no lesion to those with large lesion. The results are shown in Table 1. At the 64th week almost 90% of the rats in the control group had lesion. In contrast, about 70% of the rats had

Table 1. Endoscopic examination of the effect of ubenimex on the progression of MNNG-induced lesion.

Observation	Administration period (weeks)	Rat No.	Rats with each stomach lesion (%)				
			S_0	S_1	S_2	S_3	S_4
64th week	Not administered	23	8.7	39.1	39.1	13.0	0
	1~84	29	27.6	31.0	34.5	6.9	0
	36~84	24	33.3	25.0	29.2	12.5	0
After completion of ubenimex administration	Not administered	20	0	0	10.0	40.0	50.0
	1~84	20	15.0	10.0	30.0	35.0	10.0
	36~84	18	0	16.7	27.8	38.9	16.7

Stomachs were endoscopically examined and the lesions were classified according to the size: S_0; Not detected, S_1; minute, S_2; smaller than a pylorus ring, S_3; larger than a pylorus ring, S_4; enough covering glandular stomach.

Table 2. The effect of ubenimex on the volume and number of tumor induced by MNNG.

Administration period (weeks)	Rat No.	No. of lesion						Tumor volume per rat (mean±SD, mm³)
		Criterion[a]						
		Total	I	II	III	IV	V	
Not administered	20	36	6	1	0	4	25	3,770±4,692
1~84	17	23	0	0	1	1	21	793±1,240[b]
36~84	19	28[c]	1	0	0	1	25	722±694[b]

Tumor length (L) and width (W) were measured with calipers and tumor volume (V) was calculated by $V=LW^2/2$.

[a] According to the criteria of histological diagnosis of biopsied gastric mucosa proposed by Japanese Research Society for Gastric Cancer[18].

[b] $P<0.01$ by Student's t-test.

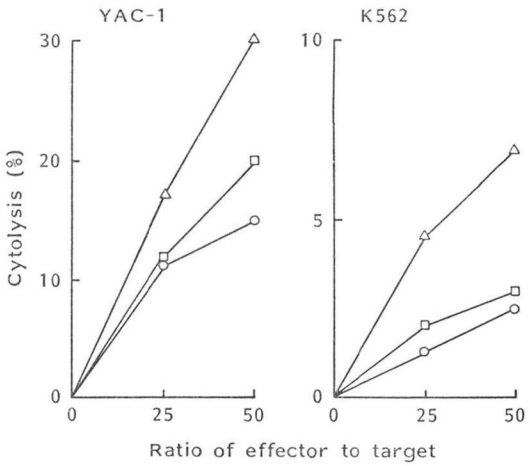
[c] The sum of the numbers of lesion under criterion I to V does not make the total number of 28, since one of the 28 lesions was histopathologically identified as fibrosarcoma and thus excluded here.

lesion in the two ubenimex administration groups. After completion of ubenimex administration, no lesion was observed in 15% of the rats in the group where ubenimex was administered from the first week. In the other two groups all the rats had lesion and a much larger number of the lesion of the rats in the control group was at S_4. The percentage of rats with the S_4 lesion was 50% in the control group but 10 to 16.7% in the ubenimex administration groups.

At the 85th week, the rats were sacrificed after the second endoscopic examination and the excised stomachs were subjected to macroscopic and microscopic examinations. The microscopic examination was carried out only for evaluation of the lesions according to the criteria proposed for histopathological diagnosis of biopsied gastric mucosa from Japanese Research Society for Gastric Cancer[18]. The results are shown in Table 2. The percentage of the lesions diagnosed as cancer of criterion V was 69.4, 91.3 and 89.3% in the control group and ubenimex administration groups starting from the 1st and 36th weeks, respectively. When the lesions of criterion IV, which are strongly suspected to be cancer, were included, the percentage became 80.6, 95.7 and 92.9%, respectively. Tumor volume was examined on the lesions diagnosed as criterion IV and V. Mean tumor volume per rat in the ubenimex administration groups from the 1st and 36th weeks was 21.0 and 19.2% of that observed for the control group rats, respectively. Tumor number per rat was less in both ubenimex administration groups than in the control group, but the differences were not statistically significant. The number of the lesion of criterion I was 6 in the control group but zero and 1 in ubenimex administration groups. Although these numbers were much less than those of criterion IV and V lesions, this result may suggest that

Fig. 1. The natural killer activity of spleen cells obtained from rats after completion of ubenimex administration.

The natural killer activity of spleen cells was determined on 4 rats of each group. The symbols in the figure were as follows: ○ for control group and □ and △ for two ubenimex administration groups where ubenimex was administered from the 1st and 36th weeks, respectively.



ubenimex has a relatively potent antigastritis activity.

Table 2 additionally shows the number of rats which survived through the 84th week. A fairly large number of rats died during the experiment. The main cause of the deaths was thought to be pneumonitis infection and accidents such as anesthetization failure at the endoscopic observation. The cause of death for 2, 2 and 1 rats in the control group and ubenimex administration groups from the 1st and 36th weeks, respectively, was possibly cancer-induced pyloric stenosis and perfolation.

Natural killer cells exhibit antitumor activity[19,20]. Ubenimex was found to indirectly exhibit antitumor activity *via* immune function[10]. Therefore, the natural killer activity of spleen cells obtained from rats that survived through the 84th week was further examined. The activity was seen to markedly increase in rats administered with ubenimex from the 36th week whatever the target cells for the activity assay was (Fig. 1). The activity appeared to increase in rats administered with ubenimex from the 1st week but the data were not demonstrative.

Discussion

MNNG is a chemical carcinogen which induces stomach cancer in rats[15]. In the present study ubenimex-inhibitory effect on the progression of MNNG-induced tumorous lesions was endoscopically demonstrated. In addition the lesions diagnosed as cancer by the histological diagnosis was markedly smaller in volume in the two ubenimex administration groups than those observed for the control group. Therefore, we conclude that ubenimex has inhibitory activity against MNNG-induced rat stomach cancer.

Ubenimex inhibited tumor progression but did not reduce tumor incidence. ISHIZUKA *et al.* reported a similar result indicating that ubenimex did not reduce the incidence of 20-methylcholanthren-induced mouse skin tumor at the end of their experiment although the reduction did occur in the midst of the experiment[7]. BRULEY-ROSSET *et al.* reported that ubenimex decreased even the incidence of spontaneous lymphoma in aged mice[21].

AOIKE *et al.*[22], BLOMGREN *et al.*[23] and ABE *et al.*[10] reported that ubenimex increased the natural killer activity of the spleen cells and peripheral blood cells of tumor-bearing mice and humans. In the present study, ubenimex also increased natural killer activity when administered from the 36th week but did not demonstratively when administered from the 1st week. Contribution of the increase of natural killer activity to the mechanism through which ubenimex exhibits the inhibitory action against MNNG-induced rat stomach cancer may be dependent on the schedule of ubenimex administration.

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