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Biochemical Response to Colloidal Bismuth Subcitrate

Dose–Time Effect

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ABSTRACT

In the present study, an investigation was undertaken to assess the efficacy on serum enzymes of colloidal bismuth subcitrate (CBS). CBS was administered with injections to male rats in 100-, 200-, 400-, 500-, and 1000-µg/L doses of bismuth. Rats were anesthetized at different intervals (24, 48, and 72 h) after CBS injections. The levels of serum enzymes were determined. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and creatine kinase (CK) levels significantly increased after all CBS treatments without dependence on time. All doses of bismuth significantly affected the lactate dehydrogenase (LDH) in serum after 72 h. The lowest doses were the most toxic on ALT and LDH. These data suggest that treatment with CBS can provide evidence for a possible marker of liver toxicity although there is no evidence of liver accumulation of bismuth in the present study.

Index Entries: Bismuth; toxicity; serum; liver; enzymatic activity.

INTRODUCTION

The mechanisms of the action of bismuth (Bi) drugs are still poorly understood. Generally, Bi compounds are relatively safe to use. Toxic effects resulting from bismuth compounds have been documented in animals and humans *(1–5)*, but they are rarely seen in normal use of bismuth citrate drugs (colloidal bismuth subcitrate [CBS] and ranitidine bismuth citrate [RBC]) because of their low absorption. Bi-induced toxicity might

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develop because of excessive ingestion or misuse when taken in very large quantities and chronic use for periods *(6).*

One metal that has received little attention with regard to biochemical response is Bi, which is somewhat suprising because Bi has been used for many years for gastrointestinal complaints and has had a revival of popularity in peptic ulcer treatment because of its anti-*Helicobacter* actions *(1).* Biochemical studies were generally conducted to evaluate the effects of Bi in the kidney *(4,7).* Bi was deposited in the kidney, brain, lung, and liver of rats after oral dosing with bismuth subcitrate *(2).*

In blood, Bi is thought to be primarily present in red blood cells, and the remaining portion is found in serum or/and plasma *(8).* Thus, it is also important to evaluate the effect of Bi on enzymes in serum in order to understand the extent of liver damage. To our knowledge, no study has been carried out to examine this issue. Therefore, in this study we were interested in whether Bi could have affected the serum enzymes depending on doses and time. Thus, rats were given a range of doses of CBS. At 24, 48, and 72 h following injections of CBS, blood samples were collected and hepatotoxicity was assessed by measuring the levels of common serum liver enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatine kinase, and lactate dehydrogenase [AST, ALT, ALP, CK and LDH, respectively]).

MATERIALS AND METHODS

Experiment was carried out on male Sprague–Dawley rats, 8 wk old, weighing 150–200 g. The animals were kept on a 12-h light–dark cycle and allowed free access to food and water. All experiments were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (1985).

The animals were randomly divided into six groups: (1) controls, which were intact with no previous experimental history; (2) rats that received a single CBS injection in a dose of 100 µg/L of Bi intraperitoneally (ip); (3) rats that received a single CBS injection in a dose of 200 µg/L of Bi ip; (4) rats that received a single CBS injection in a dose of 400 µg/L of Bi ip; (5) rats that received a single CBS injection in a dose of 500 µg/L of Bi ip; (6) rats that received a single CBS injection in a dose of 1000 µg/L of Bi ip. These investigations stem from the work of Woods and Fowler *(9)* and Szymanska et al. *(10).* There were 21 rats in each group. At 24, 48, and 72 h following injections of all doses of Bi, the animals were anesthetized with ether. Blood samples were collected into serum superator tubes (Microtainer; Becton Dickinson, Franklin Lakes, NJ, USA), allowed to stand (75–90 min), centrifuged (11,000*g*, 5 min), serum harvested, and stored at –20°C. The following parameters were measured at 37°C by an automated biochemical analyzer (ADVIA 1650) with Bayer testing kits (Bioclinica): ALP, ALT, AST, CK, and LDH. Variations of serum enzymes after CBS exposure were used as liver-injury biomarkers.

Statistical Analysis

Experimental data were analyzed using one-way analysis of variance (ANOVA) to determine whether any treatment significantly differed from the controls. Significant differences between the controls and treated samples were confirmed by Fisher's least significant difference test. Results presented as mean ±SD values < 0.05 were regarded as statistically significant.

RESULTS

The AST, ALT, ALP, and CK levels significantly increased 24 h following exposure to all Bi doses (100, 200, 400, 500, and 1000 µg/L) compared with the controls. The increase in ALT was greatest at the lowest dose (100 µg/L of Bi). Again, when compared with the control values, administration of 200, 400, 500, and 1000 µg/L doses of Bi caused similar increases of ALT and AST. Although LDH increased the most at the lowest doses (100 and 200 µg/L of Bi), its level did not show a statistically important increase for 400, 500, and 1000 µg/L of Bi (Fig. 1).

The levels of AST, ALT, ALP and CK significantly increased after 48 h following exposure to all Bi doses compared with the observations of controls. Although LDH was not significantly affected by 400 and 1000 µg/L of Bi, the mean increase reached statistical significance at 100, 200, and 500 µg/L of Bi (Fig. 2).

All of the studied enzyme samples significantly increased at all doses of Bi after 72 h compared with controls (Fig. 3).

DISCUSSION

A study of the biochemical profile of changes in certain important enzymic activities was used to assess the CBS-induced toxicity. The results of the present study clearly indicate that there is a significant increase in the serum enzymic levels (AST, ALT, ALP, and CK except for LDH) after all of CBS treatments without depending on time.

At later times, severe liver injury was evidenced by necrosis of hepatocyte causing elevation of serum enzymes (AST and ALT) *(11,12).* The liver and kidney have been shown to be the target organs of acute CBS toxicity *(2,9).* Tissue accumulation of Bi even occurred after short-course therapy with CBS *(13).* Electron microscopy revealed swollen mitochondria and distortion of mitochondrial inner membranes in rat liver at the 40- and 80-mg/kg dose levels of bismuth subnitrate ($BiONO_3$) *(9).* In vitro studies conducted within 1-h periods with Bi concentrations at 0, 0.1, 0.2, and 0.4 m*M* in reaction mixtures in rats revealed the direct action of the metal on membranal enzymes in liver. In addition, Leussink et al. *(4)* reported disorganization of functions of liver and kidney in rats injected with CBS. At

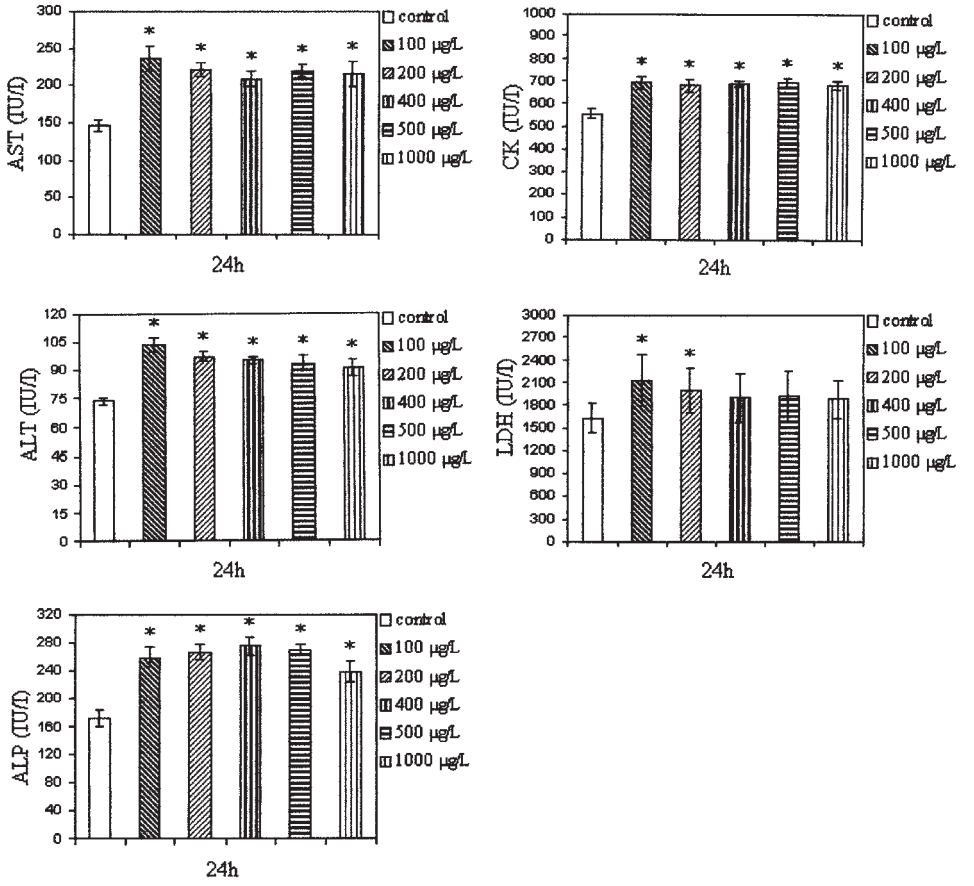

Fig. 1. The changes in AST, ALT, ALP, CK, and LDH levels in the serum of rats after 24 h following exposure to all Bi doses. Each bar represents mean ±SD of seven animals. *$p < 0.05$.

this point, our study provided biochemical evidence of CBS-induced hepatic injury. As a matter of fact, the elevation in transaminase is encountered in conditions causing hepatocellular damage, loss of functional integrity of the cell membrane, and necrosis such as in chemically induced liver injury and elevation in enzymes *(11,14).* Thus, it will be relevant to briefly mention the importance of enzymic changes as manifestations of tissue toxicity. Elevations are seen in the liver and myocardial injury and a rise in serum AST and ALT is more specific and predominant in the liver and myocardial injury, respectively. The modulations in transaminase are also influenced by the degree of hepatic decompensation of cell necrosis *(15).* A significant increase in ALP could occur in paranchymal liver disorders such as hepatitis and cirrhosis, and striking elevation is encountered with extrahepatic biliary tract (mechanical) obstruction or with intrahepatic (functional cholestasis) *(16).*

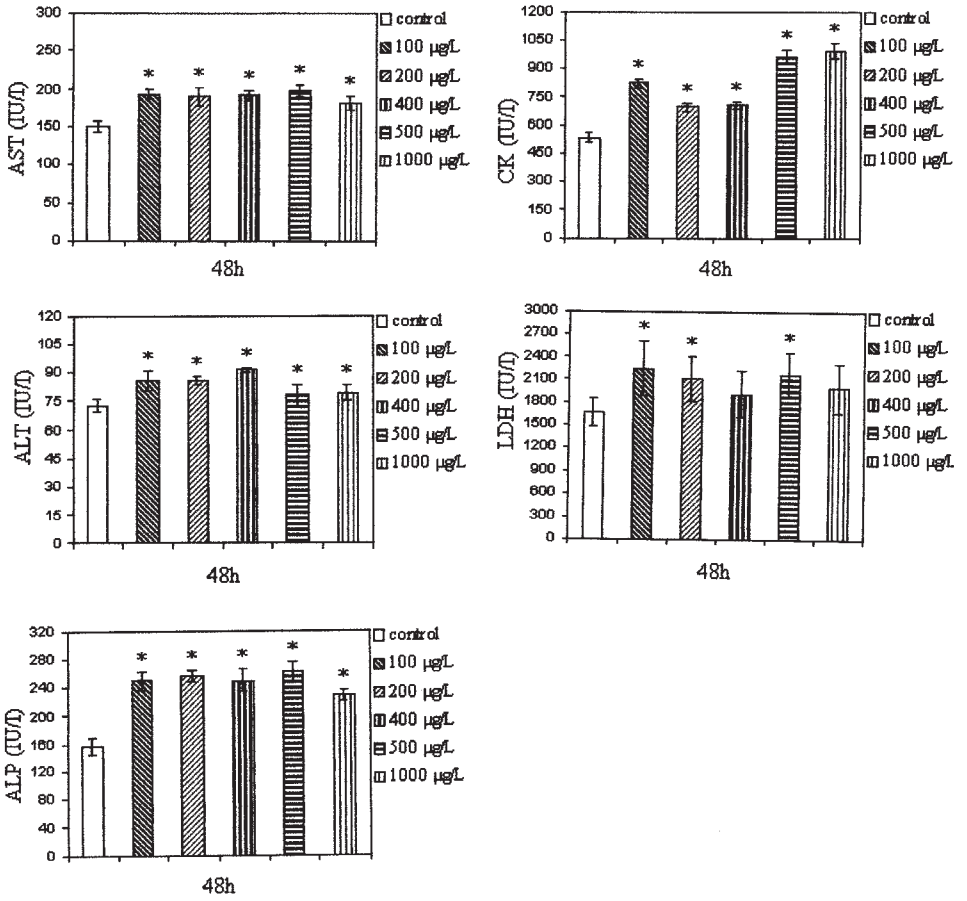

Fig. 2. The changes in AST, ALT, ALP, CK and LDH levels in the serum of rats after 48 h following exposure to all Bi doses. Each bar represents mean ±SD of seven animals. *$p < 0.05$.

Type 3 GSD (type 3 glycogen storage disease) causes increased CK. In conclusion, CK is found to be a diagnostic indicator for hepatic disorder *(17).* Again, the present investigation demonstrates that all doses of Bi significantly affected LDH in serum after 72 h, being different from other enzymes. The LDH in serum as a biological marker for liver damage increases *(18).* Cell necrosis leads to a rise in concentration of the LDH enzyme in serum and tissue *(19).* The LDH released into the medium provides an index of cell death and membrane permeability to LDH, and an increase in LDH activity in the medium occurs as a result of cell membrane disintegration and enzyme leakage *(19–21).* Thus, it was obvious that the time-dependent increase of degenerative effects of Bi become more prominent with the increase in the LDH level. In addition, our study established that Bi-related toxicity on the serum enzymes is not directly related to the

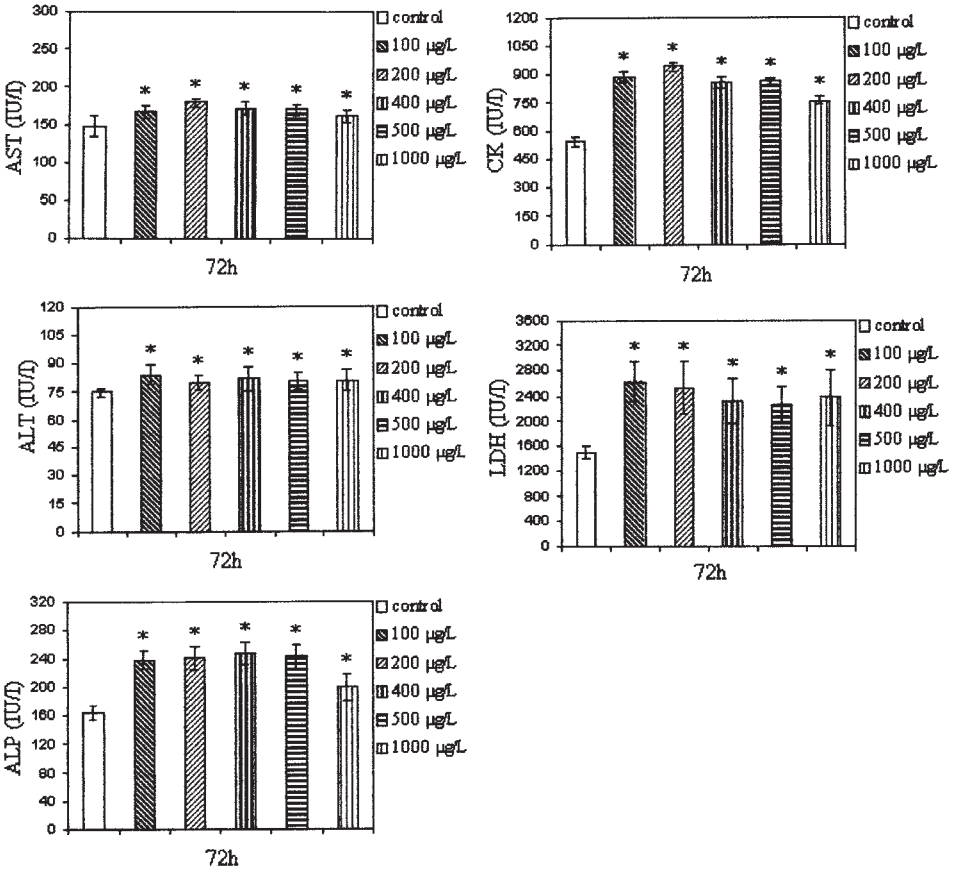

Fig. 3. The changes in AST, ALT, ALP, CK, and LDH levels in the serum of rats after 72 h following exposure to all Bi doses. Each bar represents mean ±SD of seven animals. *$p < 0.05$.

dose. From human clinical experience, it is known that Bi toxicity does not completely depend on the dose or duration of Bi exposure *(22).* The lowest doses of Bi (100 and 200 µg/L) caused the greatest increase of ALT and LDH after 24 h. LDH was significantly high even after 48 h at the same doses. A safety level of 50 µg/L of Bi and an alarm level of 100 µg/L have been suggested in the past, but no proof is available to support the choice of these levels *(1).* Thus, our study shows evidence that 100 and 200 µg/L of Bi has the most toxic effect.

As a result, the levels of enzymes in serum elevated after intoxication of CBS. Moreover, these enzymes were differently affected by doses of CBS and exposure time to CBS. These findings are comparable to those of previously reported for other heavy metals known to have toxicological potential and could represent early events in Bi-induced hepatocellular injury.

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