EFFECTS OF NEOPLURAMYCIN ON ASCITES TUMORS IMPLANTED IN EXPERIMENTAL ANIMALS

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The antitumor effects of the new antibiotic, neopluramycin, found by KONDO *et al.*[1] were studied against leukemia L-1210, EHRLICH ascites tumor and ascites hepatoma AH-130 in the present experiments.

Various doses of neopluramycin dissolved in physiological saline were administered intraperitoneally to the ascites tumor-bearing mice and rats, and the inhibitory activity of the agent against the proliferation of these ascitic tumor cells was compared with that of the antileukemic agent, daunomycin[2~5].

Materials and Methods

Animals: Both sexes of BDF_1 (C57BL♀ × DBA♂) mice weighing 20±2 g, *dd* strain mice weighing 20±2 g and Donryu strain rats weighing 100±5 g were used. These animals were kept on standard diet (CLEA-CE-2), with free supply of water.

Agents: Neopluramycin ($C_{40}H_{50}N_2O_{10}$) was supplied by the Institute of Microbial Chemistry, and daunomycin ($C_{27}H_{29}NO_{10}$) was produced by Meiji Seika Kaisha, Ltd.

Methods: The experimental groups on each tumor and the number of animals in each group were as shown in Tables 1, 2 and 3.

BDF_1 mice, *dd* strain mice and Donryu strain rats were inoculated intraperitoneally with 10^6 cells of leukemia L-1210, EHRLICH ascites tumor or ascites hepatoma AH-130 from the 6-day-old donors, respectively.

Neopluramycin was dissolved in the physiological saline adjusted to pH 4.8 with 0.1 N HCl solution, and daunomycin used as a control agent was dissolved in physiological saline.

Twenty-four hours after the implantation of each tumor, the solutions containing the various doses of these antibiotics (Tables 1 ~3) were injected intraperitoneally at 0.1 ml/animal. These injections were performed once a day during 10 successive days.

Autopsy was conducted immediately after death of animals during the experimental period, and ascites and hydrothorax volumes were measured. All the surviving animals were sacrificed on the 50th day after the beginning of the experiments, and were observed patho-anatomically.

Antitumor activities were evaluated by comparing the mean survival time of the treated animals (T) to that of the untreated control (C), *i.e.* percentage increase in life-span (ILS)=100 T/C−100 (%). Agents demonstrating ILS≧50 % are considered effective.

Results

I. Effects of Neopluramycin on Leukemia L-1210 (Table 1)

Neopluramycin was effective at doses of 250 mcg/kg, 500 mcg/kg and 1,000 mcg/kg.

Table 1. Effects of neopluramycin and daunomycin on leukemia L-1210 in BDF_1 mice.

Group		Dose mcg/kg	Sex	No. of mice	Survival days	ILS (%)	Mean ILS (%)
Neopluramycin	I	1,000	♂	5	11.0	44.7	51.1
			♀	5	12.6	57.5	
	II	500	♂	5	12.6	65.8	62.9
			♀	5	12.8	60.0	
	III	250	♂	5	11.0	44.7	67.4
			♀	5	15.2	90.0	
	IV	125	♂	5	9.8	28.9	27.0
			♀	5	10.0	25.0	
	V	62.5	♂	5	8.8	15.8	11.7
			♀	5	8.6	7.5	
	VI	15.65	♂	5	8.0	5.3	1.2
			♀	5	7.8	−3.0	
Daunomycin	VII	2,000	♂	5	12.6	65.8	69.2
			♀	5	13.8	72.5	
	VIII	1,000	♂	5	11.6	52.6	67.6
			♀	5	14.6	82.5	
	IX	500	♂	5	11.8	55.3	71.4
			♀	5	15.0	87.5	
	X	250	♂	5	9.2	21.1	26.8
			♀	5	10.6	32.5	
Control (L-1210)	XI		♂	5	7.6		
			♀	5	8.0		

However, a slight toxic influence appeared at a dose of 1,000 mcg/kg. The control agent, daunomycin was effective at doses of 500 mcg/kg, 1,000 mcg/kg and 2,000 mcg/kg. The greatest effect on the leukemia L-1210 was shown in 5 female mice administered neopluramycin at a dose of 250 mcg/kg.

On the whole, the daunomycin groups showed a higher average percentage increase in lifespan than neopluramycin.

In patho-anatomical findings, 0.05~1 ml of hydrothorax and 0.1~4 ml of ascites were recognized in treated animals. Moreover, hypertrophy of the liver and spleen, invasion of the tumor cells into the pancreas, caseous necrosis in the lung, edematization of the kidney and hypertrophy of lymph nodes *etc.* were observed in each animal of treated groups as well as in animals of the control group.

II. Effects of Neopluramycin on EHRLICH Ascites Tumor (Table 2)

Neopluramycin was effective at all doses from 15.65 mcg/kg to 500 mcg/kg, but a slight toxic influence appeared at 500 mcg/kg. Daunomycin was effective at 125 mcg/kg, 250 mcg/kg, 500 mcg/kg and 1,000 mcg/kg, but showed toxicity at 1,000 mcg/kg. The greatest effect was with daunomycin at 500 mcg/kg. The group treated with 250 mcg/kg of neopluramycin also showed considerable effect.

Five, 3 and 2 mice treated with 250 mcg/mg, 125 mcg/kg and 62.5 mcg/kg of neopluramycin, and 10, 5, 6 and 2 mice treated with 500 mcg/kg, 250 mcg/kg, 125 mcg/kg and 1,000 mcg/kg of daunomycin, respectively, survived to the 50th day after the beginning of the experiment.

Ascites and hydrothorax were unrecognizable in all survivors to the 50th day.

III. Effects of Neopluramycin on Ascites Hepatoma AH-130 (Table 3)

Differences between neopluramycin and daunomycin were distinct in this experiment. Neopluramycin was effective at 500 mcg/kg and 1,000 mcg/kg, but only slightly. Daunomycin showed a remarkably high effectiveness at 500 mcg/kg and 1,000 mcg/kg. Five and 1 animals in 1,000 mcg/kg and 500 mcg/kg groups, respectively, of these daunomycin-treated groups survived to the 50th day.

As regards the patho-anatomical findings, retention of 0.1~1 ml of hydrothorax and 1~9 ml of ascites were recognized in most

Table 2. Effects of neopluramycin and daunomycin on EHRLICH ascites tumor in *dd* strain mice.

Group		Dose mcg/kg	Sex	No. of mice	Survival days	ILS (%)	Mean ILS (%)
Neopluramycin	I	500	♂	5	26.3	95.5	67.6
			♀	5	19.0	39.7	
	II	250	♂	5	41.6	210.4	222.9
			♀	5	45.6	235.3	
	III	125	♂	5	36.0	168.7	186.6
			♀	5	41.4	204.4	
	IV	62.5	♂	5	37.0	151.3	164.6
			♀	5	37.8	177.9	
	V	15.65	♂	5	29.0	116.7	112.8
			♀	5	28.4	108.8	
Daunomycin	VI	2,000	♂	5	13.0	−3.0	8.8
			♀	5	16.4	20.6	
	VII	1,000	♂	5	34.8	159.7	171.8
			♀	5	38.6	183.8	
	VIII	500	♂	5	50.0	273.1	270.4
			♀	5	50.0	267.6	
	IX	250	♂	5	46.8	249.3	209.2
			♀	5	36.6	169.1	
	X	125	♂	5	44.6	232.8	198.0
			♀	5	35.8	163.2	
Control (EHRLICH ascites tumor)	XI		♂	5	13.4		
			♀	5	13.6		

Table 3. Effects of neopluramycin and daunomycin on ascites hepatoma AH-130 in Donryu strain rats.

Group		Dose mcg/kg	Sex	No. of rats	Survival days	ILS (%)	Mean ILS (%)
Neopluramycin	I	1,000	♂	5	10.4	67.7	62.9
			♀	5	9.8	58.1	
	II	500	♂	5	10.6	71.0	64.6
			♀	5	9.8	58.1	
	III	250	♂	5	9.6	54.8	46.8
			♀	5	8.6	38.7	
Daunomycin	IV	1,000	♂	5	39.4	435.5	387.1
			♀	5	27.2	338.7	
	V	500	♂	5	14.5	133.9	129.3
			♀	5	13.8	122.6	
	VI	250	♂	5	9.6	54.8	56.5
			♀	5	9.8	58.1	
Control (Ascites hepatoma AH-130)	VII		♂	5	6.2		
			♀	5	6.2		

animals of the neopluramycin groups. Retention of hydrothorax and ascites was hardly recognized in the daunomycin-treated animals. In this experiment, degeneration of organs in each animal was similar to experiments with leukemia L-1210 and EHRLICH ascites tumor. However, the degeneration of internal organs in animals treated with daunomycin was less than in animals administered neopluramycin.

Discussion and Conclusion

In the present experiments, antitumor effects of the new antibiotic, neopluramycin, on the experimental ascites tumors in mice and rats were compared to those of daunomycin.

The optimal effective dose of neopluramycin was 250 mcg/kg on leukemia L-1210, 250 mcg/kg on EHRLICH ascites tumor and 500 mcg/kg on ascites hepatoma AH-130.

The effectiveness of neopluramycin on each tumot appears to be lower than that of daunomycin througout the expeiments. Particularly, this fact is evident in the experiment on ascites hepatoma AH-130. However, at a dose of 250 mcg/kg, the effectiveness of neopluramycin on leukemia L-1210 is high as compared with that of daunomycin at the same dose. The inhibitory effect of both neopluramycin and daunomycin on EHRLICH ascites tumor is extremely high.

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