Journal of Applied Pharmaceutical Research
Volume 7, Issue 4, Year of Publication 2019, Page 1 – 6
DOI: 10.18231/j.joapr.2019.v.7.i.4.001

Review Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR
www.japtronline.com ***ISSN: 2348 – 0335***

MECHANISM OF HAEMATOTOXICITY INDUCED BY PHENYLHYDRAZINE: A REVIEW

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Article Information
Received: 12th March 2019
Revised: 19th August 2019
Accepted: 23rd September 2019

Keywords
Haemolytic anaemia, Erythropoietin receptors, Phenylhydrazine, Haematotoxicity

ABSTRACT
This work was carried out to show the effects of phenylhydrazine (PHZ) induced anaemic condition. Anaemic condition is defined as reduction in red blood cells (RBC) than normal number of red blood cells. The anti-anaemic activity can be studied using the changes in haematological parameters (PCV, RBC & Haemoglobin) influenced by PHZ [(40mg/kg p.o.)] in rats. PHZ, a potent chemical that causes different effects on different tissues at several levels. Administration of PHZ causes haemolytic anaemia, genotoxic effects and rose in iron absorption in spleen, liver and duodenum & causes change in iron metabolism. PHZ acts by activating immune response which triggers phagocytosis and also interfere with the binding of erythropoietin (EPO) receptors and further JAK-STAT pathway. PHZ also causes genotoxic effect by forming single strand DNA damage. In view of lipid peroxidation along with the formation of Thiobarbituric acid (TBA)-reactive malonyldialdehyde, it is recommend that PHZ induces anaemia as an outcome of peroxidation of RBC membrane lipids and this effect may be a upshot of the autoxidation of the drug and the interaction of membrane lipids and oxygen radicals

INTRODUCTION

In 1895 Hermann Emil Fischer used PHZ for various reactions in sugars. PHZ has some adverse effect on human subjects. PHZ exposure may cause red blood cell damage and in turn leads to anaemia, it may also cause complications on the other tissues like spleen and liver. PHZ is proved to be mutagenic invitro and known to exhibit genotoxicity invivo in rats [1]. PHZ is employed to make phenyl hydrazone of natural mixtures of sugars so as to render the differing sugars easily separable from one another. This Molecule is also found to induce acute haemolytic anaemia in animal models. PHZ is one of the major intermediates used in the various industries for variety of purposes. Due to the toxic effects of Phenyl hydrazine derivatives, use of them as anti-pyretics has been stopped [2].

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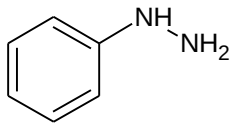
Physicochemical Characteristics [3]

Formula : $C_6H_8N_2$
Boiling point : 243.0±9.0ºc at 760mm Hg
Density : 1.1±0.1g/cm
Molecular weight : 108.14g/mol
Monoisotopic Mol. Weight : 108.068748266
Molecular Framework : Aromatic compounds
Vapor density : 4.3(vs air)
Refractive index : $\frac{n20}{D}$ 1.607
Color : Yellow to pale brown oily liquid
Structure :



Mainly it is used as a chemical intermediate in the agrochemical, chemical and pharmaceutical industries. Derivatives of PHZ are firstly used as antipyretics however due to its noxious action on red blood cells made their use vicious. PHZ used mainly for experimental purposes that is induction of anaemia in rats (animals). PHZ shows a strong drug against polycythemia Vera [4] a disorder, which will be characterized by rise in the total number of erythrocytes in the body [5].

Anaemia:

Anaemia is one of the common blood diseases that affects people of all ages, although the people at greater risk are the elderly, young women of child-bearing age and the infants are also get affected. There are over 400 forms of anaemia, many of which are rare but altogether cases there's below normal number of circulating red blood cells (i.e., 13g/dl in male & 12g/dl in female Haemoglobin levels) [6]. Anaemia is a condition which leads to dysfunctioning of red blood cells within the body and this ends up in lowered transfer of oxygen to the body organs. It remains a notable public health concern in many growing and underdeveloped countries with all age groups in risk, because it causes varying degrees of decreased work capacity, impairment in cognitive performance, reduced immunity to infections, pregnancy complications, decreased psychomotor skills and poor learning capacity [7]. Anaemia affects the lives of over 2 billion people globally, accounting for over 30% of the world's population which is the most common issue particularly in developing countries occurring in the least stages of the life cycle. The different forms of anaemia includes haemolytic anaemia, thalassemia, iron-deficiency anaemia, RBC anaemia, malignant anaemia, anaemia of chronic disease [7]. Symptoms of anaemia include shortness of breath, muscle pain, change in stool colour, fainting & fatigue, angina & heart failure, spleen enlargement and jaundice [8]. The treatment for anaemia is directed according to the reason for the anaemia [7]. WHO's Haemoglobin thresholds used to define anaemia (1g/gl=06206mmol/L) for children 11.0g/dl, teens 12.0g/dl, non-pregnant 12.0g/dl, pregnant 11.0g/dl, men 13.0g/dl [9].

Anaemia diagnosed by evaluating following parameters: complete blood count (CBC), haemoglobin (Hb), packed cell volume (PCV), ferritin, iron, white blood cells (WBC), red blood cells (RBC) [10].

MECHANISM OF PHENYLHYDRAZINE INDUCED TOXICITY

Alteration of iron metabolism

PHZ rises in the iron absorption [11],[12] and produces the expression of iron transport genes [transferrin receptor (TFR1) and haemoxygenase (HO1)]. Haemoxygenase is a very important inducible enzyme involved in heme degradation [13] and also causes iron efflux from cell [14]. Oral administration of PHZ at the dose of 40mg/kg/p.o for 7 days causes haemolytic anaemia in rats. Concentration of EPO was significantly increased by almost 5000-fold in the first couple of days followed by falling down to the basal level after 6 days after PHZ infusion. The mRNA expression of erythroferrone (ERFE), inhibits the production of hepcidin in the liver, and it helps in the synthesis of haemoglobin as this protein increases the amount of iron, was rapidly increased within the bone marrow and spleen 3 days after injection of PHZ and then gradually decreased but was still more than baseline on 6th day. Hepcidin, a regulator of iron metabolism, mRNA level was also found to be reduced by more than 8 times the basal level on 5^{th} day. Mechanistic examination manifested that the increase of serum EPO essentially determined the induction of ERFE expression particularly at the primary 3 days after PHZ treatment. Hepcidin suppressionn is restrained significantly by ERFE overthrow which is mediated by Lentiviral elements under PHZ treatment. Thus, EPO dependent ERFE expression acts as an erythropoiesis-driven regulator of iron metabolism under PHZ-induced haemolytic anaemia [15].

Haemolytic anaemia

PHZ causes haemolytic anaemia to know about EPO, pathological, regenerative response through clinical and

morphological studies. PHZ is given through oral, inhalation and dermal routs that causes oxidative stress within erythrocytes which results in oxidation of oxyhemoglobin leads to the formation of methemoglobin and later converts into irreversible hemichromes which causes precipitation of haemoglobin in the formation of Heinz bodies [16][17]. PHZ causes impairment in skeletal protein, lipid peroxidation, ATP depletion, imbalance of cation and decreased membrane deformability. All these symptoms figure outs haemolytic anaemia [18].

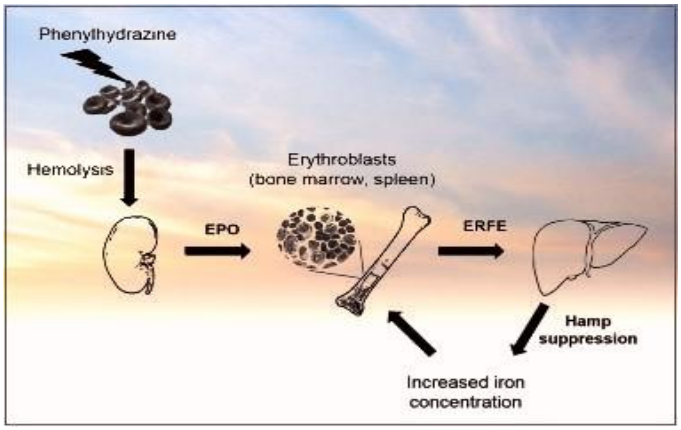


Fig no 1: Mechanism of PHZ induced anaemia

(EPO - erythropoietin, ERFE- erythroferrone, Hamp- Hepcidin antimicrobial peptide)

Effect of PHZ on JAK-STAT pathway

EPO-receptors affected by PHZ of JAK-STAT pathway and this EPO-receptor is responsible for the maturation of red blood cells. Cytokine receptor family member is EPO-receptor, upon binding to EPO, this receptor causes activation of Jak2 tyrosine kinase that results in activation of different intracellular pathways (Ras/MAP kinase, phosphatidylinositol 3-kinase and STAT transcription factors). This stimulated EPO-receptor has a role in cell survival. This EPO-defect receptor causes erythrocytosis and erythroleukemia. This Cytokine irregulation affect the growth of certain tumors [19].

A common feature of these RTKs does not have kinase activity, but this intracellular domain has binding site for tyrosine kinase JAK, upon binding to the ligands causes activation of JAK. Activation of JAK phosphorylates various proteins which are responsible for signal transduction from the extracellular to the intracellular. Cytokine disregulation causes alteration in the JAK-STAT pathway [19].

Effect of PHZ on spleen and immune system

PHZ plays role in electron transfer leading to the formation of free radicals. Stimulation of the bone marrow could also be induced by PHZ colloidal gold accumulates only within the sinusoids of the Bone marrow. Formation of meta-haemoglobin & Heinz body formation are the opposite effects of PHZ toxicity. Increase in the size of the spleen due to the excessive rush of iron results in EPO activity on the spleen, this condition is termed as splenomegaly. PHZ induced anaemia activates immune reaction which triggers phagocytosis within the spleen and binding of EPO receptor [19].

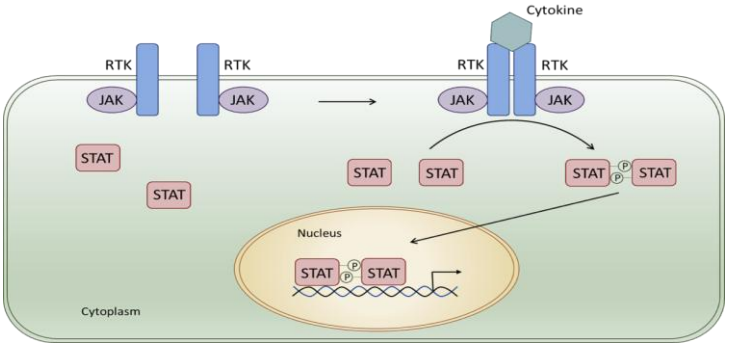


Fig no 2: JAK-STAT pathway

JAK-Janus kinase, STAT- Signal transducers and activators of transcription, RTK - Receptor tyrosine kinase, P – Phosphorylation)

Genotoxic effect of PHZ

By alkaline elution rate method we can say that PHZ causes single strand DNA damage from lung tissue extracts and mouse liver [20]. The liver DNA from PHZ treated albino rats is analysed by electrophoresis process and results in markedly fragmented. Studies have shown that there is an increase in iron absorption in liver and intestine in the PHZ induced anaemia. Fact finding evidences have also been showed that Iron metabolism completely disrupted and in few cases genotoxic effects producing single strand DNA damage have also been concluded [21].

EFFECTS IN HUMANS

Single exposure causes of acute intoxication with PHZ in man include the formation of methaemoglobin and its sequelae [22]. Repeated exposurePHZ causes polycythaemia at a dose of PHZ 100mg/day/p.o shows jaundice, anaemia and oedema as a side effects [23]. Additionally, the urine found to be dark in colour because of its content of haemoglobin, bilirubin and bile acid derivatives. It is unknown that the noticed enlargement of

kidneys, liver and spleen are also a result of polycythaemia [23][24]. PHZ contact also causes skin and eye irritation in humans [25].

DOSE

Administration of PHZ at the dose of 30mg/day (0.4mg/kg/p.o body weight) for 8 days leads to haemolysis of transfused erythrocytes at a level of 0-10% [26]. PHZ injection dose of 10 or 20mg/kg/p.o body weight into pregnant wistar rats on days 17 to 19 gestation showed in behavioural disturbances in the pups (reduced learning ability). After the administration of PHZ of 20mg/kg/i.p body weight on 18 and 19 of gestation the serum bilirubin level in the foetuses is increased than in the dams and there is increase in mortality among the pups [27].

FIRST AID

In case of eye contact, flush with large amount of water immediately. Occasionally lift upper and lower lids and continue washing without stopping for 30min and immediately seek the medical attention [28]. If exposed while breathing, take the person away from the site and use the universal precautions. If the heart beating stopped, CPR should be done mean while start rescue breathing and transfer the person to medical facility. If any symptoms occurred than the person has to be under the medical observation for several days and symptoms might delay [29]. If PHZ comes in contact with skin, immediately remove the contaminated cloths and quickly wash the contaminated skin with large amount of water & soap [30].

Discussion

PHZ is understood to cause anaemia since decades. PHZ was remarked as a potent drug to fulfil the needs of researchers to fight against blood disorders. PHZ is a very common chemical compound used in agro, pharma and in chemical industries. PHZ is toxic by oral route and also by inhalation and dermal routes. LD50 values are from 80-188mg/kg weight. The PHZ induced toxicity is attributed to the lipid peroxidation which occurs within the membrane of the RBC. PHZ has potential for skin and eye irritation properties and also has evidence for its skin-sensitizing properties in humans [31].

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CONCLUSION

Red blood cell hemolysis was induced in mice by administration of phenylhydrazine. PHZ is the principal molecule which is used to induce anaemia in the laboratory animals there by helping in studying about haemolytic anaemia. PHZ affects the JAK-STAT pathway. It also causes splenomegaly, affects iron metabolism and genotoxic effect and mainly causes haemolytic anaemia at the dose of over 40mg/kg/p.o/OD for 7 days in rats. PHZ also affects normal cell metabolism as its electron transfer reaction leads to the formation of free radicals and also deregulates hepcidin expression.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

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