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Changes in Adenosine Triphosphate (ATP) Concentration and Its Activity in Murine Tissue After Thallium Administration

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Thallium (Tl) is one of the most toxic metals known to man, causing acute and often lethal poisoning as a result of accidental, criminal, suicidal, or therapeutic administration. Ingested Tl is rapidly absorbed and widely distributed throughout the body, causing extensive tissue damage. Clinical symptomatology of Tl toxicity is usually nonspecific due to multi-organ involvement (Saddique and Peterson 1983; Chandler and Scott 1986) and the mechanisms of Tl toxicity are not fully defined. Since Tl behaves much like potassium in the body and binds to sulfhydryl groups, it causes: (1) interference with vital potassium-dependent processes, (2) substitution for potassium in (Na^+-K^+) activated ATPase, and (3) interference with the activity of the sulfhydryl group containing enzymes (Saddique and Peterson 1983; Chandler and Scott 1986; Douglas *et al.* 1990). The (Na^+-K^+)-ATPase enzyme is activated by the substitution of Tl for potassium in rabbit erythrocytes (Gehring and Hammond 1967) and rabbit kidney (Britten and Blank 1968) *in vitro,* but is competitively inhibited by high levels of Tl (Douglas *et al.* 1990). If enzyme systems involved in the production or utilization of ATP *in vivo* are influenced by administration of high doses of Tl, it is thought that changes in ATP concentration in organs such as the kidney and the liver may respond in a dose-dependent manner to increasing concentrations of Tl. In the present study we investigated the relationships between ATP concentration, (Na^+-K^+)-ATPase activity, and Tl concentration in tissues of mice treated with Tl.

MATERIALS AND METHODS

Fifty male adult ICR mice (Nippon SLC Inc., Shizuoka, Japan) weighing 30-35 g were housed in our university animal center (22°C; 55% humidity, 12-hr light-dark schedule) and had free access to a commercial diet (CE-2; CLEA Japan Inc., Tokyo) and tap water during the experimental period. A solution of thallium sulfate (purity 99.9%, Mitsuwa Chemical Co., Ltd., Tokyo) was prepared in saline and administered intraperitoneally to each of 36 mice at a single dose of 25 mg/kg body weight. Six animals were killed under ethylether anesthesia at 3, 6, 12, 24, 72 hr and 10 d after Tl administration. For control, 8 mice were treated with saline and 1 or 2 animals were killed at each time point. Liver and kidneys were removed from all mice and were quick-frozen using a clamp cooled in liquid

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nitrogen Samples were stored at -80°C until analysis

Tissues were homogenized in a Polytron homogenizer with 8% perchloric acid. Homogenates were centrifuged at 2000 x g for 10 min to yield a 10% (w/v) homogenate. Five hundred µL aliquots of supernatant were added to 2.5 mL of 0.2 *M* trisodium phosphate and 10 mL of distilled water, An aliquot was diluted 10,000 times in HEPES buffer. The ATP concentration in each sample was assayed by measuring luciferin-luciferase luminescence (Lumicounter 1000, Niti-On, Tokyo) (Strehler 1974). The absolute sensitivity of the ATP measurement using this method was 0.2 n*M*, with a coefficient of variation of 5%.

Tissues, 0.15 to 0.25 g, were then homogenized in a Polytron homogenizer with ice-cold 0.25 *M* sucrose- 5 m*M* Tris-HCl (pH 7.4) containing 1 m*M* $MgCl_2$ to yield a 10% (w/v) homogenate. Homogenate was centrifuged at 600 x g for 10 min at 4°C and the supernatants were used as the source of (Na^+-K^+)-ATPase. ATPase activity was measured using end-point phosphate analysis following Kimelberg and Papahadjiopoulos (1972). Protein determination was performed using the method described by Lowry *et al.* (1951).

Tl concentration in tissue samples was determined by atomic absorption spectrophotometry after wet digestion with a mixed acid (16 N nitric acid and 60% perchloric acid) as described by Aoyama *et al.* 1988. The absolute sensitivity of the Tl measurement was 0.1 µg, with a coefficient of variation of 4%.

Data were analyzed statistically by Wilcoxon's T-test for comparison between the non-exposed control and the exposed group and one-way ANOVA for time-dependent changes with a preset probability level of $p < 0.05$ or $p<0.01$

RESULTS AND DISCUSSION

Maximum tissue concentrations of Tl were observed 3 hr after administration, which was followed by a gradual decrease (Fig. 1). Ten days after Tl administration, tissue concentrations could not be distinguished from control animals, with levels below 0.1 µg/g.

The ATP concentrations m*M*/g tissue) in control mice averaged 2.81 ± 0.17 (SD) in the liver and 1.31 ± 0.23 in the kidney. These values were similar to those reported by Williamson and Brosnan (1974). ATP concentrations increased 3 hr after Tl administration and showed a 1.7-fold increase over controls at 6 hr (Fig. 2). After 12 hr, ATP concentrations in exposed mice were significantly lower than control animals, The activity of (Na^+-K^+)-ATPase in liver was markedly inhibited at 12 hr after Tl administration, but showed a slight increase after 24 hr. The activity of (Na^+-K^+)-ATPase in liver of treated mice returned to that of the control animals after 72 hr (Fig. 2).

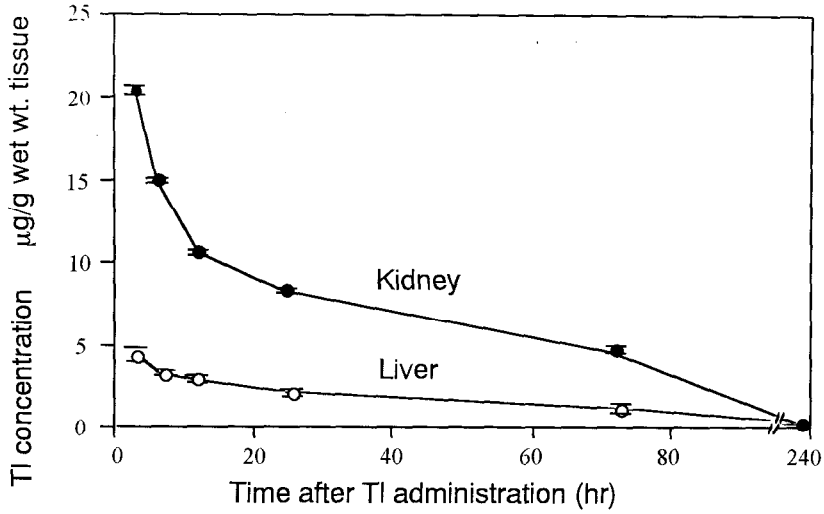

Figure 1. Time course of thallium levels in liver (○) and kidney (●) after administration of 25 mg Tl/kg body weight. Data shown are mean ± SD for 6 exposed animals at each time interval.

ATP concentration in kidney increased abruptly after Tl administration and reached a maximum level (2.8-fold increase) at 12 hr (Fig. 3). ATP concentrations showed a rapid decline after 24 hr, reaching levels that were only 20% of controls. The decrease in ATP concentration persisted at 72 hr after Tl administration, but returned to control levels after 10 d (Fig. 3). (Na^+-K^+)-ATPase activity showed a slight inhibition at 6 hr but increased in a manner similar to the change observed in the liver at 12 hr. Enzyme activity turned to control levels after 72 hr (Fig. 3).

It is known that activation of the (Na^+-K^+)-ATPase by Tl in the presence of Na and competitive inhibition (relative to Na) by high levels of Tl (Ki = 6.8 mM) occurred *in vitro.* We observed that a rapid accumulation of Tl in the liver (within 6 hr) was associated with an initial competitive inhibition of (Na^+-K^+)-ATPase activity, while the subsequent marked decrease in Tl concentration led to remarkable activation of the enzyme over the next 20 to 40 hr. Recently, Appenroth et al. (1995) found that a significant increase occurred in (Na^+-K^+)-ATPase activity in the rat renal medulla on the 2nd day after Tl administration, which subsequently returned to normal levels. Enhanced (Na^+-K^+)-ATPase activity may be a consequence of stimulation by Tl.

Increased ATP concentrations and decreased (Na^+-k^+)-ATPase activity were found simultaneously in the liver at 6 hours after Tl administration. ATP concentrations gradually returned to normal levels as Tl concentrations diminished. Changes in ATP concentration in the liver in the early stage may be related to the activity of (Na^+-K^+)-ATPase. ATP concentrations and ATPase activity in the kidney

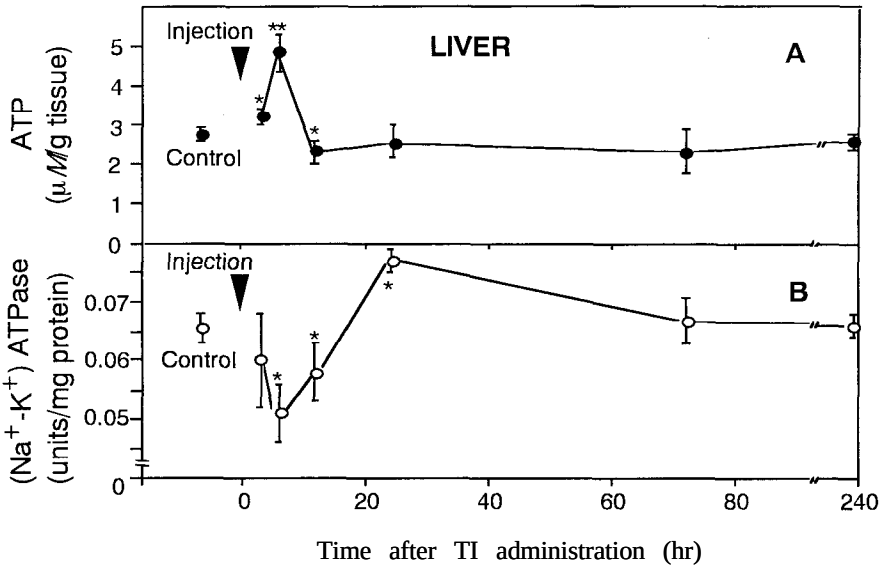

Figure 2. Changes in ATP concentrations and (Na^+-K^+)-ATPase activity in liver after thallium administration. Panel A: ATP concentration (•), Panel B: (Na^+-K^+)-ATPase activity (O). Data shown are mean ± SD for 6 exposed and 8 control animals. Significantly different from controls at **p < 0.01, and * p < 0.05.

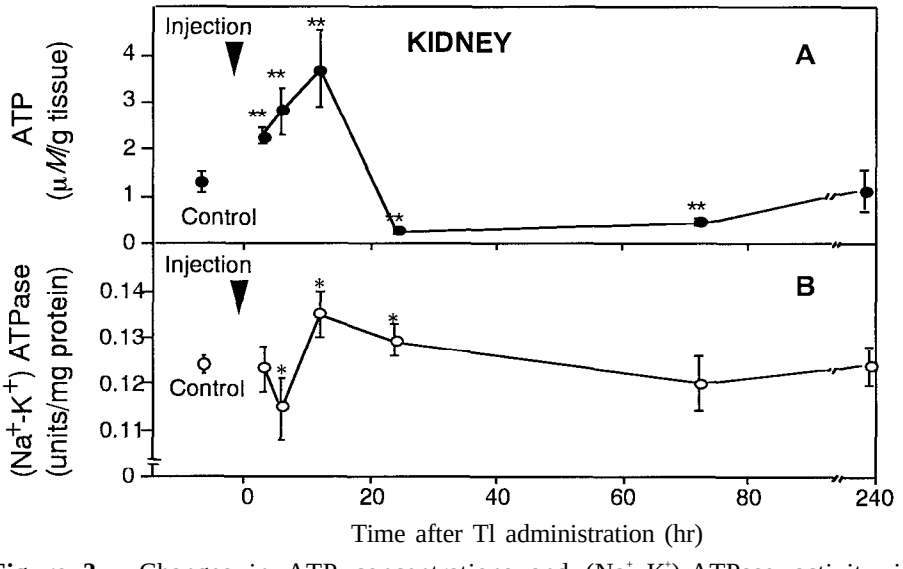

Figure 3. Changes in ATP concentrations and (Na^+-K^+)-ATPase activity in kidney after thallium administration. Panel A: ATP concentration (•) Panel B: (Na^+-K^+)-ATPase activity (O). Data shown are mean ± SD for 6 exposed and 8 control animals. Significantly different from controls at **p <0.01, and * p < 0.05.

changed in a similar manner in the liver during the initial period after Tl administration. Subsequently, a marked decrease in ATP concentrations with a significant increase in ATPase activity was found in the kidney. The decrease in ATP concentration was maintained until 72 hr despite rapid return of ATPase activities to normal levels. It has been reported that ATP concentrations decrease significantly in damaged kidney (Ghazarian *et al.* 1983). In addition, renal failure such as oliguria, diminished creatinine clearance, raised blood urea nitrogen, and albuminuria have been observed in humans poisoned with Tl (Saddique and Peterson 1983; Chandler and Scott 1986; Aoyama *et al.* 1986). This decreased ATP concentration in the kidney after 72 hr was considered to result from kidney damage.

We suggest that the changes in ATP concentration in tissue during the early period after Tl administration may be closely related to changes in (Na^+-K^+)-ATPase activity.

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