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Depression of Liver Drug Metabolism in Sarcoma-bearing Mice. Evidence for a Circulating Factor and Dissociation from Lipolytic Activity

RICCARDO BERTINI and PIETRO GHEZZI

Laboratory for Enzyme Research, Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea 62, 20157 *Milano, Italy*

Abstract—*Mice bearing the S-180 sarcoma displayed a depression of liver catalase and cytochrome P-450-dependent enzymes (ethoxycoumarin deethylase, ED) from day 6 following tumor implantation. Injection of serum obtained from tumor-bearing mice into normal mice caused depression of liver ED suggesting that a circulating factor was involved. Tumor-bearing mice did not show any significant change in serum triglycerides and food intake. By contrast, injection of endotoxin, interleukin-1 (IL-1) or tumor necrosis factor (TNF) caused not only a depression in liver ED but also a marked increase in serum triglycerides.*

To study the possible analogies between cancer-associated circulating factor and monokines, we studied the effect of dexamethasone (a known inhibitor of monokine synthesis) on liver ED activity in tumor-bearing mice. Dexamethasone (DEX) treatment increased (up to 60%) liver ED activity in tumor-bearing mice.

We conclude that: (i) *a circulating factor is involved in cancer-associated ED depression*; (ii) *that this mediator is not necessarily identical to TNF or IL-1 and* (iii) *that DEX reverses the depression of liver ED in cancer, possibly by inhibiting the synthesis, or the effects, of this factor.*

INTRODUCTION

DEPRESSION of liver cytochrome P-450-dependent drug metabolism was reported to be associated with infection, inflammation and cancer [1–3]. This is associated with other metabolic alterations including hypoferremia and hypozincemia [4, 5], induction of acute phase proteins [4, 6] and wasting and weight loss [7, 8]. As early as 1949, Nakahara and Fukuoka [9] reported that catalase was depressed in the liver of tumor-bearing animals, and presented experimental evidence that a tumor product was responsible for this effect. The tumor-derived product depressing catalase activity was termed 'toxohormone', and later other authors reported that various toxohormones reproduced some of the changes observed in tumor-bearing animals, namely depression of plasma iron levels [10], cachexia [7] and depression of liver cytochrome P-450 [11]. A lipolytic factor prepared from S-180 ascites fluid was also characterized and termed 'toxohormone-L', and was proposed to be responsible for the depletion of fat stores observed in cancer [12]. Liver drug metabolizing enzymes were reported to be depressed in mice treated with interleukin-1 (IL-1) and tumor necrosis factor (TNF) [13, 14], two macrophage-derived mediators that play an important role in the acute phase response to infection and inflammation. It was suggested that IL-1 and/or TNF (also known as cachectin) could mediate some of the metabolic alterations observed in cancer diseases [3, 8].

However, it is not clear whether a single mediator is implicated in the various metabolic changes observed in tumor-bearing animals [15], and whether these mediator(s) are identical to those implicated in the inflammatory acute phase response. However, the possibility that TNF or IL-1 might mediate cancer-associated metabolic changes is still just a hypothesis. In the present paper, using the S-180 murine sarcoma, we have examined the possible correlations between cancer-associated depression of liver drug metabolizing enzymes and other biochemical parameters associated with the acute phase response, i.e. liver catalase, plasma iron and serum triglycerides. Food intake was also monitored since previous publications indicated that anorexia is also induced in tumor-bearing animals. Since a serum factor from

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Send correspondence and reprint requests to Dr. Pietro Ghezzi at the above address.

tumor-bearing rats was demonstrated to depress liver drug metabolism [11], we have also tested the eventual ability of serum from S-180 sarcoma-bearing mice to depress liver drug metabolizing enzymes when injected into normal mice. To study the possible analogies between this putative cancer-associated factor and monokines like IL-1 and TNF we have also studied the effect of dexamethasone (DEX) that reportedly inhibits IL-1 and TNF synthesis [16, 17] and protects against inflammation-induced depression of liver drug metabolism [18].

MATERIALS AND METHODS

Materials

Endotoxin (from *E. coli* 055:B5) was obtained from Sigma, St Louis, MO. Recombinant human TNF (specific activity 1×10^7 U/mg) was a kind gift of Dr. L.S. Lin, Cetus Corporation, Emeryville, CA. Recombinant human IL-1 alpha (specific activity 2.1×10^7 U/mg) was a kind gift of Dr. P.T. Lomedico, Hoffman-La Roche, Nutley, NJ. Dexamethasone phosphate (Soldesam) was from Farmacologico Milanese, Milan.

Animals and treatment

Male, adult (30 g) CD-1 mice (Charles River, Calco, Italy) were used. Animals were housed five per cage in air conditioned quarters (60% relative humidity, 22°C) with a 12 h light–dark cycle, and were given standard laboratory chow (Altromin, Rieper, Bolzano, Italy).

The mice were injected intramuscularly in the right paw with 0.2 ml of sarcoma 180 suspension (2×10^5 cells/mouse). DEX was given i.p. at a dose of 0.3 mg/kg in 0.2 ml of sterile, pyrogen-free saline, daily, starting from day 7 after tumor implantation.

For studying the effect of cytokines on serum triglycerides, normal mice were starved overnight and then treated i.v. with endotoxin (2.5 μg/mouse), TNF or IL-1 (both 1 μg/mouse) dissolved in 0.2 ml of sterile, pyrogen-free saline. Control mice received saline alone.

In serum transfer experiments, mice were injected with 0.2 ml of serum from tumor-bearing mice. In these experiments, appropriate controls were performed by injecting 0.2 ml of serum obtained from healthy mice.

Biochemical determinations

At different times from tumor implantation, mice were killed by cervical dislocation and the livers were removed and rinsed in ice-cold phosphate-buffered saline. Livers were then homogenized (1/4, w/v) in ice-cold 0.05 M phosphate buffer, pH 7.4, and ED was measured in homogenates as previously described [19].

The catalase activity of the homogenate was determined using a titrimetric method according to Aebi [20]. Serum iron was determined with a commercially available kit (Boehringer, Mannheim). Serum triglyceride levels were determined with a commercially available kit (Farmitalia Carlo Erba, Milan).

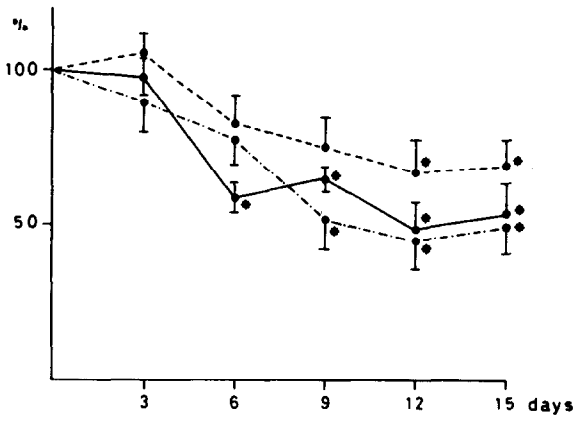

Fig. 1. Time course of plasma iron (---), liver ED (——) and catalase (·-·-·) activities after tumor implantation. Control, non-tumor-bearing mice were killed and assayed for levels of plasma iron, ED and catalase at the same time points as tumor-bearing mice. Data for each time point are shown as a percentage of control mice killed at the same time. Data are ± S.E. (five mice per group). Mean control values were: ED 23.4 ± 1 (nmoles/min/g *of liver*); *iron* 1.8 ± 0.005 (μg/ml); *catalase* 3.8 ± 0.4 (nmoles H_2O_2/min/g *of liver*). **Significantly different from control* ($P < 0.05$ *by Student's t-test*).

RESULTS

The levels of plasma iron, liver cytochrome P-450-dependent ED and catalase activities at different times after tumor implantation are reported in Fig. 1. It can be seen that from day 6 following implantation, a depression in liver enzymes activities was observed accompanied by hypoferremia. Maximal changes were observed at day 12 for all the effects. Depression of ED activity in 12-day tumor-bearing mice was not confined to the liver, but was also observed in the spleen (controls: 7.6 ± 1.3 nmol/min/g; tumor-bearing: 1.0 ± 0.2) and brain (controls: 2.6 ± 0.4 nmol/min/g; tumor-bearing: 1.1 ± 0.2).

We have also measured serum triglycerides and food intake in the same mice. No significant changes were observed in these parameters in tumor-bearing mice at any time (data not shown). By contrast, a marked hypertriglyceridemia was observed in normal mice given i.v. injections of recombinant IL-1, TNF or endotoxin (Table 1).

The ability of serum obtained from tumor-bearing mice at different times following tumor implantation to depress liver ED activity when injected into normal mice is reported in Fig. 2. Maximal liver ED-depressing activity was also detected in sera from tumor-bearing mice with a peak at 12 days after implantation, when compared with ED activity of mice injected with serum obtained from age-matched healthy mice. Since other investigators

Table 1. Effect of endotoxin, TNF IL-1 or S-180 sarcoma on the triglyceride serum concentration

	Endotoxin	TNF	IL-1	S-180*
Control	1.44 ± 0.1	1.23 ± 0.2	1.79 ± 0.1	1.39 ± 0.1
Treated†	2.66 ± 0.2‡	2.24 ± 0.3‡	2.67 ± 0.3‡	1.39 ± 0.1

*Triglyceride serum concentration in tumor-bearing mice was measured 12 days after tumor implantation and compared with normal mice. Mice were starved over night before serum collection.

†Mice (five per group) were treated intravenously with endotoxin (2.5 μg/mouse), TNF (1 μg/mouse) or IL-1 (1 μg/mouse). Mice were starved overnight before the experiment. Triglyceride serum concentration was measured 24 h after treatment. Data are expressed as mmoles/l (mean ± S.E.).

‡$P < 0.05$ versus saline controls by Student's *t*-test.

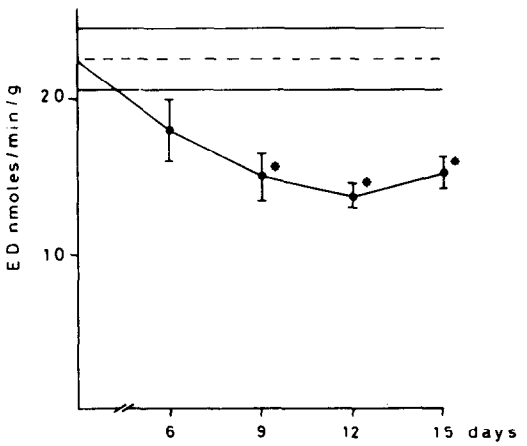

Fig. 2. Depression of liver ED activity, induced by serum obtained from tumor-bearing mice at different times following tumor implantation. Normal mice were injected with 0.2 ml *of serum killed* 24 h *later. Controls received* 0.2 ml *of serum obtained from age-matched healthy mice. Data are mean ± S.E. (five mice per group). *Significantly different from control* (P < 0.05 *by Student's* t*-test).*

used S-180 ascitic fluid as a source of a lipolytic 'toxohormone', we have also tested the effect of the injection of 0.2 ml of cell-free ascitic fluid on liver ED activity in normal mice. A significant decrease of liver ED (34%) was observed, when compared with control mice given saline (data not shown).

The effect of chronic administration of DEX (0.3 mg/kg, i.p. daily, starting from day 7 after tumor implantation) on liver ED activity of tumor-bearing mice at day 15 was studied. DEX was also given to age-matched control mice. The results of three separate experiments are reported in Fig. 3. It can be seen that DEX *per se* caused a marked depression of ED activity in normal, non-tumor-bearing mice. However when administered to tumor-bearing mice, DEX treatment resulted in an increase (+34%, +21% and +64% in the three experiments) in liver ED activity. DEX treatment had no visible effect on tumor growth. By contrast, in the same experiments DEX had no effect on tumor-associated hypoferremia (normal mice: control, 1.8 ± 01 μg/ml; DEX, 1.4 ± 0.1; tumor-bearing mice: control, 1.2 ± 0.1; DEX, 1.2 ± 0.1). The effect of DEX on catalase depression in tumor-bearing mice could not be studied because DEX had a too marked inhibitory effect on liver catalase (data not shown).

DISCUSSION

The purpose of this work was to set up an experimental model that could be used to study the role of endogenous mediators in cancer-associated metabolic alterations and particularly depression of liver drug metabolism, using the S-180 murine sarcoma. In this model, depression of liver ED was associated with depression of catalase activity and hypoferremia. Depression of liver ED could be induced in healthy mice upon injection of ascitic fluid or serum obtained from S-180-bearing mice, as previously reported with rat tumors [11]. It is important to note that when serum from tumor-bearing mice was injected into normal mice, maximal ED-depressing activity was present 12 days following tumor implantation, paralleling the depression of liver ED in the donor mice. However, no anorexia or changes in serum triglycerides were observed. This is in contrast with other tumor models where the animals develop anorexia and wasting [7]. Therefore, from the data reported here the following tentative conclusions could be drawn: (i) the metabolic alterations investigated (depression of liver ED and catalase, and hypoferremia) are not just secondary to a catabolic state or anorexia; and (ii) the endogenous mediators responsible for these effects are distinct from those inducing anorexia or lipolysis. This is in agreement with the hypotheses of other investigators that suggested that 'toxohormone' was not a single substance, and that more than one mediator could be involved in cancer-associated metabolic alterations [15]. As far as the possible relationship, or identity, between toxohormones and monokines like TNF or IL-1 is concerned it should be noted that whereas IL-1 or TNF (or endotoxin) induced a marked hypertriglyceridemia,

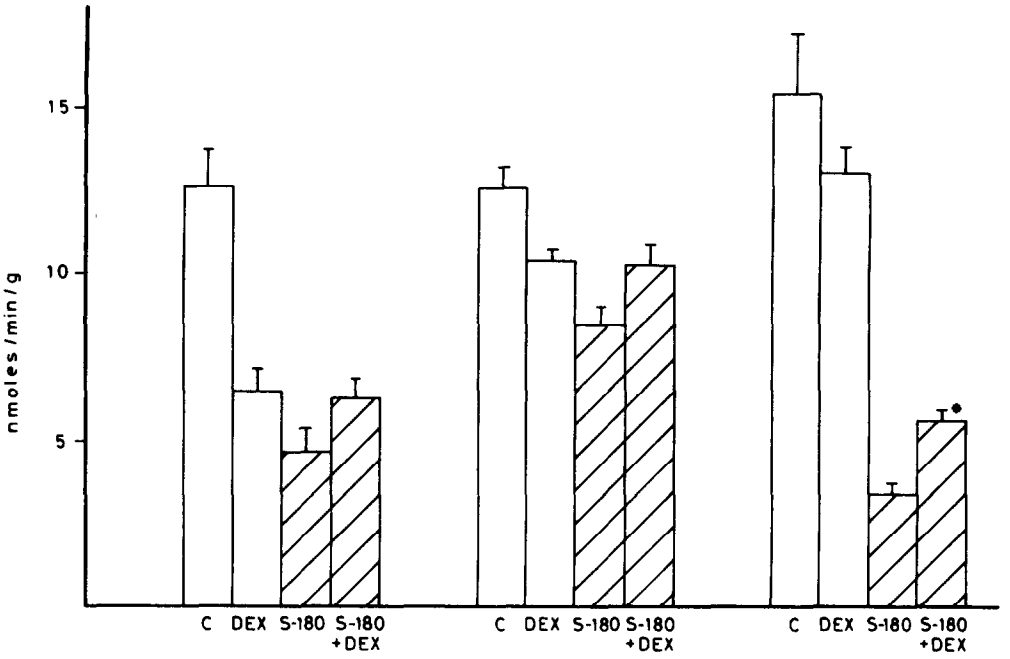

Fig. 3. Effect of chronic treatment with DEX (0.3 mg/kg *daily), starting from day 7 after tumor implantation, on liver ED activity of tumor-bearing mice or normal mice. Mice were killed* 24 h *after the last treatment and liver ED activity was measured. Data are mean ± S.E. (five mice per group). *Significantly different from untreated tumor-bearing mice* ($P < 0.01$ *by Student's t-test).*

no such effect was observed in sarcoma 180-bearing mice. Furthermore, other investigators reported that IL-1 caused anorexia [21], and sarcoma 180 in our experimental conditions did not decrease the food intake. In conclusion, only some of the effects of IL-1 and TNF *in vivo* were observed in tumor-bearing mice, indicating that these two monokines are probably not responsible for the effects induced by the tumor.

The effect of DEX on cancer-associated depression of liver ED deserves further discussion. Although a statistically significant protection was observed only in one experiment, there was consistently a trend for an increase in liver ED by DEX in tumor-bearing mice. This effect seems not to be an artifact due to an aspecific effect of DEX that, on the contrary, decreased liver ED in healthy mice. DEX is a well known inhibitor of the synthesis of various cytokines, including IL-1 [17], TNF [16] and IFN [22].

These cytokines were all reported to depress liver drug metabolism *in vivo* [13, 14 23], and inhibition of their synthesis by DEX was shown to be protective against endotoxin-induced depression of liver ED [18]. The partial reversal of the depression of liver ED observed in sarcoma 180-bearing mice would suggest that the synthesis of the putative toxohormone involved is also inhibited by DEX, although this does not necessarily indicate an identity between this factor and IL-1 or TNF. The lack of effect of DEX on S-180-associated hypoferremia suggests that a different, non DEX-inhibitable, mechanism is implicated.

In conclusion, the present paper indicates that depression of liver drug metabolism observed in sarcoma 180-bearing mice is likely to be mediated via a serum factor probably distinct from IL-1 and TNF. This experimental model can therefore be useful in studying the pharmacological modulation of impaired drug metabolism in cancer. The characterization of the factor(s) implicated in the metabolic changes observed in sarcoma 180-bearing mice is currently under investigation.

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