LIPID METABOLISM IN TUMOUR BEARING MICE TREATED WITH *AEROMONAS* L-ASPARAGINASE

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ABSTRACT

The anticancerous drug isolated in our laboratory from estuarine *Aeromonas* was characterised and is found to be an enzyme, L-asparaginase. The antileukaemic effect of this drug was studied in mice by inducing leukaemia with Ehrlich ascites cell lines. It was compared with commercially available drug, Leunase, isolated from *E. coli*. The lipid profiles in mice during leukaemia and under treatment was studied. The decreased levels of cholesterol and increased levels of triglycerides and phospholipids in serum, liver and kidney were observed in tumour bearing mice. Significant changes in the above values were observed with enzyme therapy. It could bring some of the values to near normal level. L-asparaginase from *Aeromonas* was found to be more effective.

KEYWORDS: *Aeromonas*, L-Asparaginase, Lipids, Tumour

INTRODUCTION

The enzyme, L-asparaginase isolated from *E. coli* and commercially available under the trade name 'Leunase' is used very significantly in the treatment of acute leukaemia. This enzyme catalyses the hydrolysis of L-asparagine which can not be synthesized by the sensitive tumour cells due to the deficiency of the enzyme asparagine synthetase. Normal cells, however, are able to synthesize L-asparagine from aspartic acid and ammonia with the help of asparagine synthetase and hence do not appear to have an absolute requirement for exogenous L-asparagine.

Reports available indicate that L-asparaginase isolated from different sources have different antineoplastic effect with varying immunological complications (1,2). L-asparaginase from estuarine *Aeromonas* isolated in our laboratory (3) was purified and the antileukaemic effect was studied (4). *E. coli* asparaginase contains two forms EC1 and EC2, of which EC2 is found to be antileukaemic. The advantage of the new drug over the commercially available drug is that the total enzyme is

antileukaemic. Lipid profiles in cancer and under treatment were studied by several workers (5,6). Metabolism of carbohydrates, proteins and glycoproteins have also been studied extensively in cancer (7,8). But very little work is available in the metabolism of carbohydrates and glycoproteins during enzyme therapy (9,10). Similarly a few reports are available on the metabolism of lipids during L-asparaginase therapy (11,12).

Lipid profiles viz. cholesterol, triglycerides, and phospholipids in serum and tissues (liver and kidney) were studied in tumour bearing mice and under treatment with the drug isolated from *Aeromonas* and has been compared with Leunase and the results are discussed in this paper.

MATERIALS AND METHODS

Experimental Animals

Inbred Balb/c mice of 7 weeks age, weighing 14-17 g were used for the study. The mice were obtained from the stock inbred colony which was maintained by mating of brothers and sisters. The mice were maintained on dry pellets of laboratory feed (Gold Mohur rat feed, Hindustan Lever Ltd.) and tap water.

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Tumour cell lines and their maintenance

Ehrlich ascites tumour cell line, kindly provided by Amala Cancer Research Institute, Trissur was used for the study. The tumour cell line was maintained by serial intraperitoneal (IP) transplantation in mice. Full grown tumour cells were aspirated from the mouse peritoneum, washed thrice with 0.9% saline, and suspended in saline. About 1×10^5 cells were injected intraperitoneally into a new healthy mouse. All operations were done aseptically. Solid tumour growth was obtained from the ascites cells by injecting 5×10^4 cells subcutaneously under an area of shaved skin on the flank.

Asparaginase Preparation

(a) *Aeromonas L-asparaginase*

Growing of cells, preparation of crude cell free extract and purification were done as described by Benny (4). The enzyme preparation obtained after Ammonium sulfate precipitation, Gel permeation hydroxyapatite chromatography and lyophilization was dissolved in 0.9% saline.

(b) *Leunase enzyme*

The commercially available *E. coli* L-asparaginase, manufactured by Kyowa Hakko Kogyo Co. Ltd., Japan, marketed by Biochem India Ltd. under the trade name Leunase was used to compare the effect of *Aeromonas* L-asparaginase. The lyophilized enzyme preparation was also dissolved in 0.9% saline.

Experimental groups

Mice were divided into 7 groups, consisting of 6 mice in each group and were maintained as follows:

Group 1: Normal mice maintained with normal laboratory feed.

Group 2: Control group. Ehrlich ascites tumour implanted mice. About 1×10^5 tumour cells were injected into the peritoneal cavity. These mice were not treated and were sacrificed on the 8th day of

tumour implantation.

Group 3: Tumour implanted mice treated with Leunase. 24 h after tumour implantation, Leunase was administered through the same route in 3 intermittent doses of 25 units, at an interval of 48h and were sacrificed on the 8th day of tumour implantation.

Group 4: Tumour implanted mice, treated with *Aeromonas* L-asparaginase. Injections were done as described earlier, and sacrificed on the 8th day of tumour implantation.

Group 5: Control mice bearing tumour, that were not treated. Sacrificed on the 15th day of tumour implantation.

Group 6: Tumour implanted mice treated with Leunase as in Group 3. Sacrificed on the 15th day of tumour implantation.

Group 7: Tumour implanted mice, treated with *Aeromonas* L-asparaginase preparation as in Group 4. Sacrificed on the 15th day of tumour implantation.

Blood from mice was collected, after the stipulated time by cutting the jugular vein with a sharp blade. Blood was allowed to clot and the serum was collected by centrifugation at 1,500 rpm for 10 min. The liver and kidney were removed to ice cold containers, after washing with saline to remove the blood. 500 mg each of kidney and liver were homogenated with 5 ml distilled water in a Remi homogenizer to get a 10% homogenate. This homogenate and serum were used for the estimation of lipids. Extraction of the tissues for the estimation of cholesterol was carried out by the method of Abell et al. (13). The triglycerides and phospholipids were estimated by the methods of Van and Zilversmit, and Zilversmit and Davis as described by Kurup et al. (14,15).

RESULTS AND DISCUSSION

Levels of Cholesterol, Triglycerides, phospholipids in serum and tissues (liver and kidney) of tumour bearing and L-asparaginase treated mice were studied and compared with those of normal. The results are given in Tables 1-3.

Table 1. Serum and tissue cholesterol levels in tumour bearing and L-asparaginase treated mice.

Groups	Serum	Tissues	
		Liver	Kidney
Normal	78.34±3.71	372.46±18.15	387.63±16.47
8 days post tumour implant	48.20±7.35 ^a	251.12±18.47 ^a	309.08±21.52 ^a
Treated with Leunase	56.77±4.17	439.10±24.59 ^a	353.63±27.28 ^b
Treated with Asparaginase	54.27±3.85	397.16±27.65 ^a	347.45±29.84 ^b
15 days post tumour implant	41.35±3.15 ^a	219.03±15.28 ^a	234.25±14.56 ^a
Treated with Leunase	52.74±4.29 ^a	446.11±20.73 ^a	286.01±17.75 ^a
Treated with Asparaginase	56.96±3.85 ^a	381.72±18.28 ^a	298.12±20.56 ^a

Values are expressed as mg cholesterol/100 ml serum or 100 g wet tissue

Average of 6 values in each group ± SEM

Group 2 & 5 have been compared with Group 1

Group 3 & 4 have been compared with Group 2

Group 6 & 7 have been compared with Group 5

a - p less than 0.01

b - p between 0.01 and 0.05

Table 2. Triglyceride levels in serum and tissues of tumour bearing and L-asparaginase treated mice.

Groups	Serum	Tissues	
		Liver	Kidney
Normal	5.83±0.416	452.61±20.81	62.70±4.15
8 days post tumour implant	9.33±0.681 ^a	498.64±27.19 ^b	60.01±5.91
Treated with Leunase	11.08±0.925 ^b	543.12±37.78	81.46±6.83 ^a
Treated with Asparaginase	8.71±0.546	562.38±39.45 ^b	78.43±6.24 ^a
15 days post tumour implant	19.82±1.146 ^a	578.90±32.46 ^a	65.59±4.97
Treated with Leunase	12.83±0.974 ^a	527.86±35.71 ^b	59.12±3.81 ^b
Treated with Asparaginase	7.91±0.546 ^a	514.74±38.29 ^b	61.64±4.68

Values are expressed as mg glycerol/100 ml serum or 100 g wet tissue

Average of 6 values in each group ± SEM

Group 2 & 5 have been compared with Group 1

Group 3 & 4 have been compared with Group 2

Group 6 & 7 have been compared with Group 5

a - p less than 0.01

b - p between 0.01 and 0.05

Table 3. Serum and tissue phospholipid levels in tumour bearing and L-asparaginase treated mice.

Groups	Serum	Tissues	
		Liver	Kidney
Normal	288.31±16.41	1953.54±87.65	1852.51±76.49
8 days post tumour implant	461.28±37.56 ^a	1886.17±83.78	1620.97±70.44 ^a
Treated with Leunase	547.77±39.65 ^b	1998.28±86.69	1458.86±62.64 ^b
Treated with Asparaginase	527.33±41.17 ^b	2114.15±90.52 ^b	1591.47±67.61
15 days post tumour implant	691.92±40.84 ^a	1941.35±81.26	1945.91±73.24
Treated with Leunase	634.26±48.72	2150.32±84.77 ^b	1296.78±54.77 ^a
Treated with Asparaginase	531.82±44.92 ^a	2079.46±90.55 ^b	1446.59±58.65 ^a

Values are expressed as mg phospholipids/100 ml serum or 100 g wet tissue

Average of 6 values in each group ± SEM

Group 2 & 5 have been compared with Group 1

Group 3 & 4 have been compared with Group 2

Group 6 & 7 have been compared with Group 5

a - p less than 0.01

b - p between 0.01 and 0.05

Cholesterol levels in serum, liver and kidney were found to be decreased significantly with the progression of tumour growth. Highly elevated cholesterol level was observed in liver during administration of L-asparaginase, but the increase in serum and kidney were not found to be very significant. The increase in serum was pronounced in groups treated with *Aeromonas* L-asparaginase and at a later stage L-asparaginase administration resulted in concomitant increase of liver cholesterol.

A significant elevation of triglyceride levels in serum and liver was found during the early stages of tumour growth and no change was observed in kidney. Highly elevated levels of triglycerides in liver was found during the later stages of tumour growth but the change in kidney was not very significant. Triglyceride levels in liver and kidney were found to be increased with administration of L-asparaginase. But increase was more significant in kidney. A few days after the cessation of therapy, a significant reduction in triglyceride level in serum was observed. In groups treated with *Aeromonas* L-asparaginase, serum triglyceride values returned to near normal.

Serum phospholipids increased significantly during the early stage of tumour growth. In kidney,

its level was found to be decreased. The serum phospholipids remain elevated and the liver phospholipids returned to normal at a later stage of tumour growth. Kidney phospholipids were found to be decreased during the early stage of tumour growth and increased during the later stage. In serum, its level was found to be elevated after L-asparaginase administration. Cessation of therapy resulted in significant decrease in groups treated with *Aeromonas* L-asparaginase and no significant decrease was observed in groups treated with Leunase. Phospholipids in liver was also altered significantly upon the administration of both *E. coli* and *Aeromonas* L-asparaginase. In kidney, its level was found to be decreased immediately after L-asparaginase administration, but a few days after the cessation of therapy, the level decreased significantly. This decrease was more marked in groups treated with *E. coli* L-asparaginase.

The lipid profile in patients with malignancies was studied by Kritchevsky et al. and reported that cholesterol level in patients with non localized malignancies dropped below the normal level (5). These alterations might be useful as differential markers with the more mature leukaemia having more complex glycolipids as suggested by William et al. (6). He found that the composition of neutral

glycosphingolipids in acute leukaemic cells differs significantly from that found in normal or chronic leukaemic cells. Only a few reports are available on the effect of L-asparaginase on lipid metabolism. Low serum cholesterol has been reported and has been shown to be related to decrease in the serum alpha, pre- β and β lipoproteins (10,11). Hypcholesterolemia associated with L-asparaginase therapy may be due to decreased synthesis of proteins required for the transport of cholesterol (12). Reports available suggests that there is no clear relationship between tumour growth and triglyceride pattern. However, in the present study we observed a significant elevation of serum triglyceride levels. The increased triglyceride levels observed in serum and liver during tumour growth might be due to the decreased production of enzymes concerned with the catabolism of triglycerides or may be due to increased biosynthesis. since lipoprotein lipase, triglyceride lipase and other enzymes of triglyceride/lipoprotein metabolism could not be studied, a definite conclusion could not be drawn. At a later stage of therapy, the triglyceride levels in serum decreased and came to near normal level in *Aeromonas* L-asparaginase treated groups. A decreased level in serum during therapy was an important observation.

Hypocholesterolemia associated with L-asparaginase therapy has been related to the decreased synthesis of proteins necessary for cholesterol transport (10,12). Hypcholesterolemia observed in both L-asparaginase treated and nontreated tumour bearing mice in the present study is in agreement with the views suggested by Kritchevsky et al. (5). Decreased cholesterol level may be due to the growth of tumour rather than the direct action of L-asparaginase.

Haskal et al. and Yoon et al. have suggested that L-asparaginase administration cause fat infiltration into hepato parenchymal cells of man and mice (11,12). An increase in phospholipids in fatty liver was observed by Yoon et al. (12). They also observed fat infiltration and numerous irregular size lipid droplets in the paranchymal cells of mice treated with L-asparaginase. Histological examination of the liver showed fat infiltration in man during the first two weeks of treatment which subsides upon cessation of L-asparaginase therapy (10,11). The elevated levels of phospholipids and triglycerides in liver and kidney in the present study might be due to fat infiltration.

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