BIOMEDICAL AND ENVIRONMENTAL SCIENCES **18**, 238-240 (2005)

Effect of Dermal Exposure to Paraphenylenediamine and Linear Alkylbenzene Sulphonate in Guinea Pigs

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Objective To study the effects of paraphenylenediamine (PPD) and linear alkylbenzene sulphonate (LAS) alone and in combination on the skin. **Methods** Forty-eight guinea pigs were divided equally into 4 groups and exposed to PPD (4 mg/kg), LAS (12 mg/kg) and PPD (4 mg/kg) plus LAS (12 mg/kg) for 30 days. The biochemical parameters such as acid phosphatase, gtutathione-s-transferase, glutathione peroxidase, glutathione, lipid peroxidation and histamine contents in exposed skin were estimated. The histopathological examination of the exposed skin was also carried out. **Results** The skin enzymes, lipid peroxidation, and histamine increased while glutathione decreased in skin. The simultaneously exposed group showed additive toxic effects. The histopathological examination showed severe hyperkeratosis, thickening of collagen fibres and vacuolisation of epidermal cells in PPD plus LAS exposed skin. **Conclusion** The findings of the present study suggest that simultaneous exposure to PPD and LAS has additive toxic effects.

Key words: Dermal; Paraphenylenediamine; Linear alkylbenzene sulphonate; Enzymes; Histopathology

INTRODUCTION

Paraphenylenediamine (PPD) is an important industrial chemical. Contact dermatitis in workers and populations due to prolonged and repeated skin contact with PPD have received attention[1]. PPD is the most widely used primary intermediate in permanent hair dye preparations. Direct contact with PPD may cause skin irritation, keratoconjunctivitis, swollen conjunctiva and eczema of eye lids[2]. The linear alkylbenzene sulphonate (LAS) is also used in detergents and cosmetics. Since shampoo may contain LAS and PPD used to dye the hair. Cases of dermatitis due to LAS have been reported[3]. Since there are no reports on biochemical and histopathological effects of PPD and LAS, we have reported the effect of LAS on the dermal toxicity of PPD in this paper.

MATERIALS AND METHODS

Forty-eight male guinea pigs weighing 250±10 g were procured from ITRC and housed in an air-conditioned room and maintained on standard pellet diet (Ashirwad, Chandigarh) and water. PPD was obtained from E. Merck, Germany and LAS was obtained from the IPCL. A 1.0% solution of PPD in 25% ethanol was prepared. LAS (3%) was made in distilled water, 0.1 mL vehicle was applied topically to a 2cm×2cm clipped area (control) on the skin of animals. Similarly, the animals in groups 2, 3, and 4 were treated with 4 mg/kg PPD, 12 mg/kg LAS and 4 mg/kg PPD plus 12 mg/kg LAS respectively daily for 30 days. After 30 days of treatment, the animals in the control and treated groups were sacrificed and a portion of the treated skin was taken and transferred to petri-dishes. The skin was homogenized in ice-cold 0.25 mol/L sucrose solution using ultra turrax homogeniser for biochemical studies.

Assays

The enzymatic activities of acid phosphatase (ACP)[4], glutathione-s-transferase (GST)[5], B-glucuronidase (B-glu.)[6], glutathione peroxidase (GPx)[7] and other parameters *viz.* glutathione (GSH)[8], histamine[9], protein[10], lipid peroxidation (LPO)[11] were determined using the standard methods in skin homogenates.

Small pieces of the skin were fixed in 10% buffered formalin solution and then processed for the preparation of histopathological sections. After routine processing, paraffin sections were cut at 5 μm thickness and stained with haematoxylin and eosin

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0895-3988/2005
CN 11-2816/Q

for microscopic examination[12].

RESULTS

Biochemical Findings

Effect of PPD, LAS and PPD plus LAS on skin enzymes The activity of B-glucuronidase was significantly increased by PPD and PPD plus LAS while that of ACP increased significantly following the treatment with PPD and PPD plus LAS respectively over a period of 30 days compared to the control. The activity of GST and GPx was elevated significantly by PPD and PPD plus LAS treatments.

Effect of PPD, LAS, and PPD plus LAS on glutathione,lipid peroxidation and histamine in the skin The glutathione was significantly decreased by PPD plus LAS, whereas LPO was increased significantly by PPD and PPD plus LAS. Significant increases were observed in histamine by PPD, LAS, and PPD plus LAS, respectively.

Histopathological changes in skin The repeated dermal application of PPD and LAS alone for 30 days produced damage to skin as compared to control animals. However, severe hyperkeratosis, vacuolization of epidermal cells and thickening of collagen fibres were produced when the animals were treated simultaneously with PPD and LAS (Figs. 1 and 2).

TABLE 1

Effect of Paraphenylenediamine (PPD), Linear Alkylbenzene Sulphonate (LAS) and Their Combination on the Skin of Guinea Pigs

Assays	Control	PPD	LAS	PPD+LAS
B-glu*	34.0±2	35±1.2	37±1.5	40±1.0[a]
ACP**	23.65±1.65	24.17±3.45	30.27±1.72[a]	39.17±4.25[a]
GST**	168±9	205±10[a]	173±12	250±12[a]
GPx**	2.15±0.05	5.25±0.25[a]	2.30±0.55	6.34±0.05[a]
GSH***	10.5±0.1	11.04±0.14	10.54±0.09	5.11±0.12[b]
Histamine****	4.5±0.52	7.35±0.12[b]	10.45±0.17[b]	12.6±0.12[a]
LPO*****	5.54±0.56	10.96±1.2[a]	6.89±1.00	11.89±1.2[a]

Note. *mU/mg protein; **n moles/min/mg protein; ***ug/mg protein; ****ug/g; *****ug/g; Each value represents ± of 12 animals in each group. [a] $P<0.001$, [b] $P<0.01$, [c] $P<0.05$ when compared to control (ANOVA).

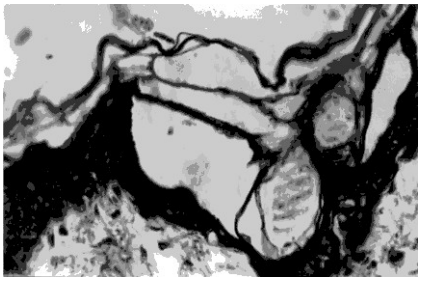

FIG. 1. Section of skin of guinea pigs treated with PPD and LAS in combination showing degree of hyperkeratosis and swollen collagen fibres. H & E × 327.

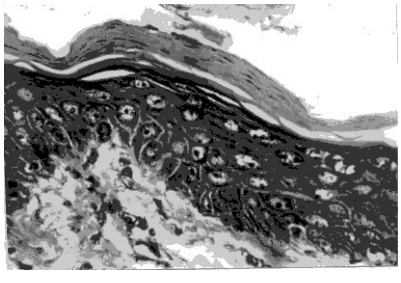

FIG. 2. Section of skin of guinea pigs treated with PPD and LAS in combination showing vacuolisation of cells of the epidermal layer. H & E × 327.

DISCUSSION

The development of *in vivo* test systems for assessing primary irritation was based on subjective observations of inflammatory response. Some more direct assessments of the measurement of common manifestations of toxicity in skin should be designed. The inflammation takes place due to absorption of PPD and LAS. The toxicity could be influenced by the rate and amount of absorption through the skin. The PPD and LAS after absorption may act on membranes and produce damaging effects[13].

The biochemical changes reflect the degree of skin injury induced by harmful stimuli and may offer a direct quantitative assessment of skin cell damage. The increased activity of lysosomal enzymes via ACP and B-glucuronidase shows the disturbance of normal metabolism. The enhanced activity of GST and GPx

enzyme shows that the defense mechanism is increased to counter the damaging effects of PPD plus LAS. The increase is related to the inflammatory response of the skin as seen histopathologically in this investigation. Decrease in glutathione may show that toxicity is increased as a result of PPD and LAS exposure[13]. The observed increase in malondihaldehyde, an index of lipid peroxidation, suggests that increased free radical formation is responsible for the tissue damage. The increase in histamine content may be due to hypersensitivity. The present study of biochemical and histopathological changes shows that simultaneous exposure to PPD and LAS may cause more cutaneous toxicity.

ACKNOWLEDGEMENT

The authors are thankful to Director of Industrial Toxicology Research Centre, Lucknow for support and encouragement in carrying out the studies.

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(*Received October* 1, 2004 *Accepted March* 2, 2005)