SPECIALIA

Changes induced by Ehrlich ascites carcinoma in hepatic fumarase and aconitase activities

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Summary. Ehrlich ascites carcinoma induced significant changes in liver fumarase (activation) and aconitase (inhibition). A significant increase in hepatic fumarase specific activity was also observed in mice inoculated with Ehrlich ascites fluid after centrifugation of cells (EAF). Hepatic aconitase was not significantly influenced by EAF.

It is well known that malignant neoplasms have a lower aconitase content than normal tissues[2,3]. These studies also revealed that fumarase occurs in tumors in amounts comparable to those in normal tissues of rats and mice. Hano and Akashi[4] reported a daily decrease in the levels of hepatic aconitase in Ehrlich tumor-bearing mice. The present study was performed to determine the liver fumarase activity in mice inoculated with Ehrlich ascites carcinoma in comparison with the aconitase levels. Studies were also undertaken in mice treated with Ehrlich ascitic fluid (EAF) free of cells.

Materials and methods. The experiments were carried out with male Swiss mice (mean b. wt 28 g) maintained in a standard balanced diet and tap water ad libitum. Animals were divided into the 4 groups shown in the table. Mice in Ehrlich group were injected, by i.p. route, with a clear non-hemorrhagic suspension of Ehrlich ascites carcinoma removed from a mouse treated similarly 10 days before. The ascitic tumor diluted in isotonic saline at the concentration of 2.0×10^6 Ehrlich viable cells (trypan blue dye exclusion)/0.1 ml was immediately administered, at this dose level, to the recipient animals. A normal group was maintained without any treatment. The 3rd group was injected i.p. with 3 ml of EAF/mouse/day during 3 days. EAF was prepared by centrifuging Ehrlich ascites carcinoma at 3000 rpm for 30 min at room temperature. This fraction does not cause the growth of tumors when injected in mice. The total dose of EAF used per mouse was in the range of the volume present in Ehrlich carcinoma at 10 days of evolution. Animals in the control group were treated, by i.p. route, with 3 ml of isotonic saline/mouse/day, during 3 days. All the animals were sacrificed by cervical traumatism. Mice in Ehrlich group were killed 10 days after inoculation and the animals in EAF and control groups were sacrificed 24 h after the last administration of EAF or saline, respectively. The livers were immediately excised and 5% homogenates were prepared in ice-cold 0.1 M phsophate buffer, pH 7.4.

Effects of Ehrlich ascites carcinoma and EAF* on liver fumarase and aconitase

Groups	Treatment	Fumarase (units/5 min/ mg protein)	Aconitase (units/10 min/ mg protein)
Ehrlich	Tumor cells	1026 ± 59 (6)	59 ± 4 (8)
Normal	None	812 ± 30 (8)	111 ± 6 (8)
EAF	Tumor supernatant	1040 ± 58 (7)	97 ± 13 (8)
Control	Saline	840 ± 54 (6)	110 ± 16 (6)

Data expressed as means ± SEM. Number of mice in parentheses.
* Ehrlich ascitic fluid free of tumor cells.

Fumarase and aconitase activities were determined by a modification[5,6] of the spectrophotometric method of Racker[7]. Incubations were carried out at 37 °C for 5 min (fumarase) or 10 min (aconitase) after the addition of 0.05 ml (fumarase) or 0.20 ml (aconitase) of the homogenates to start the reactions. 1 unit of fumarase or aconitase activity is equivalent to an increment in optical density of 0.001. Total proteins in the homogenates were determined by the biuret method of Gornall, Bardawill and David[8] using bovine plasma albumin as standard, and the enzymatic activities were expressed in units/mg of protein. The statistical analysis of the data was carried out using Student's t-test.

Results and discussion. As shown in the table, the activity of hepatic fumarase was significantly increased ($p < 0.01$ vs normal) in mice bearing Ehrlich ascites carcinoma. Similarly the levels of fumarase in EAF treated mice were found significantly higher ($p < 0.05$) in comparison with the control group. On the other hand, a significant inhibition of hepatic aconitase was found in Ehrlich carcinoma group ($p < 0.001$ vs normal). Aconitase activity was not significantly modified in EAF-treated mice ($p < 0.5$ vs controls).

The inhibition of liver aconitase activity observed by us in Ehrlich tumor-bearing mice is in accordance with the data of Hano and Akashi[4]. Low levels of this enzyme were also found by other authors[9,10] in fetal rat liver. Our results clearly show that Ehrlich ascites carcinoma induce an increase in hepatic fumarase activity. This activation is systemically induced since EAF, devoid of tumor cells, has the same effect. Ehrlich carcinoma cells probably liberate a substance or substances to ascitic fluid with the property to influence the hepatic fumarase levels. Further investigations were necessary to elucidate the mechanism of this effect.

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