Effects of dimethyl sulphoxide on the synthesis of plasma proteins in the human hepatoma HepG2

Induction of an acute-phase-like reaction

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Effects of dimethyl sulphoxide (Me_2SO) on the synthesis of plasma proteins by the human hepatoma cell line HepG2 were examined. Me_2SO treatment resulted in decreased synthesis of albumin and α -fetoprotein, and in increased synthesis of haptoglobin. Plasma-protein profiles induced by Me_2SO treatment were very similar to those seen in acute-phase reactions.

INTRODUCTION

HepG2 is a human hepatocellular carcinoma cell line which was established by Aden *et al.* [1]. These cells are known to display a cellular morphology similar to that of liver parenchymal cells. They also express well differentiated phenotypes of liver parenchymal cells, such as the synthesis of the major plasma proteins [2], receptors for insulin, transferrin, epidermal growth factor, low-density lipoprotein and asialoglycoprotein [3] and they retain the ability to proliferate in culture. For this reason the HepG2 cell line is now widely used as a model for human hepatocytes. We have reported previously that treatment of HepG2 cells with Me_2SO increases the activity of 5-aminolaevulinate dehydratase (EC 4.2.1.24), the second enzyme of the haem-biosynthetic pathway, and the cellular concentration of haem [4]. The increase of 5-aminolaevulinate dehydratase was transcription-dependent [4]. In the present paper we examine the effect of Me_2SO on plasma-protein synthesis by HepG2 cells to evaluate whether changes in haem synthesis after Me_2SO treatment are associated with changes in other hepatocyte functions. We report here that Me_2SO -induced changes in plasma-protein synthesis in HepG2 cells are similar to those known to occur in acute-phase reactions.

MATERIALS AND METHODS

Materials

Me_2SO (Spectro grade) was purchased from Fisher, Fair Lawn, NJ, U.S.A.; all tissue-culture materials were from GIBCO, Grand Island, NY, U.S.A. Rabbit anti-(human protein) antisera and IgG purified from anti-serum were purchased from Behring Diagnostics, La Jolla, CA, U.S.A. [anti-albumin, anti- α -fetoprotein (anti-AFP), anti-fibrinogen, anti-haptoglobin and anti-caeruloplasmin] or DAKO Corp., Santa Barbara, CA, U.S.A. (anti-fibrinogen and anti-transferrin); standard human proteins were from Cappel, West Chester, PA, U.S.A. (albumin) or Behring (fibrinogen, transferrin,

AFP, haptoglobin and caeruloplasmin). Recombinant mouse tumour-necrosis factor (TNF) and human interleukin (IL)-6 were generously given by Dr. S. Wolpe, the Rockefeller University and Dr. S. Clark, Genetics Institute, Cambridge, MA, U.S.A. respectively. Agarose was purchased from Bio-Rad, Richmond, CA, U.S.A.; sodium barbital was from Fisher.

Cell culture

HepG2 cells were kindly provided by Dr. Barbara Knowles of the Wistar Institute, Philadelphia, PA, U.S.A. Cell cultures and treatment with Me_2SO and with other factors were performed as previously described [4]. Conditioned media from an established mouse macrophage cell line, RAW264.7, which had been treated with bacterial lipopolysaccharide (LPS) ($5 \mu\text{g/ml}$) for 24 h, were kindly provided by Dr. S. Wolpe from The Rockefeller University.

Plasma-protein assays

Plasma protein synthesized and secreted by HepG2 cells into culture media was quantified by rocket immunoelectrophoresis [5]. For determining fibrinogen, haptoglobin and caeruloplasmin, culture media were dialysed against 10 mM-sodium phosphate buffer, pH 7.4, and concentrated (10-fold) by using a Speed-Vac concentrator (Savant, Hicksville, NY, U.S.A.). Plasma protein synthesis was expressed as μg of protein secreted/day per mg of cellular protein. Protein concentrations were determined by using the method of Lowry *et al.* [6] after cells had been solubilized by heating in 1 M-NaOH at 65°C for 1 h. Bovine serum albumin was used as standard. Statistical analyses of data were performed by using Student's unpaired *t* test.

RESULTS

HepG2 cells maintained a stable level of albumin synthesis throughout numerous passages. When these cells were treated with various concentrations of Me_2SO for 48 h, the synthesis of albumin and AFP was found to

Abbreviations used: Me_2SO , dimethyl sulphoxide; AFP, α -fetoprotein; TNF, tumour-necrosis factor; IL, interleukin; LPS, (bacterial) lipopolysaccharide; MEL, mouse Friend-virus-transformed erythroleukaemia.

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Fig. 1. Effects of Me₂SO on the synthesis of albumin and AFP in HepG2 cells

Cells were plated on 6 cm diameter dishes at a concentration of 5×10^5 cells/plate in a volume of 5 ml and incubated for 2 days before the experiment. (a) Dose-response curve. Me₂SO was added on day 0 at the concentrations indicated on the abscissa, and cultures were incubated for 48 h. ●, Albumin; ○, AFP. (b) Time course of albumin synthesis. Me₂SO was added at a final concentration of 2% (v/v) on day 0. Media were removed at each time point indicated on the abscissa, and cultures were supplemented with fresh media containing Me₂SO. ●, Control; ○, Me₂SO treatment. Concentrations of albumin and AFP secreted into the media in the last 24 h were determined by rocket immunoelectrophoresis as described in the Materials and methods section. Data are means $\pm$ S.D. for triplicate dishes. Statistical significance: * $P < 0.005$; ** $P < 0.001$ versus control.

Fig. 2. Effects of LPS-conditioned mouse macrophage media, IL-6 and TNF on the synthesis of plasma proteins in HepG2 cells

Cells were incubated in the presence of LPS-conditioned media from mouse RAW264.7 cells, IL-6 or TNF for 96 h. Plasma proteins secreted into the medium in the last 48 h were determined as described in the Materials and methods section. Values are expressed as a percentage of the control value. (a) Effects of LPS-conditioned media; (b) effects of IL-6; (c) effects of TNF. Control values were as follows: albumin, 20–29 µg/day per mg of cell protein; AFP, 6.4–17 µg/day per mg; fibrinogen, 1.0–1.7 µg/day per mg; and haptoglobin, 0.03–0.05 µg/day per mg. □, Control; ▨, 0.05% (v/v) LPS-conditioned medium (a), 1 du (dilution unit) of IL-6/ml (b) or 1 unit of TNF/ml (c); ▩, 0.25% (v/v) LPS-conditioned medium (a), 20 du of IL-6/ml (b) or 10 units of TNF/ml (c); ■, 0.5% (v/v) LPS-conditioned medium (a), 200 du of IL-6/ml (b) or 100 units of TNF/ml (c). Values are means of duplicate determinations from single experiments (a and c) or two experiments (b). An IL-6 concentration of 1 du/ml is equivalent to the concentration which gives rise to 50% of maximum response. Abbreviations: Alb, albumin; Fib, fibrinogen; Hp, haptoglobin.

be decreased in a dose-dependent fashion with Me₂SO concentrations greater than 1% (v/v) (Fig. 1a). When cells were treated with 2% Me₂SO, the synthesis of albumin was decreased within 48 h and remained at ~40% of the control level throughout the experimental period of 96 h (Fig. 1b). The amount of AFP was also decreased in cells treated with 2% Me₂SO for 96 h, compared with that in untreated cells (means $\pm$ S.D.:

2.5 ± 0.8 as against 7.0 ± 1.1 µg/day per mg of cellular protein respectively; $P < 0.05$; $n = 3$), but that of haptoglobin increased (0.227 ± 0.040 as against 0.083 ± 0.007 µg/day per mg for Me₂SO-treated and untreated cells respectively; $P < 0.005$; $n = 3$). The levels of transferrin, fibrinogen and caeruloplasmin did not change significantly (results not shown). Cellular morphology was not significantly affected, but the growth

rate of the cells was reduced to about 50% by treatment with Me₂SO [4]. Cellular protein and DNA contents remained unchanged after Me₂SO treatment (results not shown). When Me₂SO was removed from the medium at the end of 48 h treatment, the synthesis of these proteins returned to the untreated control level during the subsequent 48 h.

When HepG2 cells were treated with an LPS-conditioned medium, suppression of cell growth and alterations in plasma-protein production were observed. LPS-conditioned medium suppressed production of albumin and AFP, and increased haptoglobin production in a dose-dependent manner (Fig. 2a). Among a number of monokines produced by LPS-conditioned macrophages, IL-6, TNF and/or IL-1 have been implicated as inducers of acute-phase reactions [7]. Treatment of HepG2 cells with IL-6 resulted in a slight decrease in the synthesis of albumin and AFP and dose-dependent increases in fibrinogen and haptoglobin (Fig. 2b). Treatment with TNF resulted in a slight increase in the synthesis of haptoglobin, whereas changes in albumin, AFP and fibrinogen were insignificant (Fig. 2c). IL-6 and TNF did not suppress cell growth (results not shown). IL-1 at 20 units/ml was highly toxic to HepG2 cells and did not produce acute-phase reactions. These findings indicate that an acute-phase reaction can be induced in HepG2 cells by treatment with LPS-conditioned media, IL-6 or TNF, and the changes in plasma-protein synthesis induced by Me₂SO are strikingly similar to those observed in the acute-phase reaction.

DISCUSSION

In the present study we examined the effect of Me₂SO on plasma-protein synthesis to determine whether the Me₂SO-induced haem synthesis [4] is associated with changes in other hepatocyte-specific functions. Our results demonstrate that incubation of HepG2 cells with Me₂SO resulted in dose- and time-dependent decreases in the synthesis of albumin and AFP, and in increases in the synthesis of haptoglobin. These changes are very similar to those observed after treatment of cells with LPS-conditioned media from mouse macrophages or recombinant human IL-6, i.e., known inducers of acute-phase reactions [7]. It should be noted that AFP synthesis was decreased after treatment with LPS-conditioned media, IL-6 or Me₂SO, suggesting that AFP may also be an acute-phase protein.

In the acute-phase reaction in man, hepatic synthesis and secretion of albumin is decreased, whereas that of haptoglobin, fibrinogen, α_1 -acid glycoprotein, caeruloplasmin, serum amyloid A [8,9] and C-reactive protein [10] are increased. Acute-phase proteins have been shown to be produced in cultured liver cells [11–14], including HepG2 [15]. Activated macrophage-derived hepatocyte-stimulating factor (also known as IL-6, interferon- β_2 or B-cell stimulating factor-2 [7,16]), IL-1 and/or TNF are thought to play an important role in the induction of acute-phase-protein synthesis [11,17–19]. Although the response of HepG2 cells to IL-6 is reported to be limited to α_1 -antichymotrypsin [15], our findings indicate that changes in other acute-phase proteins, e.g., increased synthesis of haptoglobin and fibrinogen, and decreased synthesis of albumin and AFP, can be demonstrated in HepG2 cells in response to treatment with LPS-conditioned media or IL-6. These findings

indicate that the acute-phase reaction can be induced in HepG2 cells after treatment with IL-6, LPS-conditioned media and Me₂SO.

Me₂SO has been shown to induce cell differentiation of a variety of cell systems *in vitro* [20–30]. Our results in HepG2 cells demonstrate that Me₂SO induces alterations in plasma-protein synthesis which is reversible upon removal of Me₂SO. Thus Me₂SO elicits an acute-phase reaction in HepG2 cells which appears to reflect reversible modulation of cellular function, rather than the irreversible commitment observed in MEL cells [20] or HL-60 cells [22]. Reversible changes have also been observed in rat mammary-tumour cells after Me₂SO treatment [25] and in the LLC-PK1 kidney epithelial-cell line after dimethylformamide treatment [26].

Me₂SO has also been shown to maintain the level of cytochrome P-450 in primary cultures of rat hepatocytes [31] and to increase the haem content [4] and the activity of the cytochrome P-450-dependent enzyme 7-ethoxycoumarin de-ethylase, but not of aryl-hydrocarbon hydroxylase in HepG2 cells [32]. It is not known whether haem synthesis is altered during acute-phase reductions or heat shock. However, since LPS is known to induce microsomal haem oxygenase activity in rat liver [33] and the gene for rat haem oxygenase [34] is known to contain a heat-shock consensus element, it is conceivable that haem catabolism may be affected in acute-phase reactions. Although the induction of haem oxygenase is usually associated with a decrease in hepatic haem content, it may also result in an increase in hepatic microsomal cytochrome P-450 [35]. Whether the increase in haem content and certain cytochrome P-450 isoenzymes in HepG2 cells in response to Me₂SO treatment are causatively related to the acute-phase reaction is not known, and further studies are needed in this area.

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