BRIEF NOTE

Enhancement of Liver UDP-Glucose Dehydrogenase Caused by an Effective Glucosidurogenic Compound, Eugenol

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In the previous part of the present studies on glucuronidation enhanced by oral administration of eugenol, it was indicated that a living body performs a rapid glucuronide formation and excretion of administered glucosidurogenic foreign compound, and then makes subsequently occurring inductive adaptation of UDP-glucuronyltransferase (GT) in liver microsome for the compound [5]. The present paper deals with the metabolic response of the step of UDP-glucuronic acid (UDPGA) biosynthesis, catalyzed by UDP-glucose dehydrogenase (DH).

Male rats of the Donryu strain, 10–12 weeks of age, were used. They were administered orally with 200 mg of eugenol in 1.0 ml of olive oil. Controls received olive oil concurrently. After collection of urine for 12 hours, they were killed and the liver was quickly taken out.

Liver homogenate (4 g of liver and 16 ml of 0.154 M KCl) was centrifuged at 8,500 $\times$ g, for 20 minutes and the supernatant was made to volume, to form the 4:20 preparation for the assay of GT. The half volume (10 ml) of this preparation was centrifuged at 18,000 $\times$ g, for 90 minutes and

the supernatant was used for the assay of DH.

The activity of GT was measured by a modification of the method of Isselbacher et al. [1], as described in the previous paper [4]. DH was assayed by the method of Strominger et al. [3]. The determination of protein was made by the method of Lowry et al. [2].

Urinary glucuronides were separated by the reduction of NaBH₄ into ether-type O-glucuronide (O-G) and others, and O-G was determined by the modification of Fishman's method, which was described in detail in the previous paper [4].

Table 1 shows the effects of eugenol treatment on the activity of liver GT and DH, and on the amount of urinary O-G in the rat. The rats were administered orally with eugenol or olive oil, six times in total at 12 hours intervals. The amount of eugenol given at one time was 200 mg in 1.0 ml of olive oil. Control group of the rats was treated with 1.0 ml of olive oil at all times. The group of one time administration of eugenol was treated one time of eugenol after five times of olive oil, and the group of four times administration of

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Table 1. Effect of eugenol on glucuronidation system in the rat

Times of eugenol	Enzymes of liver		Glucuronide in urine
	GT	DH	O-G
	m μ moles/min /mg of protein	$\Delta OD_{340} \times 10^3$ /min /mg of protein	mg/ 12 hour/ rat
Control	0.083 $\pm$ 0.026[10]* (100)**	7.0 $\pm$ 1.1[10] (100)	4.4 $\pm$ 0.9[12] (1.0)**
1	0.143 $\pm$ 0.041[10] (172)	6.6 $\pm$ 1.1[10] (94)	30.7 $\pm$ 6.1[10] (7.0)
2	0.193 $\pm$ 0.043[11] (233)	12.1 $\pm$ 4.6[12] (173)	66.5 $\pm$ 25.3[14] (15.1)
4	0.213 $\pm$ 0.047[11] (257)	18.2 $\pm$ 5.4[10] (260)	120.9 $\pm$ 29.0[14] (27.5)
6	0.231 $\pm$ 0.060[10] (278)	16.3 $\pm$ 3.2[9] (233)	117.9 $\pm$ 29.7[12] (26.8)

Remarks.

* In brackets is shown the number of rats used.

** In parentheses is shown the percentage value of enzyme activities or times value of the amount of glucuronide in urine, compared with the data of control.

eugenol was four eugenol after two olive oil, and so on. After the last administration, the urine was collected two rats in a cage for 12 hours, and then the liver was taken out.

In this table, it is noteworthy that the DH activity in rat liver increased significantly after repeated massive administration of eugenol. In the case of once oral administration of eugenol, urinary O-G and liver GT increased to 7.0-fold and 172% of control, respectively. However, liver DH had no change. In the case of twice administration, urinary O-G and liver GT more increased to 15.1-fold and 233% of control, respectively. Moreover, liver DH increased rapidly to 173% of control. The most enhanced case of glucuronidation system in this experiment was observed in the case of four times administration. Both liver GT and DH increased to 257% and 260% of control respectively, and urinary O-G reached to 27.5-fold. In the case of six times administration, liver DH rather decreased to 233%, and urinary O-G also decreased to 26.8-fold of control. On the other hand, liver GT more in-

creased to 278% of control than that of four times administration.

From the data of this graded enhancement of urinary O-G excretion, it was considered that there was a linear relationship between the accumulative dose of eugenol and the rate of its glucuronide biosynthesis. The figure of increasing of GT activity showed the dose-response curve of hyperbolic shape, which suggesting the direct effect of eugenol on the inducement of GT [5]. On the other hand, DH activity in rat liver increased gradually by the repeated administration of eugenol, showing the dose-response curve of sigmoid shape. The lag period of the curve represents the existence of metabolic pool of UDPGA for glucuro conjugation. It is extremely probable that the state of the lack of UDPGA becomes "the trigger", and then inductive biosynthesis of DH occurs rapidly. At the linear period of the sigmoid curve, there was a linear relationship ($r=0.996$) between DH activity and the amount of urinary O-G, which showing an example of a linear relationship between the amount of catalyst and of its product in vivo. From the

case of six times administration of eugenol, it was considered that the rate-limiting step at the enhancement of glucuronidation is the step of UDPGA biosynthesis.

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