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56. Makoto Hayashi, Kunikazu Onodera, and Komei Miyaki: Effect of Amino Acids on the Growth of Ehrlich Tumor Cell. I. Inhibitory Action of D-Threonine and D-Lysine on the Growth of Ehrlich Solid Tumor.

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It has already been shown that phosphorylethanolamine and phosphoryl-N-methylethanolamine accelerate the growth of mouse Ehrlich solid tumor but have no effect on the growth of Yoshida sarcoma cells *in vitro*. It was assumed that there is a possibility that these amines form phospholipids *in vivo* and accelerate the tumor cell growth by unknown mechanism.[1]

The present paper describes the effect of serine, which constitutes the phospholipid, on the growth of Ehrlich solid tumor. The effect of threonine and lysine was additionally examined in the present work because Wallach and others[2] reported that the phospholipid in the Ehrlich ascites tumor cell contains lipopeptide consisting of alanine, lysine, proline, threonine, ornithine, and tyrosine and because it was assumed that the D-isomers of essential amino acids are hardly oxidized by D-amino acid oxidase and they might inhibit incorporation of the corresponding L-isomers into Ehrlich solid tumor.

Experimental

Reagents——All amino acids, both L- and D-isomers, were purchased from the Tanabe Seiyaku Co., Ltd., and the mixture of equal amounts of D- and L-compounds was used as DL-amino acid.

Growth Inhibition of Ehrlich Solid Tumor in the Mouse Hindleg Inguinal Region——Groups of 10 *dd*-strain mice, about 5 weeks old and weighing 18～20 g., were employed. Each mouse was inoculated with 2×10^6 cells of Ehrlich ascites tumor under the skin of inguinal region in the hindleg. From the next day, various concentration of physiological saline solution of the amino acid was injected into the peritoneum, while the control group received only physiological saline solution. On the 15 th day, each mouse was weighed, sacrificed and tumor was taken out. Each tumor was weighed, average value was calculated and the effect of these compounds was determined from statistical significance.

Effect on Prolongation of Life in Ascites Tumor-bearing Mice——A group of *dd*-strain mice, weighing 18～20 g., were inoculated intraperitoneally with 5×10^6 cells of Ehrlich ascites tumor. From the following day, physiological saline solution of the amino acid was injected intraperitoneally and effect of the amino acid on prolongation of life was examined. The control group received physiological saline solution alone.

Results and Discussion

Serine——DL-, L-, and D-Serine showed no effect on the growth of Ehrlich solid tumor in mouse.

Threonine——L-Threonine was entirely without effect but the administration of more than 0.25 mg./mouse/day of DL-threonine clearly inhibited the tumor growth. The D-isomer was effective at 0.1 mg./mouse/day and the growth-inhibitory effect of the DL-compound may be ascribed to the presence of the D-isomer (Table I). However, as shown in Fig. 1, there was no life-prolongation effect against mice bearing Ehrlich ascites tumor.

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1) K. Miyaki, M. Hayashi, T. Chiba, K. Nasu, T. Unemoto and K. Onodera: This Bulletin, 8, 900 (1960).

2) D.F.H. Wallach, J. Sodergerg and C. Bricker: Cancer Research, **20**, 397 (1960).

Lysine——Similar to threonine, L-lysine was without effect, as shown in Table II, and the administration of 0.1 mg./mouse/day of the DL-compound or D-isomer showed apparent inhibitory effect. The inhibitory effect of DL-compound was assumed to be the same as that of D-threonine. The degree of inhibition of lysine was greater than that of threonine. The prolongation of life span of mice bearing Ehrlich ascites tumor was not observed as indicated in Fig. 2.

TABLE I. Effect of Threonine on the Growth of Ehrlih Solid Tumor

Amino acid	Dose (mg./mouse/day)	Mean value of wet wt. of tumor (g.)	Significance(P)	Mean value of body wt. of mouse (g.)	Significance(P)
L-Threonine	0	0.44		23.6	
	0.1	0.49	>0.05	26.0	>0.05
	0.25	0.40	>0.05	24.6	>0.05
	0.5	0.41	>0.05	24.5	>0.05
	1.0	0.39	>0.05	24.3	>0.05
DL-Threonine	0	0.47		18.5	
	0.1	0.36	>0.05	17.6	>0.05
	0.25	0.29	<0.01	17.6	>0.05
	0.5	0.31	<0.01	17.1	>0.05
	1.0	0.26	<0.01	18.0	>0.05
D-Threonine	0	0.51		20.7	
	0.1	0.37	<0.01	19.0	>0.05
	0.25	0.33	<0.01	20.2	>0.05
	0.5	0.28	<0.01	19.4	>0.05
	1.0	0.33	<0.01	19.0	>0.05

TABLE II. Effect of Lysine on the Growth of Ehrlich Solid Tumor

Amino acid	Dose (mg./mouse/day)	Mean value of wet wt. of tumor (g.)	Significance(P)	wt. of mouse (g.) Mean value of body	Significance(P)
L-Lysine	0	0.42		19.2	
	0.1	0.39	>0.05	18.8	>0.05
	0.25	0.42	>0.05	20.3	>0.05
	0.5	0.40	>0.05	19.7	>0.05
	1.0	0.45	>0.05	20.5	>0.05
DL-Lysine	0	0.38		22.9	
	0.1	0.26	<0.02	21.0	>0.05
	0.25	0.23	<0.02	22.5	>0.05
	0.5	0.23	<0.001	21.5	>0.05
	1.0	0.25	<0.001	21.0	>0.05
D-Lysine	0	0.36		17.6	
	0.1	0.21	<0.01	17.6	>0.05
	0.25	0.17	<0.001	17.8	>0.05
	0.5	0.17	<0.001	19.0	>0.05
	1.0	0.18	<0.001	18.6	>0.05

TABLE III. Effect of D- and L-Threonine on the Growth of Ehrlich solid Tumor

Threonine	Dose (mg./mouse/day)	Mean value of wet wt. of tumor (g.)	Significance(P)
DL-Threonine	0	0.37	
	1.0	0.24	<0.02
D-Threonine + L-Threonine	0.5 1.0	0.24	<0.01
D-Threonine + L-Threonine	0.5 2.0	0.25	<0.02

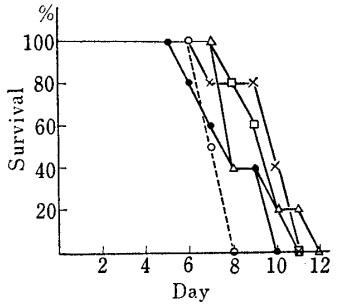

Fig. 1. Life Prolongation Effect of DL-Threonine in Ehrlich Ascites Tumor

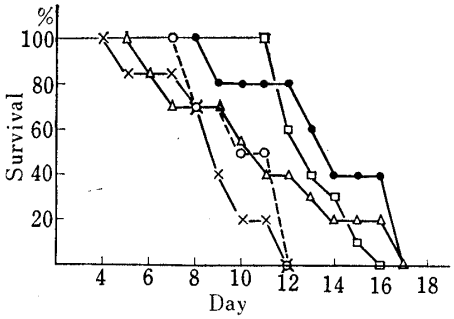

Fig. 2. Life Prolongation Effect of D-Lysine

---o---o--- : Control
—×—×— : 0.1 mg./mouse/day
—•—•— : 0.25 mg./mouse/day
—△—△— : 0.5 mg./mouse/day
—□—□— : 1.0 mg./mouse/day

As described above, inhibitory action of D-threonine and D-lysine on the growth of Ehrlich solid tumor seems to be the inhibition of the L-isomer into the tumor cell, but, at present, there is not sufficient data to prove whether the effect is specific to the tumor cell or not. As is clear from Table I and II, the body weights of the host mice was not significantly different from those of the control mice and it seems correct to consider that the D-isomer specifically acts on the tumor cell.

Assuming that the L-isomer might antagonize the action of the D-isomer, larger dose of L-threonine was administered for a definite quantity of D-threonine, as shown in Table III, but the effect of D-isomer was not affected under these conditions.

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Summary

D-Threonine and D-lysine were clearly proved to inhibit the growth of Ehrlich solid tumor in the inguinal region of mouse hindleg but its mechanism has not been clarified as yet. These amino acids showed no effect in the prolongation of life of mice bearing Ehrlich ascites tumor.

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