J. Biomed. Lab. Sci. 7 (2001) 99-102

The Beneficial Effect of Melatonin for Toluene Hepatotoxicity in Rats

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Toluene is mainly metabolized in liver by oxidative pathway. Oxigen free radicals occur through the process of toluene metabolism. Therefore it causes tissue and cell injury by the oxygen free radicals from the metabolism of toluene. Melatonin acts as a highly efficient free radical scavenger that protects cells from damage by oxygen free radicals. To test this hypothesis, toluene hepatotoxicity was induced by an abdominal injection of toluene. To see if the melatonin protects the rat's liver, melatonin was administrated orally, at the time of each toluene injection. Aspartate aminotransferase (AST), alanin aminotransferase (ALT), latic dehydrogenase (LDH) and alkaline phosphatase (ALP) levels in serum were measured to estimate hepatic function. Malondialdehyde (MDA), which gives an indirect index of oxidative injury was also measured. Hippuric acid is the last metabolic production of toluene was measured by HPLC. There were significantly higher in AST, ALT, LDH, MDA and hippuric acid in toluene group, but there were no significant difference in melatonin group except ALT and hippuric acid. There was significantly lower in ALP level in toluene group, but there was no significant difference melatonin group, suggesting a significant hepatotoxicity due to oxygen free radicals through the process of toluene metabolism. Melatonin treatment significantly protected hepatic function and free radical-mediated injury in the liver against toluene-induced changes. Accordingly, this study shows that melatonin is helpful in protecting liver injury by acute toluene intoxication.

Key Words: Toluene hepatotoxicity, Melatonin, Hippuric acid

INTRODUCTION

Industrial development gives many benefits to humans. But a laborer who works in an industrial company may obtain an occupational disease and have troubling health.

Toluene is a colorless liquid used extensively as a solvent in the chemical, rubber, paint, and pharmaceutical industries, but with much lower volatility than benzene. Toluene is a narcotic; acute symptoms from inhalation include euphoria, excitement, dizziness, headache, and nausea. Extreme acute exposure can result in coma and even death. Because toluene is a solvent for glue, it is frequently one of the solvents associated with "glue sniffing." Chronic exposure to toluene does not involve the hematologic effects that characterize benzene exposure[8]. Acute intoxication of toluene causes liver injury[11,15].

About 80% of the toluene absorbed is metabolized by an oxidative pathway through benzyl alcohol to benzoic acid. At low to moderate toluene concentrations, about 80% of the benzoic acid is conjugated with glycine to form hippuric acid and is excreted in the urine. Less than 20 of the benzoic acid produced is excreted as the glucuronide. There is evidence that this latter pathway is important only when the hippuric acid pathway is saturated, which occurs only at very high toluene exposures. About 20% of absorbed toluene is excreted in the expired air. A very small fraction of toluene is hydroxylated and excreted as cresols in urine, principally as o-cresol[7].

Melatonin, a pineal secretory product, has been reported to have antioxidant properties in addition to its known hormonal activities. Melatonin acts as a highly efficient free radical scavenger that protects cells from damage by oxygn free radicals. Melatonin may have a protective effect on the deteriorated hepatic function that results from oxygen free radicals in the toluene-induced hepatotoxicity[12,18].

To test this hypothesis, toluene hepatotoxicity was induced by an abdominal injection of toluene for 4 days into rats. To see if the melatonin protects the rat's liver, mela-

*Received: July 5, 2001
Accepted after revision: August 21, 2001
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tonin was administrated orally, at the time of each toluene injection. Aspartate aminotransferase (AST), alanin aminotransferase (ALT), latic dehydrogenase (LDH) and alkaline phosphatase (ALP) levels in serum were measured to estimate hepatic function. Malondialdehyde (MDA), which gives an indirect index of oxidative injury was also measured. The urine for hippuric acid was collected in a metabolic cage for 24 hours the last day the toluene injection was given.

MATERIALS AND METHODS

1. Animals

Normal male rats of the Sprague-Dawley strain, weighting between 320 and 350 g, were used in this experiment. All the animals were fed on a standard rat chow and water ad libitum and kept in a temperature controlled environment (20° to 22℃) with an alternating cycle of 12-hour light and dark. All animals were maintained on commercial pellets purchased from Sam Yang Food Co (Wonju, Korea).

2. Experimental Protocol and Groups

Experiments were performed in three groups. In the toluene-alone group (toluene) with 5 rats, each rat received 1.5 ml of toluene which was dissolved in the same amount of olive oil, per kg of body weight 2 times per day, both morning and evening, for 4 days by abdominal injection by method of Pathiratne et al[16]. In the melatonintreated group (melatonin) with 5 rats, each rat received 4 mg of melatonin, which was dissolved in distilled water, per kg of body weight 1 time per day by injecting toluene like the toluene-alone group, in the morning, for 4 days with oral administration. In the control group (control) with 5 rats, each rat received 1.5 ml olive oil per kg of body weight 2 times per day, both morning and evening, for 4 days by abdominal injection. Urine was collected in metabolic cage for 24 hours the last day of toluene injection. The urinary hippuric acid was measured by HPLC and it was corrected urinary creatinine concentration[4]. Blood sample were obtained from the abdominal aorta under light ether anaesthesia.

3. Biochemical Analysis

In serum samples, AST, ALT, LDH and ALP levels were measured by automated techniques using AU400 from Olympus (Japan). Hippuric acid level was measured by automated techniques using CTO-10AC HPLC from Shimazu (Japan).

4. Determination of Malondialdehyde

The amount of serum malondialdehyde was measured by the thiobarbituric acid assay, which based on how malondialdehyde reacts with thiobarbituric acid to give a red species absorbing at 535 nm[3].

5. Statistical Analysis

Values were expressed as mean ± SD. Statistical evaluation of significant difference between means was performed with the Student's t test. $P<0.05$ was considered significant.

RESULTS

The results are shown in Table 1. AST level was significantly higher in toluene group (182.60±13.90 IU/L) than in control group (131.60±20.89 IU/L, $P<0.002$). There was no significant difference between melatonin group

Table 1. Parameters for Hepatic Function and Malondialdehyde, Hippuric acid level

Palameter	Control	Toluene	Melatonin
AST	131.60±20.89	182.60±13.90*	146.60±13.37
ALT	32.00±5.24	50.00±11.22**	43.20±7.19∫
LDH	989±268	2003±508**	1338±634
ALP	379.20±175.57	154.40±18.54*	311.40±69.72
MDA	3.06±0.34	4.40±0.35***	3.33±0.50
Hippuric acid	1.10±0.41	644.60±278.25***	9.04±3.61∬

Values were expressed as mean±SD, *$P<0.05$ compared with control group, **$P<0.01$ compared with control group
***$P<0.001$ compared with control group, ∫$P<0.05$ compared with toluene group, ∬$P<0.001$ compared with toluene group

(146.60±13.37) and control group. ALT level was significantly higher in toluene group (50.00±11.22 IU/L) than in control group (32.00±5.24, $P<0.01$). Also there was significantly higher in melatonin group (43.20±7.19, $P<0.02$) than in control group. LDH level was significantly higher in toluene group (2003±508 IU/L) than in control group (983±268, $P<0.004$). There was no significant difference between melatonin group (1338±634) and control group. ALP level was significantly lower in toluene group (154.40±18.54 IU/L) than in control group (379.20±175.57 $P<0.02$). There was no significant difference between melatonin group (311.40±79.72) and control group. MDA level was significantly higher in toluene (4.40±0.35 nmol/ml) than in control group (3.60±0.34, $P<0.0003$). There was no significant difference between melatonin group (3.33± 0.50) and control group. Hippuic acid level in urine was significantly higher in toluene group (644.60±278.25 hippuric acid g/creatinine g) than in control group (1.10±0.41, $P<0.001$). Also there was significantly higher in melatonin group (9.04±3.61) than in control group ($P<0.001$).

DISCUSSION

Toluene is widely used solvent present in many combinations of chemicals, gasoline, paints, and other solvent mixtures[13]. We have much information about toxicity of toluene. But there is insufficient treatment and prevention of toluene toxicity.

Toluene is absorbed from the respiratory tract, gastrointestinal tract, and through the skin. Due to the lipophilic nature of toluene, the concentration in adipose tissue can be high[1,5,6,11]. Ataxia, tremors, visual impairment, diffuse cerebral atrophy have been known to occur in chronic toluene exposure and in intentional abusers[2]. Also acute intoxication of toluene causes liver injury[11,15].

Toluene is mainly metabolized in liver by mixed function oxidase system[10]. The metabolic products of toluene are cresol (less than 1%) and the intermediate metabolite, benzaldehyde. benzaldehyde is then metabolized to benzoic acid which is conjugated with glycine to form hippuric acid [5,6,17]. Oxigen free radicals occur through the process of toluene metabolism. Therefore it causes tissue and cell injury by the oxygen free radicals from the metabolism of toluene.

Melatonin acts as a highly efficient free radical scavenger that protects cells from damage by oxygen free radicals[12,18].

There were significantly higher in AST, ALT, LDH, MDA and hippuric acid levels in toluene group, but there were no significant difference in melatonin group except ALT and hippuric acid. There was significantly lower in ALP level in toluene group, but there was no significant difference melatonin group, suggesting a significant hepatotoxicity due to oxygen free radicals through the process of toluene metabolism. Melatonin treatment significantly protected hepatic function and free radical-mediated injury in the liver against toluene-induced changes.

The results in this experiment suggest that melatonin is effective in preventing the toluene-induced hepatotoxicity in a rat model.

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