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ANTITUMOR ACTIVITY AND BIOLOGICAL EFFECTS OF CHEMICALLY SYNTHESIZED MONOSACCHARIDE ANALOGUES OF LIPID A IN MICE

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Five synthetic monosaccharide analogues of lipid A containing two 3-acyloxytetradecanoyl and a phosphoryl group at the C-2, -3 and -4 positions of the glucosamine skelton were synthesized. The antitumor activity of these compounds against the ascites form of Ehrlich carcinoma and the biological effects (lethal toxicity and mitogenicity) in mice were compared with those of a bacterial lipopolysaccharide (LPS) or lipid A.

KEYWORDS ———— lipopolysaccharide; lipid A; glucosamine derivative; antitumor activity; lethal toxicity; mitogenicity

Lipid A, a constituent of the lipopolysaccharide (LPS) of Gram-negative bacteria, plays an important role in the various biological activities of LPS.[1] Recent chemical analyses revealed that lipid A contains a β-1,6-linked D-glucosamine disaccharide substituted by phosphate groups and by ester- and amide-bound fatty acids.[1,2] On the basis of these results, glycolipids derived from glucosamine dissacharides have been synthesized by Imoto et al.[3] and their biological activities examined.[4] A diacylated monosaccharide (lipid X), accumulated in phosphatidylglycerol-deficient mutants of Escherichia coli, induces the activation of B-lymphocytes and macrophages.[5]

Kiso et al.[6] and Charon et al.[7] synthesized various analogues of the nonreducing sugar moiety of lipid A. Matsuura et al.[8] reported that their derivatives induced the production of interferon and a tumor necrosis factor. More recently, Charon et al.[9] demonstrated that a number of monosaccharides derived 2-

deoxy-2-[(3R)-3- hydroxytetradecanamide] -D-glucopyranose possesses various biological activities including mitogenic activity for B-lymphocyte, secretion of interleukin I, and activation of the complement system. In the present paper, we report a comparative study of the antitumor activity and other biological effects (lethal toxicity and mitogenicity) of five synthetic derivatives of glucosamine monosaccharide (Fig. 1), bacterial LPS and lipid A.

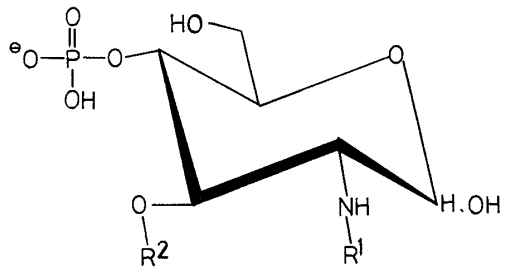

Fig. 1. Structure of Synthetic Lipid A Analogues

C14: tetradecanoyl, C14-O-C12: (R)-3-dodecanoyloxytetradecanoyl, C14-O-C14: (R)-3-tetradecanoyloxytetradecanoyl, C14-O-C16: (R)-3-hexadecanoyloxytetradecanoyl.

Compound No.	Substituent	
	R^1 (N-)	R^2 (3-O-)
101	C_{14}-O-C_{16}	C_{14}-O-C_{16}
102	C_{14}	C_{14}
103	C_{14}-O-C_{14}	C_{14}-O-C_{14}
104	C_{14}-O-C_{12}	C_{14}-O-C_{12}
105	C_{14}-O-C_{12}	C_{14}-O-C_{14}

The compounds were synthesized as described previously.[10] All compounds tested were solubilized or suspended in pyrogen-free saline (Ohtsuka Seiyaku Co., Ltd., Tokyo) supplemented with triethylamine (Wako Pure Chemicals, Osaka) in a ratio of 0.5% (v/v). Before each experiment, the solutions were sonicated for 20-30 s.

The results of the antitumor activity and biological effects are summarized in Table I. The antitumor activity of each compound was assessed against the ascites form of Ehrlich carcinoma. Tumor cells (1×10^4) were inoculated intraperitoneally (ip) into ddY mice (25 g) on day 0. Each compound was injected ip into mice (100 μg/day/mouse) on days -5, -2, +1, +3, and +5. Control mice injected with saline alone were all dead within 23 days (range, 16-23 days) after tumor inoculation.

V. anguillarum LPS as reference material[10] exhibited remarkable antitumor activity. Although the activity of the synthetic compounds was weaker than that of LPS, compounds 103 and 104 were effective ($P<0.05$) while compounds 101 and 105 produced longer survival than the control group but the difference was not significant ($P>0.05$). Compound 102, which has a single acyl group at C-2 and -3 positions, did not possess antitumor activity. These findings indicate that diacyl monosaccharide analogues of lipid A exhibit in vivo antitumor activity against the ascites form of Ehrlich carcinoma.

Table I. Biological Activities of Synthetic Monosaccharide Analogues of Lipid A in Mice

Preparation	Antitumor activity: 25-day survivors/ No. of mice tested	Lethal toxicity: 0	0.01	0.1	1.0	50 (μg/mouse)	Mitogenicity[a]: 10	50 (μg/ml)
101	2/6			0/3	0/3	1/6	-	±
102	0/6			0/3	0/3	0/3	-	-
103	4/6 ($P<0.05$)			0/3	0/3	5/6	±	±
104	3/6 ($P<0.05$)			0/3	0/3	3/6	-	-
105	1/6			0/3	0/3	6/6	±	-
E. coil 055:B5 lipid A				0/3	3/3		++	
S. typhimurium LT-2 LPS			3/3	6/6	6/6		+++	
V. anguillarum PT-514 LPS	10/10 ($P<0.001$)							
Control (saline)	0/8	0/6					- (No added)	

a) Stimulation index, -; 1.0>, ±; 1.0-3.0, +; 3.0-5.0, ++;5.0-10.0, +++;<10

The lethal toxicity of the synthetic compounds was determined in C57BL/6 mice loaded with galactosamine-HCl (16 mg/mouse) according to the method of Galanos et al.[12] The four compounds, except 102, showed lethal toxicity at a dose of 50 μg/mouse in animals which were rendered highly susceptible to LPS and lipid A by ip injection of galactosamine.

The mitogenicity of the compounds was determined on the basis of in vitro ^{3}H-thymidine (^{3}H-TdR) uptake into splenocyte in C57BL/6 mice as described in a

previous report.[13] The reference materials, LPS of *S. typhimurium* and lipid A of *E. coli* 055:B5 (Difco Laboratory, Detroit, MI, U.S.A.), were capable of increasing the incorporation of ^{3}H-TdR and three synthetic compounds (101, 103, and 105) appeared slightly mitogenic.

Further chemical modification of the monosaccharide derivatives of lipid A may produce more effective antitumor substance and immunopotentiation.

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