The effects of acute-phase inducers and dimethyl sulphoxide on δ -aminolaevulinate synthase activity in human HepG2 hepatoma cells

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The effects of acute-phase inducers and dimethyl sulphoxide (Me_2SO) on δ -aminolaevulinate (ALA) synthase in HepG2 cells were examined. Treatment of cells with Me_2SO resulted in a significant increase in ALA synthase activity. Interleukin-6 increased ALA synthase activity only slightly, but it substantially potentiated the induction of ALA synthase by Me_2SO . These data suggest that ALA synthase activity in liver is altered during acute-phase reactions.

INTRODUCTION

The acute-phase response is central in the protection of the host against bacterial invasion or environmental insults [1]. Characteristics of the acute-phase response include a rapid increase in concentration of specific plasma proteins collectively referred to as 'acute-phase reactants', whereas the concentrations of other plasma proteins, most notably albumin, decrease dramatically [1,2]. Infection and inflammation are also known to influence the metabolism and disposition of drugs and chemicals, presumably through actions on hepatic cytochrome *P*-450-dependent mixed-function oxidases [3]. It has been shown, for example, that high doses of endotoxin cause marked suppression of cytochrome *P*-450 isoenzymes and associated hydroxylase activities, whereas low doses of endotoxin have both suppressing and potentiating effects on cytochrome *P*-450, depending on the isoenzyme species [4].

Little is known, however, about the changes in haem biosynthesis that may take place during the acute-phase response. We previously demonstrated that cultured human HepG2 hepatoma cells contain detectable amounts of haem and enzymes of the haem biosynthetic pathway [5,6], and described the induction of δ -aminolaevulinate (ALA) dehydratase by dimethyl sulphoxide (Me_2SO) [5,6]. We have also reported that Me_2SO induces an acute-phase-like response in HepG2 cells [7]. These findings suggested that significant changes in haem biosynthesis might occur during the acute-phase response. In the present study, we have examined the effects of monokines, which are known to produce the acute-phase response, on ALA synthase activity in HepG2 cells, the rate-limiting enzyme for hepatic haem synthesis. Our data demonstrate that these acute-phase inducers significantly potentiate the induction of ALA synthase mediated by Me_2SO .

MATERIALS AND METHODS

Materials

Me_2SO (spectro grade) was purchased from Fisher, Fair Lawn, NJ, U.S.A.; all other tissue-culture materials

were from GIBCO, Grand Island, NY, U.S.A. [2,3- ^{14}C]Succinic acid (40–53 mCi/mmol) was obtained from Amersham, Arlington Heights, IL, U.S.A. Other reagents and chemicals were of reagent grade and obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Recombinant human interleukin (IL)-1 α (sp. activity 2.1×10^7 units/mg) was generously given by Dr. S. Wolpe, The Rockefeller University, New York; recombinant human IL-6 (sp. activity 1.0×10^6 units/mg) was kindly supplied by Dr. S. Clark, Genetics Institute, MA, U.S.A.; and recombinant human tumour-necrosis factor (TNF; sp. activity 2.0×10^6 units/mg) was kindly provided by Dr. T. Nishihara, Suntory Institute for Biomedical Research, Osaka, Japan.

Cell culture

HepG2 cells were given by Dr. Barbara Knowles of the Wistar Institute, Philadelphia, PA, U.S.A. Cells were routinely grown in minimum essential medium with Earle's salts supplemented with 10% (v/v) fetal bovine serum, 100 units of penicillin/ml, 100 μg of streptomycin/ml and 2 mM-L-glutamine, as reported previously [5]. Additions of chemicals, e.g. Me_2SO or monokines, were made directly to the culture. Cultures were incubated in the dark, and cells were harvested by treatment with 0.05% (w/v) trypsin containing 0.53 mM-EDTA for 5 min at 37 °C, and washed in Earle's salts.

ALA synthase assay

ALA synthase activity of HepG2 cells was determined by the radiochemical method described by Brooker *et al.* [8]. Cells collected from a 100 mm-diameter culture dish (Corning) were resuspended in 10 μM -pyridoxal 5'-phosphate/0.1 mM-(CaMg)EDTA [8]/0.1% (w/v) Triton X-100/5 mM-Tris/HCl, pH 7.4, and used for a single assay and for the determination of protein concentration. Enzyme activity was expressed as pmol of ALA formed/h per mg of protein.

Other assays

ALA dehydratase activity was determined by a colorimetric method [9]. Haptoglobin concentration was

determined by rocket immunoelectrophoresis [7,10]. Protein concentrations were determined by the method of Lowry *et al.* [11], after digestion of cells in 1 M-NaOH. Statistical analyses of data were performed by Student's unpaired one-tailed *t* test.

RESULTS

Effects of Me₂SO, IL-6, IL-1 α and TNF on ALA synthase activity in HepG2 cells

The effects of Me₂SO, IL-6, IL-1 α and TNF on ALA synthase activity in HepG2 cells were examined. Treatment of cells with Me₂SO for 48 h increased ALA synthase activity by approx. 50% (Fig. 1). Treatment with IL-1 α or TNF alone (results not shown) did not significantly affect ALA synthase activity. Treatment with IL-6 did not affect ALA synthase activity at 5 units/ml, but it increased the enzyme activity slightly at higher concentrations (Fig. 1). The combined addition

Fig. 1. Effects of IL-6 and Me₂SO on ALA synthase activity in HepG2 cells

Cells were incubated with either IL-6 alone (○) or IL-6+Me₂SO (2%) (●) for 48 h, and harvested for determination of ALA synthase activity. Data are means $\pm$ S.D. of three dishes: ns, not significant; **P* < 0.05, ***P* < 0.005 versus each control. *P* value for the difference between the two controls is < 0.01. *P* value for the difference between IL-6 and IL-6+Me₂SO at a concentration of 200 units of IL-6/ml is < 0.005.

Table 1. Effects of Me₂SO and IL-6 on ALA synthase activity

HepG2 cells were incubated with either Me₂SO (2%) alone, or Me₂SO (2%) and IL-6 (200 units/ml) for 72 h. Media were replenished with chemicals at 48 h. ALA synthase activity was expressed as pmol of ALA formed/h per mg of protein. *P* value for the difference between Me₂SO and Me₂SO+IL-6 is < 0.05. Data are means $\pm$ S.D. of triplicate dishes.

Treatment	ALA synthase	<i>P</i>
Control	7.62 $\pm$ 0.29	
Me ₂ SO	15.13 $\pm$ 0.27	< 0.001
Me ₂ SO + IL-6	18.38 $\pm$ 1.91	< 0.001

Table 2. Effects of Me₂SO and IL-6 on the synthesis of haptoglobin

Cells were incubated with Me₂SO (2%) and/or IL-6 for 96 h. Concentrations of haptoglobin secreted into the medium during the last 48 h were determined as described in the Materials and methods section. The means of duplicate assays are shown, with individual assay data in parentheses.

Treatment	Haptoglobin synthesis (μ g/day per mg of cell protein)
Control	0.033 (0.033, 0.033)
Me ₂ SO	0.049 (0.051, 0.047)
IL-6 (5 units/ml)	0.049 (0.051, 0.047)
IL-6 (5 units/ml) + Me ₂ SO	0.075 (0.077, 0.073)
IL-6 (20 units/ml)	0.063 (0.063, 0.063)
IL-6 (20 units/ml) + Me ₂ SO	0.108 (0.108, 0.108)

of IL-6 with Me₂SO, however, produced synergistic increases in ALA synthase activity (Fig. 1). Cells treated with Me₂SO (2%, v/v) and IL-1 α (10 units/ml), or with Me₂SO (2%) and TNF (100 units/ml), for 48 h also resulted in ALA synthase activities of 26.35 ± 2.41 (+48%; *P* < 0.05, *n* = 3) and 26.88 ± 1.16 (+51%; *P* < 0.005, *n* = 3) respectively, compared with the Me₂SO-treated control (17.76 ± 0.72 pmol/h per mg of protein; *n* = 3).

Me₂SO treatment of cells for 72 h resulted in a 2-fold increase in ALA synthase activity, which was significantly higher than its effect at 48 h (Table 1). The combined addition of IL-6 with Me₂SO for 72 h further increased the enzyme activity (121% of the Me₂SO-treated control, *P* < 0.05).

Effects of Me₂SO and IL-6 on the acute-phase response

Effects of Me₂SO and/or IL-6 on the synthesis of haptoglobin are shown in Table 2. Treatment of HepG2 cells with IL-6 showed a dose-dependent increase in haptoglobin concentrations in culture supernatants. Me₂SO also increased haptoglobin concentrations in culture supernatants, consistent with our previous findings [7]. The combined addition of IL-6 and Me₂SO resulted in significantly greater haptoglobin concentrations than with either treatment alone, indicating that their effects are additive.

Effects of IL-6, IL-1 α , TNF and Me₂SO on ALA dehydratase activity

Effects of acute-phase inducers on ALA dehydratase activity, the second enzyme of the haem-biosynthetic pathway, were also examined. Treatment of HepG2 cells with IL-6, IL-1 α or TNF resulted in little change in ALA dehydratase activity (results not shown). In contrast, Me₂SO treatment resulted, as previously demonstrated, in dose- and time-dependent increases in ALA dehydratase activity [5] and haem synthesis [5]. Additions of IL-6 with Me₂SO did not affect the Me₂SO-mediated increase in ALA dehydratase activity (results not shown).

DISCUSSION

The results of this study demonstrate that, although acute-phase inducers such as IL-1 α or TNF by themselves

had little effect on the activity of ALA synthase in HepG2 cells, they potentiated the induction of ALA synthase activity mediated by Me_2SO , a chemical known to elicit an acute-phase-like reaction [7]. IL-6, a major acute-phase inducer [12], had a marginal stimulatory effect on ALA synthase activity by itself, and a markedly potentiating effect on the Me_2SO -mediated increase of ALA synthase activity. Effects of acute-phase inducers on ALA synthase activity are similar to those observed with glucocorticoids, insulin or tri-iodothyronine on the chemical induction of ALA synthase in primary cultures of chick-embryo hepatocytes, in that hormones by themselves had little effect on the activity on ALA synthase, whereas they potentiated the induction of ALA synthase by drugs, chemicals or metabolites of C-19 and C-21 steroids [13]. These effects are expressed at the level of ALA synthase rather than ALA dehydratase, since none of these acute-phase inducers, either by itself, or IL-6 in combination with Me_2SO , affected ALA dehydratase activity.

Our previous findings demonstrated that Me_2SO elicits an acute-phase-like reaction in HepG2 cells [7]. The findings reported here also indicate that IL-6 potentiates both the Me_2SO -mediated induction of ALA synthase activity (Fig. 1, Table 1) and haptoglobin synthesis (Table 2). The lowest concentration of IL-6 potentiating the Me_2SO -mediated induction of ALA synthase was 5 units/ml, which was similar to that inducing haptoglobin synthesis (Table 2).

It has been reported that intraperitoneal injection of endotoxin brings about a marked decrease in hepatic drug oxidations catalysed by microsomal cytochrome *P*-450s [14–16]. The acute-phase response has also been implicated in the loss of hepatic microsomal cytochrome *P*-450s [4]. It is generally thought that high doses of endotoxin suppress cytochrome *P*-450 [4] and ALA synthase [14], whereas lower doses of endotoxin either suppress or increase cytochrome *P*-450s in an isoenzyme-specific manner [4]. In rodents, 3-methylcholanthrene, a potent inducer of cytochrome *P*-450_c and *P*-450_d [17], not only increases hepatic ALA synthase activity [18], but also induces an acute-phase response [4]. 3-Methylcholanthrene also increases ALA synthase activity in HepG2 cells, and its effect is significantly augmented when cells are simultaneously treated with Me_2SO (F. Iwasa, S. Sassa & A. Kappas, unpublished work). These findings indicate that the hepatic haem-synthetic pathway is significantly influenced by acute-phase inducers and

that these monokines appear to act like hormones which elicit potentiated induction responses of ALA synthase.

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