

PHENYLHYDRAZINE INDUCED TOXICITY: A REVIEW ON ITS HAEMATOTOXICITY

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ABSTRACT

Phenylhydrazine (PHZ) is known to cause anaemia since decades. Due to such effects, PHZ was remarked as a potent drug to meet blood disorders. The PHZ induced toxicity is attributed to the lipid peroxidation which occurs in the membrane of the Red Blood Cells (RBC). Formation of Metahemoglobin and Heinz body formation are the other effects of PHZ toxicity.

Key Words: Phenylhydrazine, Hemolytic Anaemia, Polycythemia vera, Lipid peroxidation, Heinz body formation

INTRODUCTION

Phenylhydrazine (PHZ) is an antipyretic drug that was first characterized by Herman Emil Fisher in 1875. This drug is well known for its ability to produce hemolysis in rats and humans (Dornfest *et al.*, 1983; Dornfest *et al.*, 1992; Ogiso *et al.*, 1989). Due to such effects, PHZ was remarked as a potent drug to meet blood disorders (Giffin and Allen, 1933). PHZ is known to decrease Hemoglobin levels, RBC (Red Blood Cell) count and PCV (Pack Cell Volume) whereas; it induces increase in MCV (Mean Cell Volume), MCH (Mean Cell Hemoglobin) and MCHC (Mean Corpuscular Hemoglobin Concentration) levels (Unami, 1996; Shukla *et al.*, 2012).

In the past years one of its derivatives, PHZ Hydrochloride was used against Polycythemia vera (Falconer E, 1933) and has been shown to induced tumor formation in mice (Toth and Shimizu, 1976). Later on the polycythemia therapy was terminated because of undesirable effects seen (Toth, 1988).

Phenylhydrazine is used worldwide mainly as a chemical intermediate in the pharmaceutical, agrochemical and chemical industries.

PHZ studies appear important from the environmental view point since many of the edible mushrooms *Agaricus bisporus* (Leverberg, 1960) and *Gyromitra esculenta* (List and Luft, 1968) have hydrazines as their basic ingredients.

Chemistry

Chemically PHZ is " $C_6H_8N_2$ " (Molecular Weight: 108). It is in the form of yellow to pale brown crystals or exist as a yellowish oily liquid. It is soluble in water (values ranging from 145 to 837 g/litre at 24 °C) and is miscible with alcohol, ether, chloroform, benzene, and acetone (Polsthettwar and Verma, 2007). It is usually liquid at room temperature with a freezing point of 19.6°C and boiling point of 243.4°C and a vapour pressure of 133 Pa at 72 °C (West and Hull, 1933). The conversion factor for phenylhydrazine is 1 ppm = 4.5 mg/m³ (at 20 °C, 101 kPa). It also exists in the form of PHZ Hydrochloride with similar properties of the original compound.

Phenylhydrazines are nucleophiles as they possess para substitution on their phenyl ring (Rosamilia *et al.*, 2008). PHZ plays role in electron transfer. During oxidation, outer sphere electrons are transferred from the PHZ species to unprotonated and one protonated species of the acceptor complex resulting in the formation of free radicals. The rate of this reduction depends upon the nature of the species (Acharya, 2004).

Toxicity

PHZ is toxic by oral route and is also known to be toxic by inhalation and dermal routes (Cary *et al.*, 2000). LD 50 values are from 80 – 188 mg/kg Body weight. It is a potential skin and eye irritant for

Review Article

humans (Rothe, 1988). It's one of the derivatives N-(α -Chlorobenzylidene) phenylhydrazine is shown to cause contact dermatitis. 5 out of 6 people got ill after coming in contact with this chemical (Rothe A, 1988). The hemolytic activity of PHZ leads directly to hemolytic anaemia in vertebrates (Jollow and McMillan, 2001; Kozlov *et al.*, 1980; Magnani *et al.*, 1986) leads to the formation of Metahemoglobin (Rifkind and Danon, 1965; Rifkind, 1965).

Skeletal protein damage, lipid peroxidation, Glutathion and ATP depletion, cation imbalances, reduced membrane deformability is contributed to the toxic effects induced by PHZ (McMillan *et al.*, 1998). It is also reported that PHZ induced anaemia is associated with activation of immune activation in the effected biological system (Naughton *et al.*, 1990). It has also been reported that PHZ oxidatively damages Hemoglobin and membrane phospholipids (Bloom and Brandt, 2001) and that damaged erythrocytes are removed from the circulatory system by the reticulo – endothelial system.

Histopathological changes following hemolytic anaemia such as erythrophagocytosis, pigmentation and extramedullary hematopoiesis in the liver and spleen are commonly observed in PHZ treated animals (Cary *et al.*, 2008). PHZ has been reported to cause vascular thrombosis as a side effect in clinical use (Reinhardt and Brittelli, 1981). PHZ is known to induce acute thrombosis in rat lung (Sato H *et al.*, 2012). PHZ induce a hyper coagulable state resulting from peroxidation and alternation in erythrocyte membrane phospholipids (Jain, 1985; Yuval and Abraham, 2007).

PHZ is demonstrated to show a remarkable specificity to oxidize cellular components and cause lysis in erythrocytes (Beutler, 1969), Increased bone marrow cells (Futamura and Matsumoto, 1994) and also enhance IL-1 α and IL - 1 β as an secondary effect.

The induced toxicity is actually genotoxicity as, Studies reported PHZ to cause single stranded DNA damage from mouse liver and lung tissue extracts via alkaline elution rate method (Parodi *et al.*, 1981). In similar experiments, liver DNA from PHZ treated rats was analyzed by electrophoresis and found to be markedly fragmented (Ferrali, 1997).

Mechanism

Anaemia is a disease characterized by the reduction in the concentration of Hemoglobin, circulating RBC and its indices (MCV, MCH and MCHC) and PCV per unit of the peripheral blood below the normal (Aguwa, 1997; Oma, 1991). Anaemia impairs normal development in children and it constitutes a major public health problem in the young children in the developing countries with wide social and economic implications (Montalembek and Girot, 1996). Main function of RBC is the transportation of oxygen into the tissues of body. At such, any pathological or physiological condition affects the RBC alters its function and this may be detrimental to the body.

A significant correlation with diagnostic values has been demonstrated between RCB, Hemoglobin, PCV and the other RBC indices (MCV, MCH, MCHC) in both humans and rats (Archer *et al.*, 1982; Bain, 1989). Sub chronic intoxication of rats with PHZ (10 mg/kg/ day for 8 days) resulted in a marked haemolytic anaemia characterized by decreased RBC, Hemoglobin and PCV (Unami *et al.*, 1996).

Studies suggest that phenylhydrazine induces anemia as a consequence of peroxidation of RBC membrane lipids and this effect may be a result of the auto-oxidation of the drug and the interaction of oxygen radicals with membrane lipids (Jain and Subhramanyam 2004).

In experiments with PHZ it was observed that hydrazine has the capacity to generate superoxide anion radical and hydrogen peroxide to cause the formation of lipid peroxidation and Heinz body formation (Jain SK and Hochstein P, 1979). The PHZ induced toxicity has long been associated with the drug induced oxidative stress occurring in the erythrocytes (Kinuta *et al.*, 1995; Jain and Hochstein, 1980).

PHZ is known to enhance the levels of hydrogen peroxide in the erythrocytes, exceeding the levels with which glutathione and catalase can cope. This is a contributory factor to the overall toxicity of PHZ towards erythrocytes particularly where inherited defects or aging give diminished activity of Glucose – 6 – Phosphate Dehydrogenase (Hoschtein, 1971).

Phenylhydrazine produces both aryl and hydroxyl radicals when incubated with rat liver microsomes (Gannett *et al.*, 1997) and oxidized by hydrogen peroxide at pH 7.4 and at 37°C (Rehse *et al.*, 1998). Other

Review Article

than these radicals, the involvement of superoxide (Goldberg and Stern, 1976) hydroxyl (Misra and Fridovich, 1972) and phenyl radicals (Hill and Thornally, 1981) in PHZ induced toxicity is proposed. The radicals induced oxidative stress on the red cell membrane resulting in haemolysis by lipid peroxidation (Cighetti et al., 1999; Zimmermann et al., 1997; Nelson et al., 1997).

Formation of Metahemoglobin which is secondary cause of PHZ induced toxicity is contributed to the oxidation of oxyhemoglobin. Metahemoglobin prior to its formation is converted into hemichromes which in turn, leads to the denaturation and precipitation of Hemoglobin in the formation of Heinz bodies (Rifkind and Danon, 1965; Rifkind 1965). The effected RBC's are eliminated from the system through murine like macrophages. Galactosyl residues are exposed on RBC membrane during oxidation leading to their uptake by murine macrophages (Horn et al., 1990).

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CONCLUSION

As described, phenylhydrazine interacts with the membrane lipid of RBC in oxidation reactions, resulting in the generation of destructive free radicals, which are responsible for subsequent haemolysis and hemolytic anaemia.

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