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ORIGINAL ARTICLE

Lactate dehydrogenase isoenzyme pattern in the liver tissue of chemically-injured rats treated by combinations of diphenyl dimethyl bicarboxylate

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Summary

The purpose of our study is to evaluate the protective effect of diphenyl dimethyl bicarboxylate (DDB) in combination with some antioxidants, namely vitamin C (V.C), vitamin E (V.E), and selenium (Se), in liver damage induced by carbon tetrachloride (0.2 ml/kg body weight). This was done by monitoring the liver total and fractional lactate dehydrogenase (LDH) activities. The results revealed a significant increase in the activity of liver total LDH activity in CCl4 – intoxicated rats with a significant increase in both LDH3 and 4 and a significant decrease in LDH5. LDH2 disappeared after CCl4 treatment and neither DDB nor its combinations could restore this permanent change. DDB alone significantly decreased the CCl4-raised total LDH and LDH4, but still far from the control and failed to correct LDH3 and 5 variations. A combination of DDB and V.C, V.E and Se showed the best corrective potential in both total LDH and LDH3 activities, without correcting the increased LDH4, nor the decreased LDH5 isoenzyme. Although this combination was previously reported to correct liver function disturbances, it seems that CCl4 and consequently hepatitis C may induce some irreversible, non-curable changes by DDB or even by additional antioxidants. Its clinical usefulness seems to be through different metabolic pathways, not including correction of LDH disturbances, which necessitates additional investigation for other adjunct medicines for treating liver fibrosis in clinical practice.

Keywords: LDH isoenzymes – liver fibrosis – DDB – CCI4 – rats

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INTRODUCTION

DDB has been used in the treatment of abnormal liver functions without noticeable side effects (Qing and Liu 1992). It was originally used in China in 1977 in the treatment of hepatoma and chronic hepatitis. The main pharmacological actions of DDB are protection of hepatocytes against injury and increase in the detoxication

capacity of the liver, so it reverses the effect of CCl4 on transaminases in rats (Zhu et al. 1999). LDH is an oxidoreductase enzyme in the glycolytic pathway whose activity is necessary for the reversible reaction in which pyruvate and lactate are inter converted (Kory and Susan 1993). Its isoenzyme patterns are characteristic of many tissues such as: LDH1, LDH2 found in the heart erythrocytes and kidney; LDH4, LDH5 found in the liver, and LDH3 in lymphatic tissue, platelets and many malignant tissues (Henderson 1986). Tissue LDH concentration is much higher than in serum or plasma, so the leakage of LDH from damaged cells is reflected in the serum LDH pattern. Changes in the serum LDH pattern have been employed for the detection of physiological changes in humans (Rodrigue 1995). LDH isoenzyme ratios in relation to total LDH seem to be species and even organ dependent (Salplachta and Necas 2000).

The purpose of our study is to evaluate the protective effect of DDB in combination with vitamin C, E and/ or selenium in carbon tetrachloride intoxicated rats. This was done by monitoring total LDH activity and separation of LDH isoenzymes by electrophoresisin a trial to explore the mechanism of the drug on the liver tissue level.

MATERIALS AND METHODS

Drugs and animals

DDB was imported from China, ground into powder, then suspended in water using gum accacia just prior to administration. CCl4 was obtained from Prolabo, UK. Eighty four male Wistar albino rats of age ranging from 7–8 weeks and weighing 110–115 g, were classified into seven groups; 12 rats each. They were given standard feed, allowed free access of water, kept at temperature range of 20–22 °C and left in a moderately humid room to acclimatize for ten days before dosing. Rats were kept in polyethylene cages of 50 cm length, 35 cm width and 30 cm heights, under a controlled 12 hours dark/12 hours light cycle. The first untreated group served as control and was given saline daily orally. The second group was given single pure CCl4, 0.2 ml/kg b.w. (Kim et al. 1999). The remaining five groups were given CC14 as mentioned before and were divided as follows: The third group was treated with DDB (300 mg/kg b. w.) (Fu and Liu, 1992). The fourth was given DDB + V.C (250 mg/kg, b.w.), orally (Palmer et al 1998). The fifth group was given DDB + V.E (300 mg/kg, b.w., orally, daily), (Liang et al 1995) .The sixth group was given DDB +Se (0.3 mg/kg, p.o., daily) (Berne et al 1991). The seventh group was given DDB + V.C + V.E + Se daily. All treatments were for one month.

METHODS

Liver and heart tissues were excised immediately after 30 days of treatment. Pending analysis, the tissues were stored frozen at –20 ºC. They were homogenized by a high speed blinder in a solution of 0.9% NaCl, 5 mM TRIS–HCl buffer (pH 7.4). Extracts were collected after centrifugation for 30 minutes at 20 ºC, 15000xg, then LDH activity was analyzed in the clear supernatant, (Babson and Babson 1973). Polyacryamide gel was prepared as 5.5% in TRIS–HCl buffer, pH 8.6 (3mm inner diameter, 5cm length). Liver extracts were electrophortetically analyzed against heart extracts as a standard control for LDH isoenzymes pattern, (Dietz and Lubrano 1967). Data were analyzed by one way ANOVA and mean values ± SEM were calculated using Duncan 's new multiple range test (Duncan 1955).

RESULTS

CCl4 injection produced a significant increase in total lactate dehydrogenase activity in liver tissue, inhibited the synthesis of LDH2, significantly elevated both LDH3 and LDH4 and depressed LDH5, compared to the control value. Administration of DDB alone significantly decreased total LDH, and did not change LDH3, 4 and 5 values induced by CCl4. The combination with vitamin C or E and Se, significantly decreased the total LDH activity induced by CCl4. It also significantly depressed CCl4-elevated LDH3 but didn't affect changes in LDH4 and LDH5 (Table 1).

DISCUSSION

LDH is an enzyme present in all human cells catalyzing the pH dependent interconversion of lactate into pyruvate. Characteristically, human LDH can be separated into five different isoenzymes (LDH1 through LDH5), based on their electrophoretic mobility (Kory and Susan 1993). In the present study, CCl4 was used to induce liver damage in rats. It significantly elevated hepatic enzyme activity of total LDH, LHD3, 4, depressed LDH5 and abolished LDH2. The effect on LDH was significantly improved by treatment with DDB, but not recovered to normal.This effect was reported on the serum level, as DDB in a dose of 300 mg/kg body weight could alleviate the CCl4 effect on total LDH activity while lower doses failed to show this protective action (El-Sawy et al 2002). Liver necrosis is known to be associated with decreased LDH1, and 2 and increased LDH3 and 4 (Yasuda et al. 1989).

Table 1. **Effect of DDB and its combinations with vitamins C (V.C), E (V.E) and Se on LDH activities and isoenzyme pattern in CCl4-intoxicated rats after 30 days of treatment** (n=12)

	Total LDH	LDH1	LDH2	LDH3	LDH4	LDH5
Normal control	1.82 ± 0.08	-	1.1 ± 0.1	1.20 ± 0.05	1.20 ± 0.05	96.50 ± 0.20
CCl4-intoxicated	3.46 ± 0.08 *	-	-	2.20 ± 0.05 *	55.30 ± 0.80*	42.50 ± 0.70 *
CCl4 + DDB	2.93 ± 0.08 *	-	-	2.70 ± 0.30 *	47.80 ± 0.40 *	49.50 ± 0.50 *
CCl4 + DDB + V.C	1.90 ± 0.08 *	-	-	2.90 ± 0.06 *	50.80 ± 0.90 *	46.30 ± 0.52 *
CCl4 + DDB + V.E	2.18 ± 0.11 *	-	-	1.98 ± 0.30 *	56.10 ± 0.40 *	41.90 ± 0.40 *
CCl4 + DDB + Se	2.82 ± 0.06 *	-	-	1.50 ± 0.14 *	56.50 ± 0.50	42.00 ± 0.40 *
CCl4 + DDB + V.C +V.E + Se	1.88 ± 0.03 *	-	-	1.65 ± 0.16 *	49.20 ± 2.60 *	42.80 ± 0.40

* Significantly different from control

Hepatic LDH activity was significantly lower in the DDB treated groups supplemented with both vitamins and Se when compared to the corresponding CCl4 treated rats. Supplementation with vitamins could be considered as a possible mechanism for potentiating DDB antioxidant power. Moreover, it was speculated that it would increase the availability of hepatic levels of both V.C and V.E, which would explain the non–enzymatic antioxidant potential of the drug (Ip and Ko 1996).

In our study, the normal control group was found to have four LDH isoenzymes in liver tissue demonstrable by electrophoresis, as LDH1 was absent. The electrophoresis of the LDH isoenzyme in the DDB treated groups supplemented with the three vitamins and Se showed an improvement in LDH3 without any benefit on both LDH4 and LDH5.However, LDH2 is still absent after this treatment. DDB was reported as directly protecting hepatocyte DNA from fragmentation and oxidative damage due to chemical-induced liver injury. This was manifested by its potential in reducing the elevated serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, total bilirubin, serum total bile acid production and tumor necrosis factor α and reducing its messenger RNA expression in liver tissue, both *in* and *ex vivo* (Gao et al. 2005).

CONCLUSION

DDB was proved to have an important hepatoprotective role against CCl4-intoxication through improvement of normal hepatic indices and the antioxidant properties. The results of our study revealed that the level of hepatic LDH in the CCl4 intoxicated rat group is significantly higher than that of normal control, and elevation of isoenzymes LDH3, LDH4 and depressed LDH5, with the disappearance of isoenzyme LDH2. After DDB treatment with vitamins and Se, a significant improvement was noticed. Only total LDH returned near to normal, while LDH isoenzyme still disturbed. So it is clear that DDB supplemented with the mentioned antioxidants only restored total LDH activity near to normal without any role on individual isoenzymes. This mechanism mostly needs further clarification on the sub cellular level to study the possible role of DDB on transcriptional events before the synthesis of individual LDH isoenzymes. Moreover, the corrective action of the drug and its additives doesn't rely on the LDH pathway.

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