Effect of lead acetate and carbon particles on the expression of glutathione S-transferase YfYf in rat liver

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Administration of intracardiac lead acetate produces a complex response in glutathione S-transferase (GST) YfYf expression in rat liver. The earliest response, an elevation of GST expression in Kupffer cells, can be mimicked by administering a suspension of carbon particles. The second effect of lead acetate administration

is a marked elevation of GST YfYf in some but not all hepatocytes (the 'patchy' response). This effect is most marked 48–76 h after administration of a single dose of lead acetate and is easily detected by immunoblotting. Dexamethasone down-regulates the lead response in hepatocytes.

INTRODUCTION

Glutathione S-transferases (GSTs) exist in multiple forms that can be classified into four families, termed Alpha, Mu, Pi and Theta (Mannervik et al., 1985; Meyer et al., 1991). In most species that have been studied the individual families have continued to diverge; however, there appears to be some evolutionary restraint on the Pi family, which generally exists as one form (Pacifi et al., 1985; Okuda et al., 1987). The description by Sato and co-workers that the rat Pi-form GST YpYp (also termed GST YfYf or 7-7; see Kitahara et al., 1984; Jensson et al., 1985; Hayes, 1986) is overexpressed in pre-neoplastic foci in the liver (Sato et al., 1985) stimulated work to define the mechanism of activation and also to determine whether the human orthologue, GST- π , has any value as a cancer marker (see Tsuchida and Sato, 1992, for a recent review). The promise of the latter, at least in plasma, is probably restricted (see Beckett et al., 1990). However, the realization that in the rat the regulation of expression involved the *fos/jun* trans-acting AP1 complex and a uniquely sensitive *cis* element (the GPE1 enhancer) has provided an insight into the mechanism of action of this class of oncogene (Cowell et al., 1988; Sakai et al., 1988; Okuda et al., 1989). Interestingly, Pi-class genes, although highly sequence-related in the protein coding domain, exhibit distinct regulatory mechanisms. Thus, while the rat is extremely sensitive via the GPE1 enhancer, the human AP1 site appears to be essential only for basal promoter activity (Xia et al., 1991). Transcription of human GST- π is activated by insulin and suppressed by retinoic acid (Xia et al., 1993), while expression of the mouse enzyme is under androgenic control (Hatayama et al., 1986).

Several groups have reported that the administration of lead salts induces expression of GST YfYf in the rat (Roomi et al., 1986; Columbano et al., 1987; Di Ilio et al., 1989). However only a limited number of hepatocytes respond to lead and the resulting 'patchy' expression of GST YfYf mimics that seen in foetal rat and human liver (Hiley et al., 1988; McCusker et al., 1989a). We have shown that a more detailed examination of the time course reveals that the first response is an induction of GST YfYf in sinusoidal cells and that this can be mimicked using carbon particles. The subsequent, quantitatively more significant, increase in GST YfYf in hepatocytes is down-regulated by dexamethasone, and details of this work are presented in the current study.

MATERIALS AND METHODS

GST YfYf was purified from rat kidney as previously described (Meyer et al., 1985). A polyclonal antiserum was raised in New Zealand White rabbits. The antiserum was monospecific as demonstrated by Western blotting, and in preliminary immunocytochemical experiments it gave significant positive staining in normal untreated rat liver sections only of biliary epithelial cells. In rat kidney sections it stained only proximal tubules of the outer medullary rays, as previously reported (Boyce et al., 1987).

Male and female Wistar rats were anaesthetized using halothane gas and then given a single intracardiac injection of lead acetate (100 μ mol/kg) dissolved in distilled deionized water. Rats were then killed after 6, 12, 24, 30, 35, 48, 72 or 76 h, 7 days or 1 month. Rats which were treated with colloidal carbon (prepared by the method of Cowper et al., 1990) were given a single intraperitoneal injection (500 μ l/kg body weight). The livers were removed, and a sample of approx. 1 cm $\times$ 2 mm was immediately fixed in Bouin's solution and prepared for immunocytochemistry, while the remainder was rinsed in buffer A (0.25 M sucrose/10 mM Tris/HCl/1 mM EDTA, pH 7.2) and stored at -20°C . Cytosols were prepared by homogenizing the tissue in 3 vol. of buffer A and centrifuging at 48000 *g* for 40 min. SDS/PAGE was carried out by the method of Laemmli (1970) in 15% polyacrylamide gels containing 0.09% (w/v) bisacrylamide (Hayes and Mantle, 1986). Proteins were transferred electrophoretically to nitrocellulose and probed with antisera against rat GST YfYf by the method of Towbin et al. (1979). In all of these experiments three animals were used for each time point. We examined tissue sections and cytosols from individual animals and obtained similar results at each time point. Representative examples are shown in Figures 1–4. The fields shown in Figures 1 and 2 are representative of the entire tissue section. In one set of experiments rats (200 g) were pretreated with dexamethasone (0.0625, 0.125 or 0.25 mg/kg body weight; dissolved in 0.9% NaCl) 24 h prior to the administration of lead acetate or carbon. Immunocytochemistry was carried out using the avidin/biotin technique of Hsu et al. (1981).

RESULTS AND DISCUSSION

An examination of the time course of GST YfYf induction by

Figure 1 Effect of lead acetate administration on the expression of Yf in rat liver

Rats were given a single intracardiac injection of lead acetate ($100 \mu\text{mol/kg}$ body wt.) and killed after 6 h (a), 12 h (b), 24 h (c), 30 h (d), 35 h (e), 48 h (f), 76 h (g), 1 week (h) or 1 month (i). Control rat liver (j) received no injection. Livers were removed and fixed in Bouin's fixative followed by 70% ethanol, embedded in paraffin and cut into $5\text{-}\mu\text{m}$ -thick sections. They were then immunocytochemically stained using the avidin/biotin method and anti-Yf antiserum as the primary antibody. The scale bar represents $20 \mu\text{m}$.

Figure 2 Effect of carbon administration on the expression of YfYf in rat liver

In (a), a rat was given a single intraperitoneal injection of dexamethasone (0.25 mg/kg body wt.) 24 h prior to injection of a colloidal carbon suspension (500 μ l/kg body wt.). The rat was killed 6 h after the latter injection. In (b), a rat was given a single intraperitoneal injection of a colloidal carbon suspension and killed 6 h later. The scale bar represents 40 μ m.

Figure 3 Immunoblot analysis of the Yf subunit in rat liver cytosols after treatment with lead acetate

A Western blot was carried out on rat liver cytosol fractions as follows. Track 1, rat liver affinity-purified 'Y fraction' (prepared by step elution from S-hexylglutathione-Sepharose); track 2, normal rat liver cytosol; tracks 3–10, rat liver cytosols obtained from animals which had been pretreated with lead acetate and killed after 11 h (3), 24 h (4), 36 h (5), 48 h (6), 60 h (7), 8 days (8), 2 months (9) and 5 months (10).

lead acetate in rat liver reveals an increased labelling of sinusoids that is apparent at 6 h and maintained for 12 h after a single intracardiac injection of lead acetate (Figure 1). An increased staining of hepatocytes is not seen until 24 h, with a peak level at between 48 and 76 h. The hepatocyte staining is 'patchy' and a detailed examination reveals that there are at least two levels of intensity in the hepatocytes overexpressing GST YfYf. We have previously suggested that the 'patchy' expression in hepatocytes may be mediated by a suspension of lead phosphate (McCusker et al., 1989b). However, while the administration of a suspension of colloidal carbon mimics the early effect, namely the increased staining of the sinusoids (Figure 2), we were unable to see initiation of the 'patchy' hepatocyte response using carbon particles (for a period up to 48 h). When cytosols from carbon-treated livers were examined by Western blotting it was not possible to detect any measurable increases in expression using this technique (results not shown). By contrast when cytosols from the livers of rats treated with lead acetate were examined by immunoblotting with anti-Yf the major response occurred between 48 and 60 h (Figure 3) and is clearly related to the staining of hepatocytes that occurs at this time (Figures 1f–1h). The lead-induced expression in hepatocytes is down-regulated by

Figure 4 Immunoblot analysis of the Yf subunit in lead acetate-treated and lead acetate- plus dexamethasone-treated rat liver cytosols

Western blots were carried out using rat liver cytosols as follows. (a) Track 1, normal rat liver; tracks 2, 3, 4 and 5, 6 h, 24 h, 30 h and 48 h respectively after lead acetate administration. (b)–(d) Rats were given a prior administration of dexamethasone of 0.0625 (b), 0.125 (c) or 0.25 (d) mg/kg body weight. Lead acetate was then administered and samples were prepared after 6 h (track 2), 24 h (track 3), 30 h (track 4) or 48 h (track 5).

pretreatment with dexamethasone and this is clearly seen at 48 h (Figures 4a–4c, track 5). Sakai et al. (1992) have shown that suppression of GST YfYf by dexamethasone is achieved through a negative interaction between the activated glucocorticoid receptor and the *fos/jun* complex. This suggests that the up-regulation seen with lead treatment is probably mediated via the palindromic TRE-like GPE1 enhancer. Further work is clearly required to establish details of the 'phagocytic' and 'patchy' signal transduction pathways we have described (e.g. the possible involvement of the protein kinase C pathway; Markovac and Goldstein, 1988), but it seems clear that the use of glucocorticoid suppression is a useful way to distinguish between them. Our results also suggest that colloidal carbon stimulation of GST YfYf in Kupffer cells (which is not blocked by dexamethasone [see Figure 2b]) is apparently not coupled to the subsequent 'patchy' overexpression seen in hepatocytes and therefore represents further evidence for the involvement of distinct mechanisms.

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REFERENCES

- Beckett, G. J., Howie, A. F., Hussey, A. J., Hayes, P. C., Miller, W. R. and Hayes, J. D. (1990) in *Glutathione S-Transferases and Drug Resistance* (Hayes, J. D., Pickett, C. B. and Mantle, T. J., eds.), pp. 399–408, Taylor & Francis, London
- Boyce, S. J., McBenett, S. M., Mantle, T. J. and Hayes, J. D. (1987) in *Glutathione S-Transferases and Carcinogenesis* (Mantle, T. J., Pickett, C. B. and Hayes, J. D., eds.), pp. 69–72, Taylor & Francis, London
- Columbano, A., Ledda, G. M., Sirigu, P., Perra, T. and Panni, P. (1987) *Am. J. Pathol.* **110**, 83–88
- Cowell, I. G., Dixon, K. H., Pemble, S. E., Ketterer, B. K. and Taylor, J. B. (1988) *Biochem. J.* **255**, 79–83
- Cowper, K. B., Currin, R. T., Dawson, T. L., Lindert, K. A., Lemasters, J. J. and Thurman, R. G. (1990) *Biochem. J.* **266**, 141–147
- Di Ilio, C., Aceto, A., Columbano, A., Ledda-Columbano, G. M. and Federici, G. (1989) *Cancer Lett.* **46**, 167–171
- Hayes, J. D. (1986) *Biochem. J.* **233**, 789–798
- Hayes, J. D. and Mantle, T. J. (1986) *Biochem. J.* **237**, 731–740
- Hatayama, I., Satoh, K. and Sato, K. (1986) *Biochem. Biophys. Res. Commun.* **140**, 581–588
- Hiley, C., Fryer, A., Bell, J., Hume, R. and Strange, R. C. (1988) *Biochem. J.* **254**, 255–259
- Hsu, S. M., Raine, L. and Fanger, H. (1981) *Am. J. Clin. Pathol.* **75**, 816–821
- Jensson, H., Eriksson, L. C. and Mannervik, B. (1985) *FEBS Lett.* **187**, 115–120
- Kitahara, A., Satoh, K., Nishimura, K., Ishikawa, T., Ruike, K., Sato, K., Tsuda, H. and Ito, N. (1984) *Cancer Res.* **44**, 2698–2703
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Mannervik, B., Ålin, P., Guthenberg, C., Jensson, H., Tahir, M. K., Warholm, M. and Jörnvall, H. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7202–7206
- Markovac, J. and Goldstein, G. W. (1988) *Nature (London)* **334**, 71–73
- McCusker, F. M. G., Boyce, S. J. and Mantle, T. J. (1989a) *Biochem. J.* **262**, 463–467
- McCusker, F. M., Phillips, M. F., Boyce, S. J. and Mantle, T. J. (1989b) in *Glutathione S-Transferases and Drug Resistance* (Hayes, J. D., Pickett, C. B. and Mantle, T. J., eds.), pp. 419–429, Taylor & Francis, London
- Meyer, D. J., Beale, D., Hong Tan, K., Coles, G. and Ketterer, B. (1985) *FEBS Lett.* **184**, 139–143
- Meyer, D. J., Coles, B., Pemble, S. E., Gilmore, K. S., Fraser, G. M. and Ketterer, B. (1991) *Biochem. J.* **274**, 409–414
- Okuda, A., Sakai, M. and Muramatsu, M. (1987) *J. Biol. Chem.* **262**, 3858–3863
- Okuda, A., Imagawa, M., Maeda, Y., Sakai, M. and Muramatsu, M. (1989) *J. Biol. Chem.* **264**, 16919–16926
- Pacifici, G. M., Warholm, M., Guthenberg, C., Mannervik, B. and Rane, A. (1985) *Biochem. Pharmacol.* **35**, 1616–1619
- Roomi, M. W., Columbano, A., Ledda-Columbano, G. M. and Sarma, D. S. R. (1986) *Carcinogenesis* **7**, 1643–1646
- Sakai, M., Okuda, A. and Muramatsu, M. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 9456–9460
- Sakai, M., Muramatsu, M. and Nishi, S. (1992) *Biochem. Biophys. Res. Commun.* **187**, 976–983
- Satoh, K., Kitahara, A., Soma, Y., Inaba, Y., Hatayama, I. and Sato, K. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 3964–3968
- Towbin, H., Staehelin, T. and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354
- Tsuchida, S. and Sato, K. (1992) *Crit. Rev. Biochem. Mol. Biol.* **27**, 337–384
- Xia, C. L., Cowell, I. G., Dixon, K. H., Pemble, S. E., Ketterer, B. and Taylor, J. B. (1991) *Biochem. Biophys. Res. Commun.* **176**, 233–240
- Xia, C. L., Taylor, J. B., Spencer, S. R. and Ketterer, B. (1993) *Biochem. J.* **292**, 845–850