The Effects of Tungstate on Skin Lesions Caused by PPD in Rats

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p-Phenylenediamine (PPD) has been used as one of the ingredients in hair dye. The purpose of this study is to investigate the skin toxicity of PPD application in a tungstate-induced xanthine oxidase (XO) deficient animal model. PPD (2.5% PPD in 2% NH4OH) was applied to rat skin (25 mg/16.5 cm²) five times every other day in rats fed a standard diet (SD) or a tungstate supplemented diet (TD). The skin structure in the SD and the TD group was intact, whereas XO activity was not detected in the TD group during experimental periods. Furthermore, there were no differences between the SD and the TD group in dermal reactive oxygen species (ROS) scavenging enzymes. In these experimental conditions, although XO activity was not detected in the applied PPD rats fed a tungstate supplemented diet (PTD) group, it showed more severe tissue damage compared with the applied PPD rats fed a standard diet (PSD) group. In addition, the PTD group showed higher increased rates of ROS scavenging enzyme activity and lipid peroxide (LPO) content, and decreased glutathione (GSH) content than in the PSD group. In conclusion, the increase of PPD dermal toxicity in tungstate-induced XO deficient animals may be due to excessive ROS via ROS imbalance during PPD skin application.

Key words  p-phenylenediamine; reactive oxygen species; tungstate; xanthine oxidase

Red brownish p-phenylenediamine (PPD) has been widely used as one of the ingredients in hair dyes. The use by consumers of hair dye results in exposure of the hair, scalp skin, eyes, nail, and hands to PPD. PPD studies have found that women using dye with a formulation that included PPD suffered acute sensitive responses such as headaches, dizziness, and systemic anaphylaxis for the long time after using the product.1 And in patch tests, PPD caused erythema, rash, and systemic anaphylaxis for the long time after using the product. Therefore, the present study was designed to investigate the effect of tungstate on PPD skin toxicity as well as the relationship to XO activity. Activities of skin XO and ROS scavenging enzymes, glutathione and lipid peroxide contents, and histopathological findings were determined in rats applied PPD during a tungstate supplemented diet feeding.

MATERIALS AND METHODS

Animals and PPD Treatment Male Sprague-Dawley rats with a mean body weight of 230±10 g were purchased from the Oriental Co., Ltd. (Busan, Republic of Korea). Rats were provided with food (Purina chow, Korea) and water ad libitum. The experimental animals were divided into four groups (six rats per group). The experimental groups were rats fed a standard diet (SD), rats fed a tungstate supplemented diet (TD), applied PPD rats fed a standard diet (PSD) and applied PPD rats fed a tungstate supplemented diet.
The hairs of the ventral part of the experimental animals were removed before the treatments of PPD on skin. PPD (25 mg/16.5 cm²) with 2.5% PPD in 2% NH₄OH was applied to ventral part five times every other day and one treatment per one time was conducted for 2 h on a specific part. In order to delineate the part of applied PPD, glass O-ring (diameter: 46 mm, height: 15 mm) was adhered to the ventral part with harmless adhesive before application and the top of the glass O-ring was sealed to prevent evaporation of the treated substance after application. The PPD non-treated groups (SD and TD) were applied with only 2% NH₄OH. During PPD application, a tungstate (0.72 g/kg) supplemented diet or a standard diet had been fed for 10 d. The animals fasted 24 h before euthenization by drawing blood from the abdominal aorta under ether anesthesia and the ventral part delineated by glass O-ring was used for the experiments. Rats were individually housed in stainless steel wire-bottom cages in a room maintained at 20±2°C and 60±5% relative humidity. The room was exposed to alternating 12-h periods of light and dark. The experimental protocols were conducted in accordance with internationally accepted principles for laboratory animal use and care as found in the Korea Food and Drug Administration guidelines.

Preparations of Sample  Skin tissue was rinsed twice with ice-cold physiological saline and homogenized with 0.25M sucrose by using biohomogenizer (Biospec products Inc., M133). The 20% homogenate was centrifuged at 6000g for 10 min and the supernatant was recentrifuged at 10000g for 20 min and the mitochondrial fraction was used for measuring of catalase (CAT) activity. The supernatant was ultracentrifuged at 105000g for 1 h. The cytosolic and microsomal fractions were used for acid phosphatase (ACP), glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD) and xanthine oxidase type O (XO) activities.

Biochemical Assay  ACP activity was estimated by the method of Bessey et al. and expressed as units per mg protein. A unit of SOD activity was the amount of the enzyme producing 50% inhibition of hematoxylin autooxidation. XO activity was measured by the methods of Stirpe and Della Corte, and expressed as formed uric acid nmol per min per mg protein. The protein content was determined by the method of Lowry et al.

Histological Evaluation  After PPD treatment while feeding a tungstate supplemented diet for 10 d, small pieces of the skin tissue taken from the experimental animals were fixed in 10% neutral formalin, alcohol-dehydrated, paraffin-embedded, and sectioned to a mean thickness of 4 μm. The sections were stained with hematoxylin and eosin according to standard procedures and observed under a light microscope.

Statistical Analysis  The results were expressed as mean±S.D. values for the six animals. Statistical comparison of differences between the different groups was carried out using a one-way analysis of variance test followed by a ANOVA Test (SPSS statistical software package, version 12.0, SPSS, Chicago, IL, U.S.A.).

RESULTS

Skin Injury by Applied PPD in Rats Fed a Tungstate Supplemented Diet  The data of applied PPD in rats fed a tungstate supplemented diet is shown in Figs. 1 and 2. The PTD group compared with the PSD group showed more severe skin injury.
vere skin injury as indicated by the micrographs of skin tissue and markedly increased ACP activity (Fig. 1). In histological findings, the layers of stratum germinativum and stratum spinosum are firmly attached to the basement membrane in both the SD and the TD group. The basal cells and spinoous cells have clear nuclei but the granular cells rarely have nuclei in those groups (Figs. 2a, 2b). The PTD group compared with the PSD group showed irregular granular keratin in granular cells and immature keratin formation. Whereas there were no differences between the SD and the TD group in ACP activity. While in the PTD group, it was significantly higher (60%) than in the PSD group.

**XOD in Skin**  
XO activity, one of ROS generating enzymes, was described in Fig. 3. While XO activity was the value of 2.18 nmol/mg protein/min in the SD group, was not detected in the TD group. In addition, the PSD group compared with PTD represented approximately 80% of massive elevated value.

**DISCUSSION**

It is well known that xenobiotics and its metabolic intermediates as well generates ROS in the process of xenobiotics metabolism that leads to various skin toxicities. In this study, we observed that the degree of skin damage was related to PPD in a tungstate-induced XO deficient animal model. Skin tissue in PPD non-treated groups (SD & TD) showed no discernible alteration in histological findings, whereas in PPD-treated groups (PTD & PSD) there appeared marked damage. And the PTD group compared with the PSD group showed more irregular granular keratin in granular cells and immature keratin formation. It is well documented that ACP activity is increased in damaged tissues, and ACP activity in PTD group was significantly higher than that of PSD group. Therefore, these results suggest that PPD skin toxicity in a tungstate supplemented diet is enhanced.

Meanwhile, XO activity in the PSD group was higher than that of the SD group. Several studies reported that XO activity is increased by tissue damage. This result suggests that increased XO activity in the PSD group is associated with PPD skin toxicity.

It is established that tissue damage was alleviated due to reduced ROS generation via inhibition of XO activity by allopurinol and tungstate. Furthermore, it has been well studied that various tissue damages were induced by excessive ROS via imbalance between ROS generating and scavenging systems. Although, XO activity in the PTD group was not detected, the PTD group compared with the PSD group showed more severe damage with skin tissue. In addition, whereas the ROS scavenging system in the PTD group decreased, LPO was increased compared with the PSD group.
group. It is widely accepted that GSH and LPO are parameters as ROS levels in tissue, and GSH is decreased, but LPO is increased by ROS. It is revealed that activities of ROS scavenging enzymes such as SOD and Gpx are inactivated by ROS. These results indicate that the levels of ROS and oxidized PPD in the PTD group are higher than those of the PSD group, and increased ROS levels by other ROS generating systems except XO and/or oxidative products of PPD influence PPD skin toxicity.

In conclusion, although the definite mechanism on the increase of PPD skin toxicity by tonguestate in this paper is unknown, our study provides experimental evidence that PPD skin toxicity would not be related to XO activity, and tonguestate, an inhibitor of XO, could not alleviate PPD skin toxicity.

Additionally, we will consider that other mechanisms such as NADPH oxidase, and/or immune systems could be related to the PPD skin toxicity along with ROS systems. Further studies in this field are needed to confirm this.

REFERENCES