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Studies on Hypokalemia Induced by Trimethyltin Chloride[1]

TANG XIAO-JIANG[#,*,2], LAI GUAN-CHAO[#], HUANG JIAN-XUN[#],
LI LAI-YU[#], DENG YING-YU[#], YUE FEI[#], AND ZHANG QING[*]

[#] Department of Toxicology, Guangdong Provincial Center for Occupational Disease Prevention and Treatment, Guangzhou, 510300, China; [*] The School of Life Science, Zhongshan University, Guangzhou, 510275, China

Objectives To determine the possible relationship between plasma potassium concentration and severity of acute trimethyltin chloride (TMT) poisoning and to assess the mechanism of TMT induced hypokalemia. **Methods** SD rats were treated with various dosages of TMT (*ip*). All the indices were measured and analysed for determing their possible relations with plasma K^+. **Results** With increase of dosage, the plasma K^+ level dropped rapidly, and deaths appeared more quickly. The LD_{50} of TMT (*ip*) was 14.7 mg/kgbw. In the low dosage group (10 mg/kgbw), the plasma K^+ level dropped slowly with the lowest dosage on day 6 (4.85 mmol/L). It rose again on day 11 (5.06 mmol/L), and recoverd on day 28. The poisoning signs corresponded with decline of the span of K^+ level. The plasma Na^+ level dropped half an hour after TMT treatment, but recovered 24 h later. In the high dosage group (46.4 mg/kgbw), the levels of plasma K^+ and Na^+ fell rapidly within half an hour ($P<0.05$), the intracellular potassium concentration of RBC did not decrerase obviously ($P>0.05$), the activities of Na^+-K^+-ATPase and Mg^{2+}-ATPase in RBC membrane were depressed remarkably ($P<0.01$, $P<0.05$, respectively), the plasma aldosterone concentrations rose as high as tenfold ($P<0.01$), the arterial blood pH fell from 7.434 to 7.258 ($P<0.01$), pCO_2 was raised from 29.62 to 45.33 mmHg ($P<0.01$). In the 24 h urine test, when rats were treated with TMT (21.5 mg/kgbw, *ip*), urine volume, urinary potassium, sodium and chloride increased significantly in comparison with those in the controls ($P<0.01$). **Conclusion** TMT could induce hypokalemia in SD rats. The available evidence suggests that TMT can induce acute renal leakage of potassium. At the same time, a significant rise of plasma aldosterone may play an important role in promoting potassium leakage from kidney to result in severe hypokalemia with inhaling acid-base abnormalities produced, which aggravate the poisoning symptoms. In the end the rats would die of respiratory failure.

Key words: Trimethyltin chloride; Hypokalemia; Animal model; Mechanism

INTRODUCTION

Organotin compounds are widely used as plastic stabilizers, catalytic and biocidal agents. About 3 000 tons of dimethytin (DMT) are used in China every year. The synthesis of DMT from inorganic tin and methyl chloride under four atmospheric pressure produces

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[2] Biography of the first author: Tang Xiao-Jiang (1967-), male, doctoral student of Zhongshan University, main research field is toxicology and cancer pharmacology.

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88% dimethytin chloride (DMT-Cl), 8% trimethyltin chloride (TMT), and 4% monomethyltin. It is well known that DMT-Cl is of low toxicity. But as one of the main byproducts, TMT is highly toxic, and its neurotoxicity was recognized many years ago [1-6]. Since 1978, 7 accidents of TMT intoxication have been reported, and caused poisoning of 193 people and 4 deaths[7-12]. In addition, it was reported in 1998 that more than 1 000 people in Jiangxi Province of China who ate TMT polluted fat were seriously poisoned. In 1987, Besser, *et al.* reported an acute limbic-cerebellar syndrome in six industrial workers who inhaled TMT[7]. Severe hypokalemia (2.5, 2.9, 2.7 mmol/L, respectively) was found in 3 cases, one of them died. In 1999, Xie, *et al.* reported two occupational intoxications occurred in Guangdong of China[9]. Most of the 39 patients had hypokalemia and two of them died of severe hypokalemia. In the same year, Peng, *et al.* described 123 cases of TMT intoxications, 54 cases of hypokalemia were identified from 60 cases studied[10]. The serum potassium level in some patients was very low (1.7-2.0 mmol/L), but the serum Na^+ and Cl^- levels were normal. It is obvious that hypokalemia plays a very important role in TMT poisoning. However, these abnormalities and their interrelations both in TMT poisoning patients and animals are to be analysed in detail. The purpose of the present study is to investigate the toxicological mechanisms responsible for the development of hypokalemia in rats due to acute TMT intoxication.

METHODS

Animals and Treatment

SD rats of clean grade weight 180-220g, were provided by Medical Animal Center, Health Bureau of Goungdong Province, China. They were housed in an animal room at temperature 23 ± 1.5℃ and relative humidity 55 ± 10% with a 12 h day/night cycle. Except for the 24 h urine test, the animals were fed with a standard rat diet, and tap water. TMT was dissolved in 0.9% saline solution to the concentration needed before injection (*ip*) to keep the volume to 10 ml/kgbw.

Test Material

Trimethyltin chloride was purchased from Acros organics (New Jersey, USA). Sodium heparin was obtained from Xuzhou Wanban Biochemical Medicine Co., Ltd. (Xuzhou, China), which was dissolved at the concentration of 1×10^6 units/L in distilled water, and then used to treat the test tubes (50 μl/tube) and dried at 37℃. *25i-STAT G3*+ test cartridges were obtained from i-STAT Co., Ltd. (New Jersey, USA). ATPase cartridges were purchased from Nanjing Jiancheng Biochemical Institute (Nanjing, China). Aldosterone radioimmunologic cartridges were provided by Sino-USA Joint Venture, Tianjin Jiuding Medical Biochemical Corporation (Tianjin, China).

Plasma Preparation

For measuring the plasma electrolytes, blood samples were collected from vein sinus of one eye (1ml/rat) in sodium heparin treated test tubes and centrifuged 1 000 × *g* for 10 min to separate plasma.

Biochemical Analyses

Sodium, potassium and chlorine concentrations in plasma, intracellular erythrocytes and urine were measured by EasyLyte plus Na^+, K^+, Cl^- Analyser (*Medica Co., USA*). Plasma aldosterone concentrations were analyzed by radioimmunoassay (Diagnostic Products Cop, Los Angeles, USA). The activities of Na^+-K^+-ATPase and Mg^{2+}-ATPase in erythrocyte membrane were measured at wavelengh 600 by CL-8000 Clinical Chemiatry Analyzer (Shimadzu Co., Japan)

Acute Intraperitoneal Toxicity (LD_{50}) of TMT in Rats

Animals of 5 groups (4 males and 4 females each group) were given a single intraperitoneal injection of 4.64, 10, 21.5, 46.4 and 100 mg TMT /kgbw, respectively. The clinical signs and number of deaths were observed.

Dose-Response Test for Hypokalemia Animal Model

TMT was given to rats (6 males and 6 females each group) by a single *ip* injection of 10, 21.5 and 46.4 mg/kgbw, respectively. Half an hour after injection, blood was collected and plasma was analyzed for Na^+, K^+ and Cl^- levels.

Time-Response Test for Hypokalemia Animal Model

TMT was given to rats (6 males and 6 females each group) by a single *ip* injection of 0, 10 and 21.5 mg/kgbw, respectively. Plasma was collected and analyzed half an hour and 24 h and on 3 d, 4 d, 6 d, 11 d, 18 d and 28 d after the injection.

Plasma Aldosterone Concentration Analysis

TMT was given to animals (6 males and 6 females) by an *ip* injection of 0, 46.4 mg/kgbw. Arterial blood was collected from femoral artery half an hour after injection of TMT, and then plasma aldosterone concentrations were measured by radioimmunoassay.

Activity of ATPase in RBC Membrane and Measurement of the Intracellular K^+ Level

TMT was given to animals (5 males and 5 females) by an *ip* injection of 0, 46.4 mg/kgbw. Blood samples were collected half an hour later with heparinized tubes and then centrifuged immediately at 20 000 × *g* for 10 min at 4℃. To measure the intracellular K^+, erythrocyte suspension was added into 3 tubes (0.2 ml/tube) containing 3.8 ml of distilled water, and then centrifuged for 10 min at 2 000 × *g*. Then K^+ concentration in lysates was measured as described[13]. To measure the activity of ATPase in RBC membrane, the erythrocytes were lysed by adding 15 volumes of 10 mmol/L Tris-HCl (pH 7.6). RBC membrane was separated from erythrocytes by centrifugation at 20 000 × *g* for 10 min at 4℃ and washed three times with 10 mmol/L Tris-HCl, pH 7.6. The activities of the three ATPases (Na^+-K^+-ATPases, Mg^{2+}-ATPase and Ca^{2+}-ATPase) in the isolated membrane fraction were analyzed as described[14].

Arterial Blood Gas Measurement

TMT was given to animals (6 males and 6 females) by an *ip* injection of 0, 46.4 mg/kgbw. Half an hour later, 0.2 ml of arterial blood was collected from heart with a

heparinized injecter and immediatly put into the cartridge and analyzed by *i-STAT Portable Clinical Analyzer* according to the test procedure. The measurement included arterial pH, pCO_2, pO_2, HCO_3^-, BEecf and sO_2.

24-Hour Urine Test

TMT was given to animals (6 males and 6 females) by an *ip* injection of 0, 21.5 mg/kgbw and then the animals were put into metabolic cages (1 rat/cage), respectively. Food was taken away and they were only supplied with distilled water. Urine was collected for 24 hours before it was analyzed. The urine volume and the concentration of urinary potassium, sodium and chloride were measured.

Presentation of Data and Statistical Analysis

All data were analyzed by SPSS software. Apart from the LD_{50} test which was analyzed by Horn's method, the data were presented as means + standard deviation with (N) indicating the number of measurements. Comparisons of data of two groups were performed by two-sided *t*-test for paired or unpaired data accordingly. Differences with *P* value less than 0. 05 were regarded as significant. If more than two groups had to be compared, analysis of variance was carried out in the first step. Differences between individual groups were then tested in the second step by *t* test using appropriately adjusted significance levels. As for plasma aldosterone concentrations, rank test was used.

RESULTS

LD_{50} (ip)

In the 100 mg/kgbw group, animals were found to be paralysed and vermiculated about 2 min after the injection and then all died within 3-20 min. In the 46.4 mg/kgbw group, paralysis, trembling and vermiculation were found about 5 minutes after the injection and all animals died within 1.5-20 h. In the 21.5 mg/kgbw group, the animals were seen to have hypopraxia 4 h later. Matted hair and slight trembling were also observed 24 hours later. 48 hours later all animals showed trembling cap-a-pie especially in head. Some of the animals fought with each other and jumped accidentally. The animals died of paralysis on day 3-6. Slight hypopraxia was seen in the 10 mg/kgbw group 4 h later, and the 4.64 mg/kgbw group had no signs of intoxication. The number of deaths in each group is listed in Table 1. Lethal $Dose_{50}$ (LD_{50}) values for females and males were depicted by using Horn's method as 14.7 mg/kgbw (95% confidence limit not found).

TABLE 1

The Deaths of Animals Treated With TMT Injection (*ip*)

Dosage (mg/kgbw)	Female		Male	
	Number of Animals	Number of Death	Number of Animals	Number of Death
4.64	4	0	4	0
10.00	4	0	4	0
21.50	4	4	4	4
46.40	4	4	4	4

Normal Levels of Plasma Electrolytes

Plasma K^+, Na^+ and Cl^- levels of 52 normal rats are listed in Table 2. There is no remarkable difference between females and males ($P > 0.05$). In addition, no significant difference was found in plasma K^+, Na^+ and Cl^- levels between the two sampling time.

TABLE 2

The Plasma Electrolyte Concentration in Normal SD Rats

Plasma Electrolyte Concentration	Number of Animals	Mean ($\bar{x} \pm s$, mmol/L)	95% Normal Range (mmol/L)
Plasma K^+	52	5.86 ± 0.61	4.66 -7.06
Plasma Na^+	52	147.56 ± 2.90	141.88 - 153.24
Plasma Cl^-	52	108.76 ± 5.21	98.55 -118.97

Dose- and Time-Response Curves of Hypokalemia

The results of dose-response test are shown in Fig. 1. With increase of the dosage, plasma K^+ level fell rapidly, and death became sooner. Plasma Na^+ level dropped half an hour after TMT treatment, but it recovered 24 h later. In rats treated with TMT (46.4 mg/kgbw), plasma K^+, Na^+ levels fell rapidly ($P < 0.05$). Fig. 2 shows the time-response curves of plasma potassium and sodium. In the low dosage group (10 mg/kgbw), plasma K^+ level dropped slowly. The lowest plasma K^+ level (4.85 mmol/L) was detected on day 6. Plasma K^+ level rose again on day 11 (5.06 mmol/L), and recovered on day 28. It is obvious that poisoning signs correspond with the decline in speed and the span of K^+ level.

Intraerythrocyte Potassium Concentration

Compared with the control group, intraerythrocyte potassium concentration of TMT in the testing group (46.4 mg/kgbw) did not descend obviously ($P > 0.05$, Table 3).

Activity of ATPase in Erythrocyte Membrane

Table 3 demonstrates that in the testing group the activities of Na^+-K^+-ATPase and

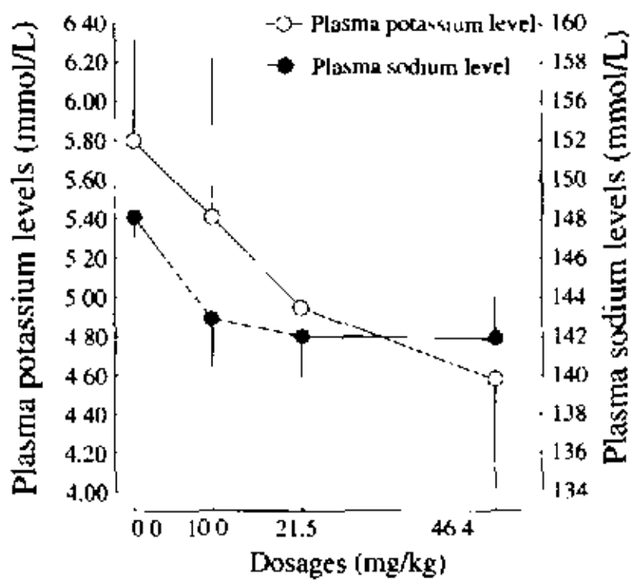

FIG. 1. Dose-response curves of plasma potassium and sodium after TMT treatment.

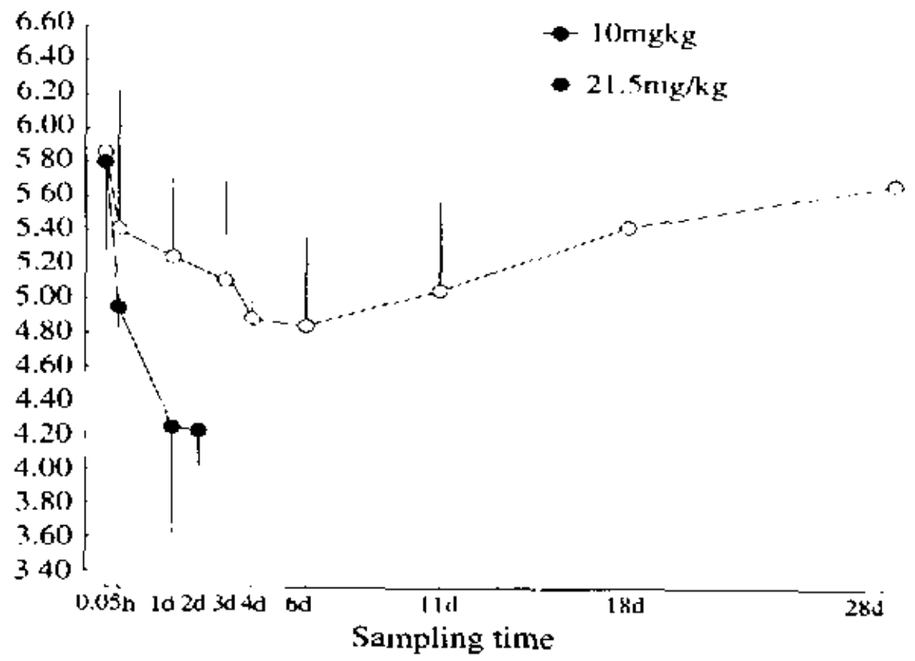

FIG 2. Dose-response curves of plasma potassium levels after TMT treatment.

Mg^{2+}-ATPase in RBC membrane are depressed remarkably ($P < 0.01$, $P < 0.05$, respectively), and that on the other hand, Ca^{2+}-ATPase does not change significantly ($P > 0.05$).

Plasma Aldosterone Concentrations

In the control group, plasma aldosterone concentration varied among the normal rats within the range of 16.57~130.99 pg/ml. Half an hour after TMT treatment, plasma aldosterone concentration raised as high as tenfold ($P < 0.01$) with the range of 318.72~735.85 pg/ml (Table 3).

Arterial Blood Gases Analysis

The results are listed in Table 3. The arterial pH fell from 7.434 to 7.258 ($P < 0.01$), and pCO_2 raised from 29.62 to 45.33 mmHg ($P < 0.01$). These results suggest acid-base disturbance and induction of respiratory acidosis.

24-Hour Urine Test

The results of the 24 h urine test are listed in Table 4. Compared with those of the controls, urine volume, urinary potassium, sodium and chloride increased significantly ($P < 0.01$). It is suggested that renal leakage is increased enormously.

DISCUSSION

The results indicated that TMT could induce acute hypokalemia in SD rats. The higher the dosage was, the lower the potassium level and more serious clinical signs would occur. Hypokalemia could be found half an hour after treatment, and lasted for more than 10 days, which was very similar to the cases of TMT poisoning. It is suggested that acute TMT poisoning in SD rats may be a suitable animal model for studying the mechanism of TMT intoxication in human beings, and hypokalemia may be a very important indicator for TMT poisoning.

The causes of hypokalemia are known to include depression of absorption, increasing

loss and redistribution of potassium in bodies, such as transportation of plasma potassium into cells[15]. In this experiment, the rats were fed with normal food, the plasma potassium levels fell below the lower limits of normal range within a very short time, so absorption depression would not be the main mechanism of hypokalemia.

It is well known that active transport of K^+ and Na^+ in and out of cells is mediated by Na^+-K^+-ATPase in cell membrane. In our experiments, the Na^+-K^+-ATPase activity in erythrocyte membrane was inhibited with no change in intracellular K^+ concentration, and respiratory acidosis was found simultaneously. It is suggested that redistribution of extracellular K^+ into intracellular is not the cause of hypokalemia.

The 24-hour urine test showed a remarkablely increase in excretion of urinary potassium, sodium, chloride and urine volume, it is suggested that TMT could greatly increase electrolyte leakage from the kidney. The results also showed that TMT might increase plasma aldosterone by 10 times as high as the controls. This indicates that renal loss of potassium might be the most likely mechanism of hypokalemia.

In conclusion, the available evidences suggest that TMT could induce rapid leakage of potassium from the kidney. At the same time, a significant increase of plasma aldosterone might play an important role in promoting potassium leakage from the kidney, and a serious hypokalemia could eventually be induced. As a result, respiratory acidosis might aggravate the poisoning signs. In the end, the rats would die of respiratory failure.

TABLE 3

Plasma Electrolytes, Aldosterone, Intracellular Potassium Levels, Activities of ATPase in RBC and Arterial Blood Gases

Indexes	Control Group		Test Group[a]	
	n	$\bar{x} \pm s$	n	$\bar{x} \pm s$
Plasma K^+ (mmol/L)	52	5.86 ± 0.61	12	4.58 ± 0.58**
Plasma Na^+ (mmol/L)	52	147.56 ± 2.90	12	141.92 ± 2.32**
Plasma Cl^- (mmol/L)	52	108.76 ± 5.21	12	110.26 ± 1.98
RBC intracellular K^+ (mmol/L)	10	67.2 ± 8.76	10	65.34 ± 10.25
Na^+-K^+-ATPase activity (μmolPi/10^9RBC/h)	10	1.140 ± 0.245	10	0.567 ± 0.113**
Mg^{2+}-ATPase activity (μmolPi/10^9RBC/h)	10	1.000 ± 0.343	10	0.635 ± 0.331*
Ca^{2+}-ATPase activity (μmolPi/10^9RBC/h)	10	0.753 ± 0.277	10	0.525 ± 0.221
pH	9	7.434 ± 0.048	6	7.258 ± 0.015**
pCO_2 (mmHg)	9	29.62 ± 6.03	6	45.33 ± 6.96**
pO_2 (mmHg)	9	84.33 ± 16.12	6	67.67 ± 18.11
HCO_3 (mmol/L)	9	19.78 ± 3.11	6	20.17 ± 2.40
BEecf (mmol/L)	9	-4.44 ± 2.79	6	-7.00 ± 2.28**
sO_2 (%)	9	95.89 ± 3.55	6	86.83 ± 9.91*
Plasma aldosterone (pg/ml)	11	50.63 ± 34.28	12	513.51 ± 162.75**[b]

[a] The dosage of TMT is 46.4 mg/kgbw (*ip*); * $P < 0.05$, ** $P < 0.01$, compared with control group (two-sided *t* test),

[b] Rank test.

TABLE 4

Urine Volume, Urinary Potassium. Sodium, Chloride in the 24 h Metabolic Test ($\bar{x} \pm s$)

Indexes	Contral Group		Test Group [a]	
	n	$\bar{x} \pm s$	n	$\bar{x} \pm s$
Urine volume (ml)	10	6.80 ± 1.99	10	23 68 ± 9.38**
Concentration of urinary Na^+ (mmol/L)	10	57.73 ± 44.93	10	48.64 ± 8.59
Urinary sodium (μmol)	10	379.44 ± 254.17	10	1120.88 ± 416.19**
Concentration of urinary K^+ (mmol/L)	10	151.60 ± 60.32	10	81.38 ± 58.68
Urinary potassium (μmol)	10	978.67 ± 279.98	10	1481.84 ± 151.13**
Concentration of urinary (mmol/L)	10	126.98 ± 32.82	10	58.13 ± 22.07
Urinary chloride (μmol)	10	840.42 ± 234.13	10	1227.57 ± 366.65*
Sum of urinary sodium and potassium (μmol)	10	1358.11 ± 491.94	10	2602.72 ± 453.77**
Sum of urinary sodium and potassium / Urinary chloride	10	1.59 ± 0.29	10	2.22 ± 0.40**

[a] The dosage of TMT was 46. 4 mg/kgbw (*ip*) ; * $P < 0.05$, ** $P < 0.01$, compare with contral group (two-sided t test).

Many biochemical and neurochemical studies have made on organotin compounds, but the mechanism of toxicity remains unknown[5,6]. As it has been shown that the clinical signs of hypokalemia in animals appeare to be very similar to those of TMT poisoning in human beings, and futher studies on mechanism of TMT hypokalemia should be conducted.

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