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The effect of estriol on the production of alpha-fetoprotein by the liver in adult mice

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Summary. A single intraperitoneal injection with a 10 mg estriol (E_3) in aqueous suspension induced a large and prolonged elevation of serum alpha-fetoprotein (AFP) in adult mice. E_3 also raised the mitotic activity of hepatocytes in the absence of liver injury. Although both the AFP concentration and hepatocyte proliferation reached the peak on day 5 after E_3 administration, a high level (about 12500 ng/ml) of serum AFP persisted for a long period after hepatocyte proliferation declined. Five mg E_3 showed a remarkable threshold effect on AFP elevation and 3 mg E_3 on hepatocyte proliferation. Immunohistochemical studies indicated AFP production by hepatocytes in adult mice after the E_3 administration.

Key words: Alpha-Fetoprotein – Estrogen – Liver – Mouse – Adult

Introduction

alpha-Fetoprotein (AFP) is a serum protein produced mainly by hepatocytes in the fetal and neonatal stages. The AFP level in adults is normally very low (Abelev 1971; Ruoslahti and Seppälä 1971) but AFP is produced in association with the hepatocyte proliferation induced by partial hepatectomy or hepatotoxic chemicals (see for example Abelev 1971; Sell et al. 1974; Smuckler et al. 1976; Watanabe et al. 1976). Recently, we have reported that a large dose of estrogens provokes active hepatocyte proliferation in adult mice (Fujii et al. 1985; Fujii and Kotani 1986). The aim of the present work is to determine whether such a large dose of estrogens induces AFP production, associated with the hepatocyte proliferation. Since estriol (E_3) was the best steroid in terms of inducing hepatocyte proliferation in the mouse (Fujii et al. 1985), E_3 was chosen to examine the effectiveness of the production of AFP in this study.

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Materials and methods

Male (C57BL/6 × DBA/2) F_1 mice, 10 weeks old and approximately 28 g in body weight, were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu. They were maintained in our laboratory under conventional conditions and fed a standard laboratory chow.

Mice were injected intraperitoneally with 0.1–10 mg E_3 in 1.0 ml aqueous suspension (Estriel, Mochida Pharm. Co., Tokyo, Japan). Control mice were given 1.0 ml medium (50 mg arabic gum and 1 mg polysolvent 80 in 100 ml isotonic saline) only, without E_3, in the same manner. The E_3 concentration was measured on a pooled serum from 3–5 animals at each time point by radioimmunoassay in the Kitazato-Bristol Laboratories, Kanagawa.

Blood was withdrawn from the inferior vena cava under ether anesthesia and allowed to clot for 2 h at 37° C. The serum was stored at −20° C until assayed. Purified mouse AFP and anti-mouse AFP were prepared by the method using immunoadsorbent columns of Sepharose described elsewhere (Nishi and Hirai 1972; Taga 1983). Serum AFP concentrations were determined by so-called rocket electrophoresis (Taga 1983).

Colchicine was injected subcutaneously at a dose of 0.1 mg/100 g body weight 6 h before sacrifice. Small pieces of the liver from 3 mice in each time point after E_3 administration were fixed in Carnoy's fluid, embedded in paraffin, sectioned serially at 6 μm-thickness and stained with haematoxylin and eosin (HE) or methyl-green/pyronin (MGP). The ratio of the number of hepatocytes in the metaphase of mitosis to 5000 hepatocytes was calculated in each animal.

Cellular localization of AFP was studied by the immunoperoxidase reaction on the tissue sections. Small pieces of liver (about 2 × 2 × 2 mm) were fixed in Zamboni's fluid (Stefanini et al. 1967) for 2–3 days and embedded in paraffin. Serial sections at 6 μm-thickness were dewaxed, hydrated and rinsed in distilled water. After inhibition of endogenous peroxidase activity by a treatment with periodic acid and sodium borohydride according to a method described by Heyderman and Neville (1977), the sections were treated with rabbit anti-mouse AFP

serum at 1/100 dilution for 40 min at room temperature, washed throughly with PBS, treated with swine anti-rabbit IgG (Dakopatts, Copenhagen, Denmark) conjugated with horseradish peroxidase for 30 min and washed again. Peroxidase activity in the sections was located after reaction with 3,3′-diaminobenzidine tetrahydrochloride and H_2O_2 at room temperature. The sections were counterstained with haematoxylin.

Results

E_3 was not detected in the serum of the control mice that received the vehicle only. In experimental mice, the serum E_3 concentration increased greatly soon after the intraperitoneal injection with a 10 mg E_3 suspension and fell gradually with time (Fig. 1). However, a considerable E_3 concentration was still observed at the end of this experiment, 30 days after E_3 administration. The time course of hepatocyte proliferation and serum AFP concentration after the 10 mg E_3 administration is shown in Fig. 2. The mitotic index of the hepatocytes rose sharply from 3 days after the administration of 10 mg E_3 and reached its peak on day 5. After the peak on day 5, the mitotic index fell rather rapidly during the next 5 days and returned to normal by day 15. The serum AFP concentration increased from day 3 (2060 ng/ml ± 720 ng/ml) after the 10 mg E_3 administration and reached a high level (12500 ng/ml ± 1000 ng/ml) on day 5. This high level of AFP persisted to day 15, while the proliferation of hepatocytes ceased by day 15. There was a sharp drop from 12100 ng/ml ± 133 ng/ml to 4150 ng/ml ± 3920 ng/ml between day 15 and day 20. Small amounts of AFP (110 ng/ml ± 20 ng/ml) were still detected on day 30. The effect of different doses of E_3 on the hepatocyte proliferation and serum AFP concentration 5 days after the E_3 administration is shown in Table 1. Three mg E_3 was a critical threshold dose which sparked off hepatocyte proliferation. No significant differences between 3–10 mg E_3 in the mitotic index were found. On the other hand, 5 mg of E_3 showed a remarkable threshold effect on the elevation of the serum AFP level.

Histologically, the cytoplasm of the hepatocytes showed prominent basophilia in sections stained with HE and pyroninophilia in sections stained with MGP in 3 days after the administration of 10 mg E_3. No necrotic hepatocytes were found by light microscopy throughout this study. Immunoperoxidase staining on tissue sections of the liver 5 days after the 10 mg E_3 administration revealed many AFP positive hepatocytes (Fig. 3a), found scattered or in small groups. Among the ordinary sized AFP positive hepatocytes, smaller hepatocytes containing AFP were frequently observed (Fig. 3b). Their nuclei were often oval in shape. No definitely positive hepatocytes were found in the control mice.

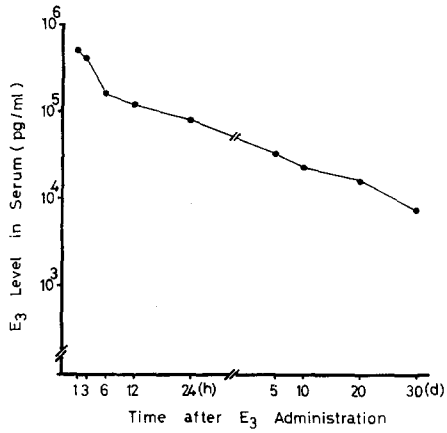

Fig. 1. Time course of serum E_3 concentration after a single intraperitoneal injection with 10 mg of a E_3 suspension. Each point represents the value measured on a pooled serum from 3–5 animals. h = hours; d = days

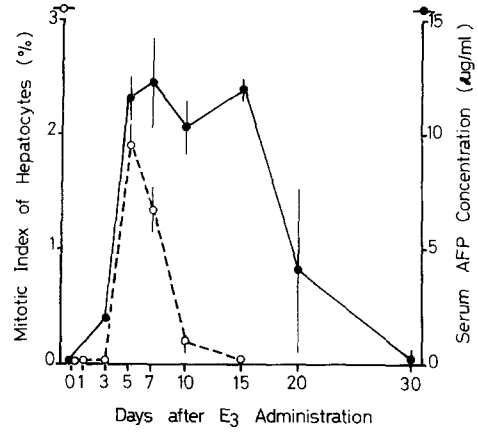

Fig. 2. Time course of the mitotic index of hepatocytes and AFP concentration after the administration of 10 mg E_3. Each point represents a mean value of 3–6 animals and vertical bar shows standard error

Table 1. The mitotic index (MI) and serum α-fetoprotein (AFP) concentration 5 days after the E_3 administration

Dose of E_3 (mg)	MI (%)	AFP (ng/ml)
(–)	0.01 ± 0.03	28 ± 2[a]
1	0.71 ± 0.25	373 ± 59
3	2.51 ± 0.25	580 ± 197
5	2.07 ± 0.15	6300 ± 1300
10	2.10 ± 0.20	12500 ± 1000

[a] Mean ± SE (3–6 mice)

Discussion

The reproductive tract is the major target organ for estrogens. However, large doses of estrogens

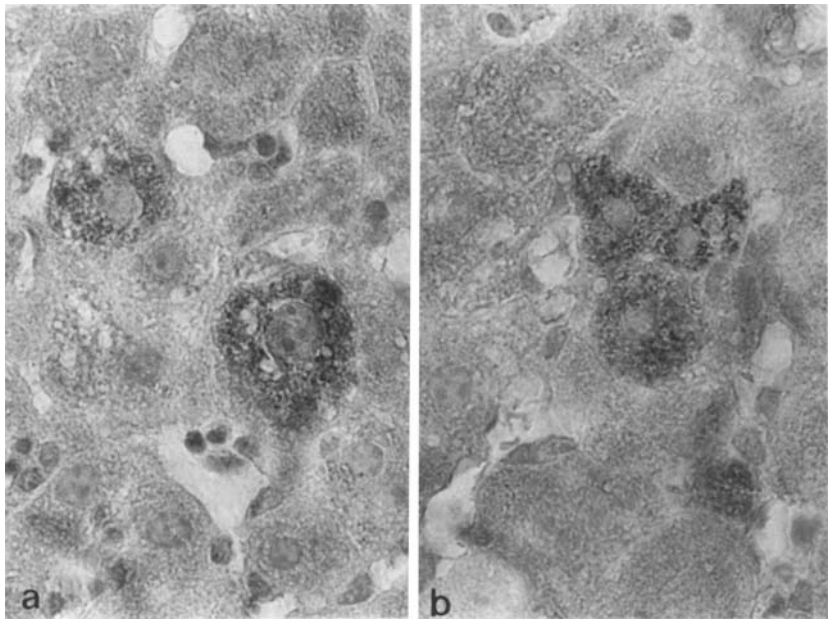

Fig. 3a, b. Immunoperoxidase staining of AFP in the liver of a adult mouse on day 5 after the administration of 10 mg E_3. **a** Two hepatocytes containing AFP are seen; **b** Three smaller AFP positive hepatocytes compared to ordinary sized hepatocytes in Fig. 3a are found in a group. Their nuclei are oval. × 700

have effects on the lymphoid tissues and the reticuloendothelial system, resulting in involution of the thymus (Dougherty 1952), stimulation of the phagocytic activity of macrophages (Nicol et al. 1964), and inhibition of immune responses (Ezaki et al. 1982). The present study reported that the exposure of adult mice to a large dose of 10 mg E_3 induced a highly elevated serum concentration of AFP. This level was about 10-fold higher in comparison with the maximum serum concentration produced by partial hepatectomy (Watanabe et al. 1976). It was also 2- to 3-fold higher than that produced after treatment with CCl_4 (Watanabe et al. 1976). Ten mg E_3 simultaneously induced an active proliferation of hepatocytes. The elevation of serum AFP concentration was grossly associated with the proliferation of hepatocytes, but not as precisely as reported by other investigators in hepatectomy or chemically induced liver necrosis (Alpert 1972; Engelhardt et al. 1976; Smuckler et al. 1976; Watanabe et al. 1976; Sell and Becker 1978). A rise of serum AFP concentration was detectable prior to the onset of hepatocyte proliferation and a high serum AFP persisted for a long period after hepatocyte proliferation declined. Such prolonged elevation of AFP is a characteristic feature of E_3 treatment in comparison with that which occurs in hepatic injury. It should be noted that E_3 induces AFP elevation and hepatocyte proliferation in the absence of liver injury by light microscopy. Three mg E_3 showed a remarkable threshold effect on hepatocyte proliferation and 5 mg E_3 on AFP elevation.

Immunohistochemical studies provide clear evidence that AFP can be produced by hepatocytes in adults after E_3 administration. We have previously reported the appearance of many haemopoietic foci in the adult mouse liver after the administration of 10 mg E_3 (Hayama et al. 1983). Granulocyte-macrophage colony-stimulating activity in the serum of E_3-treated adult mice has also been reported (Hayama et al. 1985). Changes such as the proliferation of hepatocytes, AFP production by hepatocytes and hepatic haemopoiesis, in the adult liver after the E_3 administration may represent a transient reversion to the fetal liver. These changes may also suggest a possible physiological role for the high level of E_3 in human embroys (Roy and Brown 1960; Diczfalusy 1969).

However, there is general agreement that elevated serum AFP in adults may be associated with hepatocellular carcinoma in animals (Abelev et al. 1963; Taga 1983) and man (McIntire et al. 1972; Ruoslahti et al. 1974). The elevated level of serum AFP concentration after E_3 administration in this study was as high as the AFP concentration in some hepatoma-bearing animals (Watabe et al. 1972; Sell and Morris 1974; Taga 1983). The problem of whether elevated serum AFP after E_3 administration is an indicator of potential liver malignancy remains to be established. The present study demonstrated an appearance of small AFP positive hepatocytes with oval nuclei in the liver 5 days after the 10 mg E_3 administration. An appearance of the so-called oval cells which are responsible for AFP production has been reported in the early

stages of carcinogenesis with azo-dye and 2-acetylaminofluorene (Dempo et al. 1975; Tchipysheva et al. 1977) or with N-nitrosomorpholine (Kuhlmann 1978). Cirrhosis in humans, which is considered to be regarded as a premalignant condition, is often accompanied by elevated estrogen concentrations (Kley et al. 1976) and by elevated AFP concentrations (Ruoslahti et al. 1974; Okuda et al. 1975; Lehmann 1976). The present study may provide a new approach in solving problems related to the possible correlation between estrogen and AFP production in carcinogenesis. There is no available information, to our knowledge, about the production of AFP by liver-cell adenoma following long-term use of oral contraceptives (Baum et al. 1973).

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