ORIGINAL ARTICLE

EFFECT OF PHENYL MERCURIC ACETATE ON HUMAN T AND B LYMPHOCYTES

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ABSTRACT

Background: The use of phenyl mercuric acetate as fungicides and herbicides has much increased in the recent years hence there are greater chances of mercury uptake by humans. The objective of this study was to determine the effect of phenyl mercuric acetate on human immune system.

Material & Methods: This experimental study was conducted in PhD Research Laboratory, Faculty of Pharmacy, Gomal University, D.I.Khan, Pakistan, from December 2012 to January 2013. Terasaki technique was employed to isolate components of white blood cells which is a density gradient separation technique carried out with suitable medium. This medium separate the components of WBC's density-wise through centrifugation process.

Results: In case of T-cells, the drop in T-cells GSH contents by all used concentrations (0.0001-2.0mM) of phenyl mercuric acetate (PMA) with respect to T-cells GSH control was 76.71% (2.306 μM), 73.52% (2.210 μM), 70.99% (2.134 μM), 64.64% (1.943μM), 61.68% (1.854μM) and 59.31% (1.783μM) respectively. The level of GSH content was depleted significantly ($p<0.001$) in both T and B-cells.

Conclusion: Our study suggests that mercury is able to deplete GSH in T and B lymphocytes cells resulting in a compromised immune system. Increasing use of these metals in the environment is a serious threat to human beings.

KEY WORDS: Immune system; Fungicides; Herbicides; Phenyl mercuric acetate; Terasaki technique.

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INTRODUCTION

Depletion of antioxidant glutathione results in a weak immune system.[1] T-lymphocytes and B-lymphocytes are involved in cell-mediated and humoral immune response respectively.[2] Reduced glutathione (GSH) is necessary for various functions of both innate and adaptive immune systems including T-lymphocytes proliferation,[3,4] pathocytic activity of polymorphonuclear neutrophils.[5,6] GSH, the major intracellular antioxidant against oxidative stress when depleted in the lymphocytes results in impaired function of WBCs.[7] The decrease in GSH content of these cells ultimately weaken the immune system hence it was of interest and value to investigate effect of Hg^{II} in these cells of immune systems.

Due to industrialization and changes in the environment during the twentieth century, humans and animals are exposed to numerous chemical forms of mercury, including elemental mercury vapor (Hg^0), inorganic mercurous (Hg^+) and mercuric (Hg^{2+}) compounds, and organic mercuric compounds.[8] All forms of mercury cause toxic effects in a number of tissues and organs, depending on the chemical form of mercury, the level of exposure, the duration of exposure and the route of exposure. Organic mercuric compounds are also nephrotoxic but to a lesser degree than inorganic forms, and they affect other target organs, including hematopoietic and neural tissues.[9-11] Inorganic form of mercury is more harsh then organic form of mercury.[12] Mercury, as well as cadmium, generates highly toxic hydroxyl radicals from the breakdown of hydrogen peroxide, which depletes glutathione stores.[13,14] There is evidence that glutathione depletion can lead to neurological damage; low levels of glutathione have been found in Parkinson's disease and cerebral ischemia-reperfusion injury.[15] The transport mechanism is unclear, but complexes of glutathione and mercury are the predominant form of mercury in both bile and urine.[16] Stability constants are very high for mercury and glutathione so Hg^{II} binds to GSH freely which is in the highest concentrations in cells.[17]

The objective of this study was to determine the effect of phenyl mercuric acetate on human immune system.

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MATERIAL AND METHODS

This experimental study was conducted in PhD Research Laboratory, Faculty of Pharmacy, Gomal University, D.I.Khan, Pakistan, from December 2012 to January 2013. Terasaki technique was employed to isolate components of WBCs which is a density gradient separation technique carried out with medium known as Histopaque. This medium separates the components of WBC's density-wise through centrifugation process. Following chemicals and apparatus were used in this research. Glutathione (Fluka), Ellman's reagent (5,5di-thiobis-2-nitrobenzoic acid, DTNB), RPMI-1640, Ficolpaque plus, Fetal calf serum, (>98%; agarose gel electrophoresis lyophilized) purchased from Sigma Aldrich. Lymphocytes isolation media (Fluka), Sodium Dihydrogen Phosphate (Merk), Sodium Hydroxide, HCl 35% (Kolchlight) purchased from (fluka), (10M Phercloric Acid 70% (fluka), Sodium chloride, NaCl (Merck), Potassium dihydrogen phosphate, KH_2PO_4 (Merck), Chloroform (Merck), Ethanol (Merck), Sodium hydroxide (Fluka AG) , Sodium Edetate (Riedel Dehean AG Sleeze Hannover), Dextrose were purchased from (Merck). Distilled Water (Double Refined), Rubber gloves (Disposable). U.V-visible Spectrophotometer (Schimadzu, 1601 Japan), pH Meter (NOV-210, Nova Scientific Co. Ltd, Korea), Magnetic Stirrer, Oven: Memmert Model U-30, 854 Schwabach (Germany), Hot plate-400 (England), Potter-eveljhem homogenizer (Japan), Micropipettes of 100 μl, 200 μl, 500 μl, 1000 μl (Socorex Swiss, Finland), Eppendolf's tubes (Plastic, 10l), Centrifuge (H-200, Kokusan Ensink company Japan), Rubber gloves (Disposable). Sterile pyrogen free disposable syringes (B.D) (Surge Pharmaceuticals), Siliconized Glass test tubes, Analytical weighing Balance AX 200 (Schimadzu, Japan), Pyrex (Germany) glassware were used during these experiments. All the glassware were carefully and properly washed with detergent, washing powder, chromic mixture, distilled water (double refined) and organic solvents etc. All apparatus were dried at 110°C for two hours in oven. Chromatographic column, Glass pastuer pippets (disposable), Pipette tips (10µl, 200µl, 1000µl).

RESULTS

Absorbance of each sample mixture was recorded under UV-visible spectrophotometer at fixed wave length λmax: 412 nm and each absorbance was converted to concentration of T-cells/ B-cells GSH. This concentration of unknown T-cells /B-cells GSH left after the interaction of various concentrations of phenyl mercuric acetate with T-cells/B-cells GSH was calculated by using standard curve of known concentration for GSH. The level of GSH contents was depleted significantly ($p<0.001$) in both the fraction. In case of T-cells, the drop in T-cells GSH contents by all used concentrations (0.0001-2.0mM) of phenyl mercuric acetate (PMA) with respect to T-cells GSH control was 76.71% (2.306 μM), 73.52% (2.210 μM),

Table 1: Result of various concentrations of phenyl mercuric acetate on the modulation and chemical status of T-lymphocytes (After separation).

Parameters	**0.003**			**0.03**	**0.33**	**3.33**	**33.33**	**66.66**
Time (Minutes)	Conc			Conc	Conc	Conc	Conc	Conc
Remaining concentration of GSH at 0 mint	2.306			2.210	2.134	1.943	1.854	1.783
pH		7.0	2.331	2.223	2.146	2.032	1.981	1.892
		7.5	1.847	1.764	1.675	1.580	1.503	1.401
		8.0	2.000	1.917	1.834	1.732	1.662	1.573
		8.5	2.146	2.057	1.975	1.873	1.815	1.713
Temperature (°C)		25	2.363	2.261	2.197	2.070	1.987	1.904
		37	2.223	2.146	2.057	1.898	1.885	1.796
		45	2.306	2.217	2.159	2.038	1.975	1.898
Remaining concentration of GSH at 20 mint	1.936			1.860	1.777	1.599	1.522	1.459
Remaining concentration of GSH at 40 mint	1.764			1.707	1.605	1.433	1.357	1.287
Remaining concentration of GSH at 60 mint	1.643			1.561	1.484	1.484	1.299	1.146
Remaining concentration of GSH at 90 mint	1.535			1.446	1.363	1.255	1.140	1.045
Remaining concentration of GSH at 120 min	1.465			1.395	1.261	1.108	1.051	0.968
T-lymphocytes GSH Ctrl	3.006			3.006	3.006	3.006	3.006	3.006

Table 2: Result of various concentrations of phenyl mercuric acetate on the modulation and chemical status of B-lymphocytes with time (After separation).

Parameters	0.003			0.03	0.33	3.33	33.33	66.66
Time (Minutes)	Conc			Conc	Conc	Conc	Conc	Conc
Remaining concentration of GSH at 0 mint	2.108			1.981	1.701	1.548	1.478	1.414
pH		7.0	1.854	1.758	1.720	1.637	1.605	1.548
		7.5	1.363	1.299	1.248	1.191	1.134	1.013
		8.0	1.522	1.459	1.408	1.344	1.287	1.236
		8.5	1.662	1.605	1.548	1.478	1.433	1.363
Temperature (°C)		25	1.879	1.796	1.771	1.675	1.605	1.567
		37	1.745	1.682	1.637	1.573	1.516	1.459
		45	1.822	1.752	1.726	1.643	1.599	1.561
Remaining concentration of GSH at 20 mint	1.892			1.713	1.624	1.490	1.420	1.369
Remaining concentration of GSH at 40 mint	1.739			1.573	1.433	1.350	1.287	1.242
Remaining concentration of GSH at 60 mint	1.592			1.484	1.363	1.255	1.210	1.127
Remaining concentration of GSH at 90 mint	1.427			1.325	1.268	1.166	1.115	1.006
Remaining concentration of GSH at 120 min	1.318			1.236	1.153	1.064	0.987	0.898
B-lymphocytes GSH Ctrl	3.038			3.038	3.038	3.038	3.038	3.038

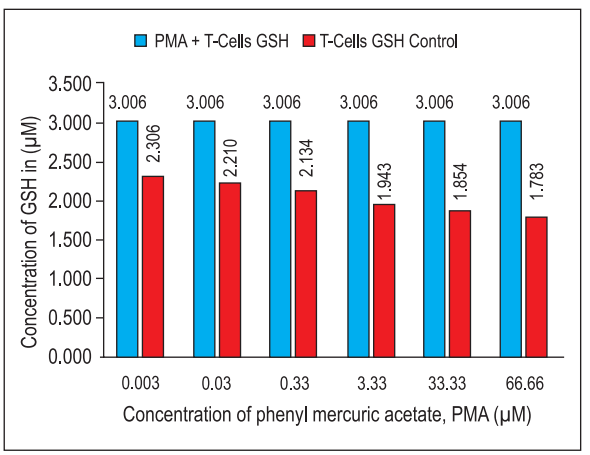

Figure 1.Effect of different concentrations of phenyl mercuric acetate (PMA) on the chemical status of T-lymphocytes-GSH level (concentration effect). Results are the mean ±SE of 3 experiments of T-lymphocytes fraction.

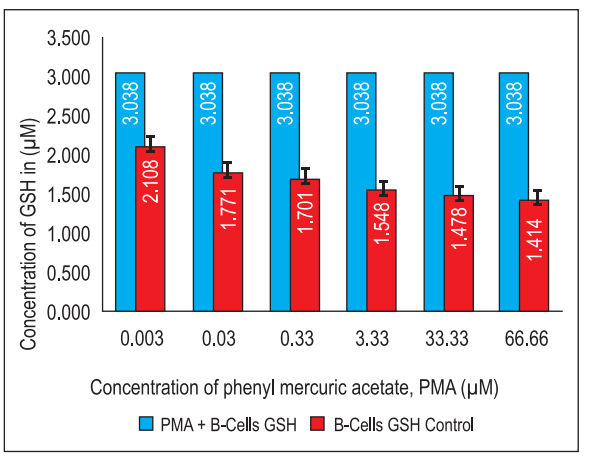

Figure 2.Effect of different concentrations of phenyl mercuric acetate (PMA) on the chemical status of B-lymphocytes-GSH level (concentration effect). Results are the mean ±SE of 3 experiments of T-lymphocytes fraction

70.99% (2.134 μM), 64.64% (1.943 μM), 61.68% (1.854 μM) and 59.31% (1.783μM) respectively. In case of B-cells, the drop in B-cells GSH contents with respect to B-cells GSH control by all used concentrations of phenyl mercuric acetate (PMA) was 69.39% (2.108 μM), 65.21% (1.981 μM), 55.99% (1.701 μM), 50.95% (1.548 μM), 48.65% (1.478 μM) and 46.54% (1.414 μM). The decrease in B-cells GSH level is greater than decrease in T-cells GSH level also showing that phenyl mercuric acetate has more penetrating capability into the semi permeable membrane of B-cells of human blood. It was found that there is further drop in GSH level of both T and B-cells with the passage of time. (Table 1,2 and Fig. 1,2)

DISCUSSION

Proliferation, growth and differentiation of

immune cells are largely dependent on GSH. For differentiation process both T-cells as well as B-cells need sufficient amount of intracellular GSH while intracellular GSH is further required for T-lymphocytes proliferative response to mitogenic stimulation to activate cytotoxic T-killer cells[18] and also for a large number of particular cytotoxic T-cells functions for example DNA synthesis for cell replication, metabolism of interleukin-2 which is very important for the mitogenic response.[19] Sometime experimental depletion of GSH decreases greatly the functions of immune cells.[18,20] It is reported that in various different experimental systems the lymphocytes intracellular GSH was shown to determine the magnitude of immunological capacity.[18] These and other findings show that intracellular GSH status plays a key and central role in the immune cell's functions. Our study is in accordance with these studies and our study suggest that heavy metal including cadmium, mercury are able to deplete GSH in T-Cells and B-Cells resulting in a compromise immune system thus increasing use and involvement of these metals in human environment is a serious threat to human beings. In our study it was noted that various concentrations of organic compound of mercury has the affinity to decrease GSH in these compartments. The time dependent effect of all these compounds in this study have shown further decrease in GSH level of T-cells and B-cells which shows that with the increase in time, the presence of heavy metals is more harmful and depletes more and more GSH resulting in serious consequences in the form of these cells functions impairment and finally death of cells. Heavy metals are foreign agents and our results are in accordance with these reports which states that GSH is depleted in blood components due to the entry of foreign particles including metals and insurant diseases including cancer, HIV, rheumatoid arthritis.[20]

CONCLUSION

Our study suggests that mercury is able to deplete GSH in T and B lymphocytes cells resulting in a compromised immune system. Increasing use of these metals in the environment is a serious threat to human beings.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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None declared.