Effect of zinc supplementation on oxidative drug metabolism in patients with hepatic cirrhosis

M. G. BARRY¹, P. MACMATHUNA¹, K. YOUNGER², P. W. N. KEELING² & J. FEELY¹

¹Department of Pharmacology and Therapeutics, Trinity College, Dublin, and ²Department of Clinical Medicine, St James's Hospital, Dublin

The pharmacokinetics of antipyrine were studied in seven zinc deficient patients with hepatic cirrhosis, before and after zinc supplementation. Each patient received zinc sulphate 660 mg daily for 30 days, restoring zinc status to normal as assessed by leucocyte zinc concentration. Antipyrine clearance was significantly reduced ($P < 0.05$) and antipyrine elimination half-life increased ($P < 0.05$) following administration of zinc sulphate without significant alteration in the apparent volume of distribution. It is concluded that supplementation of the zinc deficiency associated with hepatic cirrhosis impaired the hepatic oxidative metabolism of antipyrine.

Keywords antipyrine metabolism hepatic cirrhosis zinc supplementation

Introduction

More than 200 zinc metalloenzymes have been described and experimental data in animals suggest that zinc is essential for the activity of some drug metabolizing enzymes (Peters & Fouts, 1970). *In vitro* studies on hepatic drug metabolism in zinc deficient rats demonstrated a reduced ability to metabolize drugs such as pentobarbitone, aminopyrine and *p*-nitrobenzoic acid (Becking & Morrison, 1970). Zinc supplementation reversed the above findings, thus suggesting a facilitatory role for zinc in drug metabolism. Zinc deficiency is well recognised in patients with cirrhosis of the liver (Keeling *et al.*, 1980). Previous studies have demonstrated a significant non linear relationship between zinc status, assessed by serum zinc concentration, and drug metabolizing ability assessed by cytochrome P450 content of liver tissue and antipyrine elimination half-life in patients with alcoholic liver disease, suggesting a reduction in antipyrine metabolism associated with zinc deficiency (Hartoma *et al.*, 1977; Sotaniemi *et al.*, 1977). Therefore zinc deficiency in patients with hepatic cirrhosis may contribute to their impaired ability to metabolize drugs (Williams & Mamelock, 1980). Some of the clinical manifestations associated with cirrhosis of the liver, e.g. hypogonadism and hypoguesia are consistent with a conditioned zinc deficiency and respond to zinc therapy (Weismann *et al.*, 1979). Recently, zinc supplementation has resulted in the improvement of patients with hepatic encephalopathy complicating cirrhosis (Reding *et al.*, 1984). In this study we investigated the effect of zinc supplementation on hepatic drug metabolizing ability assessed by changes in antipyrine kinetics before and

after administration of zinc sulphate to seven patients with hepatic cirrhosis.

Methods

Seven patients (mean age 52 years; range 19–65 years and mean bodyweight 67 kg; range 58–75 kg) with histologically diagnosed alcoholic cirrhosis, participated in the study which had local ethics committee approval. The zinc status of the patients was determined before and after zinc supplementation. Zinc status was represented by both serum and leucocyte zinc concentrations. Zinc levels were measured by atomic absorption spectrophotometry (Whitehouse *et al.*, 1982). Fasting samples of blood taken with minimal stasis into trace element free Vacutainers were used. Centrifugation removed erythrocytes and a mixed leucocyte population was achieved by dextran sedimentation. The cells were counted under ultraviolet light using fluorescent dye. White cell pellets were dried at 100° C for 16 h and weighed to the nearest 0.05 mg within 2 h of removal from the oven. Dried pellets were extracted with 0.3 ml 1 M hydrochloric acid for 20 h prior to analysis for trace elements using a Perkin Elmer atomic absorption spectrophotometer. Each patient received 660 mg of zinc sulphate daily for 30 days. Drug metabolizing enzyme activity was determined before and after zinc administration using the model drug antipyrine. There was no evidence of clinical or biochemical deterioration in the

patient's condition during the study period. Antipyrine clearance was determined prior to and after zinc administration following a single intravenous dose of antipyrine 10 mg kg^{-1} . Blood samples were taken from an indwelling cannula at times 0, 3, 6, 9, 12 and 24 h. Antipyrine concentration was measured by high performance liquid chromatography (Shargel *et al.*, 1979). Antipyrine clearance was calculated from the dose (D) of antipyrine/area under the plasma concentration-time curve (AUC), calculated by the trapezoidal rule with extrapolation to infinity by the addition of C_{last}/k .

$$\text{Antipyrine clearance} = D/\text{AUC ml min}^{-1}.$$

Antipyrine elimination half-life ($t_{1/2}$) was obtained following determination of the elimination rate constant k , estimated from the slope of the log plasma concentration-time plot.

$$t_{1/2} = 0.693/k$$

The apparent volume of distribution (V_d) was calculated thus

$$V_d = (\text{Antipyrine clearance} \times t_{1/2})/0.693.$$

Values of zinc concentrations and parameters of antipyrine kinetics are given in the text and in Table 1 as means $\pm$ s.d. Differences between values pre and post zinc supplementation were tested for significance using Student's t -test for paired data. A value of $P < 0.05$ was taken as being statistically significant.

Results

The mean values of serum and leucocyte zinc concentrations, together with the pharmacokinetic parameters of antipyrine pre and post zinc supplementation are shown in Table 1. A significant increase in both serum and leucocyte zinc concentrations followed zinc sup-

Figure 1 Antipyrine plasma concentrations in seven patients with hepatic cirrhosis (mean $\pm$ s.e. mean) following a single intravenous dose of antipyrine 10 mg kg^{-1} , before $\bullet\text{---}\bullet$ and after $\bullet\text{---}\bullet$ zinc supplementation 660 mg daily for 30 days. (* = $P < 0.05$).

plementation. Figure 1 demonstrates the increase in the area under the antipyrine plasma concentration time curve following zinc supplementation. The mean antipyrine elimination half-life was significantly prolonged post zinc ($26.0 \pm 19.2 \text{ h}$) compared with pre zinc ($15.9 \pm 11.4 \text{ h}$). A significant decrease in mean antipyrine clearance also resulted following zinc treatment ($24.9 \pm 15.0 \text{ ml min}^{-1}$) compared with pre zinc ($37.7 \pm 17.5 \text{ ml min}^{-1}$). There was no significant difference in the apparent volume of distribution of antipyrine as a result of zinc therapy.

Discussion

Vallee *et al.* (1956) demonstrated a marked reduction in serum zinc concentrations in patients with post alcoholic cirrhosis and found that the extent of reduction bore a significant relationship to the disease activity and its fluctuating course. The reduction in serum zinc levels in patients with cirrhosis has been confirmed by subsequent studies (Davies *et al.*, 1968; Halsted & Smith, 1970). A disadvantage of using serum zinc concentrations to indicate zinc status is the interpretation of marginally lowered levels of $7\text{--}13 \text{ } \mu\text{mol l}^{-1}$, which may occur as a result of zinc depletion or as a consequence of a variety of metabolic and pathological stimuli known to reduce serum zinc. These stimuli include infection, starvation, corticosteroids, oestrogens, hypothermia and exercise (Webb & Cain, 1982). Leucocyte zinc content is also reduced in patients with cirrhosis (Keeling *et al.*, 1980) and is considered a more reliable index of zinc status as it shows less fluctuation than serum zinc under such conditions (Sandstead *et al.*, 1976). In this study the mean serum zinc concentration of patients with hepatic cirrhosis was marginally lowered at $11.2 \text{ } \mu\text{mol l}^{-1}$ whereas the mean leucocyte zinc concentration of $39.3 \text{ } \mu\text{g g}^{-1}$ was well below the normal range of $55\text{--}90 \text{ } \mu\text{g g}^{-1}$ seen in

Table 1 Zinc concentration in serum and leucocytes and pharmacokinetic parameters of antipyrine (mean $\pm$ s.d.) in seven patients with hepatic cirrhosis pre- and post-zinc supplementation (zinc sulphate 660 mg daily for 30 days)

	Pre zinc	Post zinc	
Serum zinc ($\mu\text{mol l}^{-1}$)	11.2 ± 4.4	15.6 ± 4.6	$P < 0.05$
Leucocyte zinc ($\mu\text{g g}^{-1}$)	39.3 ± 7.2	58.8 ± 15.3	$P < 0.05$
Antipyrine $t_{1/2}$ (h)	15.9 ± 11.4	26.0 ± 19.2	$P < 0.05$
Antipyrine V (l)	39.5 ± 13.7	39.6 ± 11.4	$P > 0.05$
Antipyrine clearance (ml min^{-1})	37.7 ± 17.5	24.9 ± 15.0	$P < 0.05$

healthy controls (Keeling *et al.*, 1982), confirming that the patients studied were zinc deficient.

Experimental data derived from studies on the role of trace metals in chemical carcinogenesis, indicated that zinc facilitated the activity of the microsomal enzyme system involved in drug metabolism as zinc enhanced the aromatic hydroxylation of benzo[a]pyrene by rat liver microsomes (Dixon *et al.*, 1968). Hartoma *et al.* (1977) conducted the first study to investigate the relationship between zinc status and drug metabolizing ability of patients with chronic liver disease. In that study zinc status was represented by serum zinc and drug metabolizing ability was represented *in vitro* by the cytochrome P450 content of liver tissue and *in vivo* by antipyrine elimination half-life. A significant positive correlation between serum zinc concentration and cytochrome P450 content of liver tissue was found. A significant non linear relationship between serum zinc and antipyrine elimination half-life was also demonstrated. However, correlation does not necessarily imply causation and the use of antipyrine elimination half-life as an index of oxidative drug metabolism in patients with cirrhosis may be a source of error as changes in volume of distribution are not considered (Wilkinson & Shand, 1975). Therefore, in this study the antipyrine test was used to investigate the effect of zinc supplementation on hepatic drug metabolism in patients with cirrhosis. The antipyrine test was devised to determine quantitatively the effect of individual environmental factors on drug metabolizing capacity (Vesell & Page, 1968). Antipyrine undergoes almost complete hepatic metabolism with a low hepatic extraction ratio and shows negligible renal elimination (< 5%) or plasma protein binding (< 10%) (Stevenson, 1977). These characteristics render antipyrine clearance from plasma following a single dose a reliable reflection of antipyrine metabolism (Vesell & Page, 1969).

Following supplementation with zinc sulphate 660 mg daily for 30 days, both serum and leucocyte zinc concentrations increased significantly. Zinc supplementation resulted in a significant reduction in antipyrine clearance and a prolongation in the elimination half-life without a

change in the apparent volume of distribution. In contrast to previous studies these results suggest that zinc supplementation in patients with hepatic cirrhosis may further impair their drug metabolizing ability. There is evidence, however, that zinc may inhibit the monooxygenase system. Zinc has been found to inhibit *N*-dealkylation of benzphetamine and ring hydroxylation of aniline in rat liver microsomes (Peters & Fouts, 1970). Inhibition of epoxide hydase activity *in vitro* following acute doses of zinc has also been described (Parkki, 1980). The initial step in hepatic microsomal electron transport involves the oxidation of NADPH. Chvapil *et al.* (1976) demonstrated the ability of zinc, in concentrations ranging from 3–26 μM , to inhibit competitively the oxidation of NADPH. Therefore, the ability of zinc to inhibit oxidation of NADPH and subsequently alter the rate of reduction of the cytochrome P450 substrate complex would reduce the rate of metabolism for some xenobiotics (Jenner & Testa, 1976). This may explain the reduction in antipyrine clearance found in this study. The limitations of the antipyrine test would suggest that the metabolism of some but not all drugs would be impaired following zinc therapy. For this reason several test drugs used simultaneously are used to predict rates of drug metabolism (Hepner & Vesell, 1976).

Zinc has been shown to be an essential co-factor for many enzyme systems and is involved in membrane protection against oxidants, in RNA and DNA synthesis and in glycoprotein metabolism (Prasad, 1985). In view of the reduction in zinc status observed in patients with hepatic cirrhosis the therapeutic administration of this trace element has been suggested in such patients (Bode *et al.*, 1988). Indeed, supplementation of zinc has been shown to improve some of the clinical features of cirrhosis, e.g. hypogonadism and hypoguesia (Weismann *et al.*, 1979) and hepatic encephalopathy (Reding *et al.*, 1984). However, this study indicates that while zinc supplementation may improve certain features of hepatic cirrhosis, the impaired ability of these patients to metabolize drugs may be further compromised following zinc therapy.

References

- Becking, G. C. & Morrison, A. B. (1970). Hepatic drug metabolism in zinc deficient rats. *Biochem. Pharmacol.*, **19**, 895–902.
- Bode, J. C., Hanisch, P., Henning, H., Koenig, W., Richter, F. W. & Bode, C. (1988). Hepatic zinc content in patients with various stages of alcoholic liver disease and in patients with chronic active and chronic persistent hepatitis. *Hepatology*, **8**, 1605–1609.
- Chvapil, M., Ludwig, J. C., Sipes, G. & Misiorowski, R. (1976). Inhibition of NADPH oxidation and related drug oxidation in liver microsomes by zinc. *Biochem. Pharmacol.*, **21**, 359–365.
- Davies, I. J. T., Musa, M. & Dormandy, T. L. (1968). Measurements of plasma zinc in health and disease, Part I. *J. clin. Pathol.*, **25**, 1787–1791.
- Dixon, J. R., Lowe, D. B., Richards, D. E. & Staking, H. E. (1968). Role of trace metals in chemical carcinogenesis. In *Trace substances in environmental health II*, ed. Hemphill, D. D., pp 144–159, New York: Academic Press.
- Halsted, J. A. & Smith, J. C. (1970). Plasma zinc in health and disease. *Lancet*, **i**, 322.
- Hartoma, T. R., Sotaniemi, E. A., Pelkonen, R. O. & Ahlqvist, J. (1977). Serum zinc and serum copper and indices of drug metabolism in alcoholics. *Eur. J. clin. Pharmacol.*, **12**, 147–151.
- Hepner, G. W. & Vesell, E. S. (1976). Aminopyrine disposition: studies on breath, saliva and urine of normal subjects and patients with liver disease. *Clin. Pharmacol. Ther.*, **20**, 654–661.
- Jenner, P. & Testa, B. (1976). Drug metabolizing enzyme systems. In *Drug metabolism, chemical and biochemical aspects*, pp 290–291. New York: Marcel Dekker.
- Keeling, P. W. N., O'Day, J., Ruse, W. & Thompson, R. P. H. (1982). Zinc deficiency and photoreceptor dysfunction in chronic liver disease. *Clin. Sci.*, **62**, 109–111.
- Keeling, P. W. N., Ruse, W., Jones, R. B., Hilton, P. J. & Thompson, R. P. H. (1980). Tissue zinc status in patients with chronic liver disease. *Gut*, **21**, 561–564.

- Parkki, M. G. (1980). Inhibition of rat hepatic microsomal styrene oxidase hydration by mercury and zinc *in vitro*. *Xenobiotica*, **10**, 307–310.
- Peters, M. A. & Fouts, J. R. (1970). The influence of magnesium and some other divalent cations on hepatic microsomal drug metabolism *in vitro*. *Biochem. Pharmac.*, **19**, 533–544.
- Prasad, A. S. (1985). Clinical endocrinological and biochemical effects of zinc deficiency. *Clin. Endocrinol. Metab.*, **14**, 567–589.
- Reding, P., Duchateau, J. & Bataille, C. (1984). Oral zinc supplementation improves hepatic encephalopathy. *Lancet*, **ii**, 493–495.
- Sandstead, H. H., Bo-Khactic, K. P. & Solomans, N. (1976). Conditioned zinc deficiency. In *Trace elements in human health and disease*, Vol. 1, ed. Prasad, A. S., pp 33–49. New York: Academic Press.
- Shargel, L., Cheung, M. W. & Yu, A. B. C. (1979). High pressure liquid chromatographic analysis of antipyrine in small plasma samples. *J. pharm. Sci.*, **68**, 1052–1054.
- Sotaniemi, E. A., Ahlqvist, J., Pelkonen, R. O., Pirttiäho, H. & Luoma, P. V. (1977). Liver parenchymal changes and indices of drug metabolism in alcoholics. *Eur. J. clin. Pharmac.*, **2**, 295–303.
- Stevenson, I. (1977). Factors influencing antipyrine elimination. *Br. J. clin. Pharmac.*, **4**, 261–265.
- Vallee, B. L., Wacker, W. E. C., Bartholomay, A. F. & Robin, E. D. (1956). Zinc metabolism in hepatic dysfunction I. Serum zinc concentrations in laennec's cirrhosis and their validation by sequential analysis. *New Engl. J. Med.*, **255**, 403–408.
- Vesell, E. S. & Page, J. G. (1968). Genetic control of drug levels in man. Antipyrine. *Science*, **161**, 72–73.
- Vesell, E. S. & Page, J. G. (1969). Genetic control of the phenobarbital induced shortening of plasma antipyrine half lives in man. *J. clin. Invest.*, **48**, 2202–2209.
- Webb, M. & Cain, K. (1982). Functions of metallothionein. *Biochem. Pharmac.*, **31**, 137–142.
- Weismann, K., Christensen, E. & Drayer, V. (1979). Zinc supplementation in alcoholic cirrhosis. *Acta med. Scand.*, **205**, 361–366.
- Whitehouse, R. C., Prasad, A. S., Rabbani, P. I. & Cossack, Z. T. (1982). Zinc in plasma, neutrophils, lymphocytes and erythrocytes as determined by flameless atomic absorption spectrophotometry. *Clin. Chem.*, **28**, 475–480.
- Wilkinson, G. R. & Shand, D. G. (1975). A physiological approach to hepatic drug clearance. *Clin. Pharmac. Ther.*, **18**, 377–390.
- Williams, R. L. & Mamelock, R. D. (1980). Hepatic disease and drug pharmacokinetics. *Clin. Pharmacokin.*, **5**, 528–547.

(Received 2 August 1990,
accepted 6 December 1990)