The Effect of L-Asparaginase on Cholesterol Biosynthesis

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TAKEDA, T. and MATSUURA, N. *The Effect of L-Asparaginase on Cholesterol Biosynthesis.* Tohoku J. exp. Med., 1981, 133 (3), 289-291 — The liver from the Wistar rat was incubated either in the solution of 1 μ Ci acetate-1- 14 C or 0.1 μ Ci mevalonic acid-2- 14 C, and incorporations of radioactivity to phospholipid and cholesterol were estimated respectively. The incorporation of labeled acetate to cholesterol in the L-asparaginase-treated rat was significantly lower than that in the controls. However, there were no differences of the incorporation into the mevalonic acid between the study group and the controls. These results suggest that the inhibitory mechanism may exist between the steps of acetate and mevalonic acid. ——— L-asparaginase; cholesterol biosynthesis; acute lymphocytic leukemia

Since the first report of successful L-asparaginase treatment of acute lymphocytic leukemia and malignant lymphoma (Oettgen et al. 1967) the clinical evaluation of this drug has been established. There, various side effects of the drug were also described. Among these, the decrease in serum lipids, especially cholesterol, is the most remarkable one (Oettgen et al. 1970). The serum protein decreases by L-asparaginase, which may be due to the suppression of protein biosynthesis (Bettigole et al. 1970). However, the cause of the change in cholesterol level was not clearly understood. The decrease in cholesterol may be derived from the disturbances of cholesterol synthesis or the acceleration of cholesterol catabolism and another possibility may be depending upon the decrement of lipoprotein, which works as a carrier of cholesterol. To know whether L-asparaginase influences the cholesterol biosynthesis, we carried out the following studies.

MATERIALS AND METHODS

Male Wistar rats weighing 150-200 g were used. L-Asparaginase (Kyowa Hakko Co.) was injected intravenously in a single dose of 2000 U/kg. The rats were anesthetized with ether and the abdominal cavity was explored. Soon after exsanguination from the abdominal aorta, the liver was extirpated and was sliced with a hand slicer. The liver slices were incubated in an oxygen filled flask with culture medium, which was put on a shaker at 37°C for 90 min. 0.25 g of the liver slices was weighed and incubated in 3 ml of phosphate buffer (pH 7.2) with 0.3 ml substrate (30 μ moles Na acetate+1 μ Ci acetate-1- 14 C or 0.1 μ Ci mevalonic acid-2- 14 C). Filter paper dipped in 2 N NaOH was used for CO₂ absorption, instead of using 1 M Hyamin in methanol solution which brought about

insufficient results. At the end of incubation, the slices were washed in the buffer and ground in mortar. Lipids were extracted by using Folch's method (1957), which were washed twice in 0.154 M KCl solution and dried. 1 ml of CHCl_3 was added and radioactivity of 0.4 ml of this solution was measured to find the incorporation to total lipid. The remainder was precipitated by acetone, which was phospholipid. The supernatant was saponified and digitonin was used to separate cholesterol from glycerol fatty acid. Each fraction was put in toluene solution or Bray's counting solution to measure in a liquid scintillation counter. Results were calculated by the following formula:

$$\frac{A}{2.2 \times 10} \times 30 \text{ } \mu\text{moles/g wet liver weight/90 min, where}$$

A is dpm of each fraction/g of liver tissue.

RESULTS AND DISCUSSION

The rats were sacrificed on the 7th day after administration of 2000 U/kg of L-asparaginase when the serum cholesterol reached the lowest level (Table 1).

The incorporation of labeled acetate into $^{14}\text{CO}_2$, total lipid, phospholipid or glycerol fatty acid was not apparently different in L-asparaginase treated and control rats. But, the L-asparaginase treated rats showed a decreased incorporation of radioactivity into cholesterol (Table 2).

There were no differences in incorporation of mevalonic acid-2- ^{14}C into cholesterol between the two groups of rats (Table 3).

CO_2 is produced in the first step from acetate, which is metabolized to acetyl CoA (Brodie et al. 1963). As far as CO_2 formation was concerned, there were no differences between the experimental groups and the controls. From these results, the L-asparaginase treatment may not inhibit cholesterol biosynthesis further beyond mevalonic acid. This suggests that the site of disturbance

TABLE 1. *Effect of L-asparaginase on serum cholesterol levels in fasted rats*

	Serum cholesterol (mg/100 ml)	t
Before	82.0 ± 24.0	
2nd day	48.6 ± 8.9	$p < 0.001$
4th	63.9 ± 9.5	$p < 0.05$
7th	46.5 ± 10.2	$p < 0.001$
11th	44.5 ± 10.9	$p < 0.001$

TABLE 2. *Incorporation of labeled acetate into lipids ($\mu\text{moles acetate incorporated/g wet liver wt./90 min}$)*

	$^{14}\text{CO}_2$	Total lipid	P-L	Cholesterol	Glycerol F.A.
Control ($n=8$)	7.61 ± 3.05	0.392 ± 0.109	0.040 ± 0.016	0.164 ± 0.057	0.093 ± 0.019
Asparaginase ($n=13$)	5.98 ± 1.03	0.504 ± 0.254	0.063 ± 0.042	0.115 ± 0.048	0.146 ± 0.089
t	$p > 0.05$	$p > 0.2$	$p > 0.1$	$p < 0.05$	$p > 0.1$

TABLE 3. *Incorporation of labeled mevalonate into cholesterol*

	%
Control (<i>n</i> =8)	27.8±5.1
Asparaginase (<i>n</i> =8)	23.6±3.6
<i>t</i>	<i>p</i> >0.05

(% mevalonic acid incorporated/g wet liver
wt./90 min)

exists in the process between acetyl CoA and mevalonic acid. The rate limiting step in this metabolism is from HMG-CoA to mevalonic acid (Tavarmina et al. 1956; Goldfarb 1978) and therefore, probably L-asparaginase suppresses this step. This step is carried out in the microsomal fraction, and protein synthesis, which is inhibited by L-asparaginase, is also performed in the microsome fraction.

Whether L-asparaginase administration disturbs only cholesterol synthesis or microsome itself remains to be solved.

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