Review Article

Molecular Mechanism of Phenylhydrazine Induced Haematotoxicity: A Review

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ABSTRACT

Phenylhydrazine (PHZ), a potent chemical causes toxicity on various tissues at various levels. Administration of phenylhydrazine mainly causes haematotoxicity which leads to the haemolytic anemia. In mammals PHZ induced anemia increased the iron absorption in spleen, liver and duodenum and finally iron metabolism was altered. Local demand and supply of Fe would increase erythropoeitic activity of the spleen so the size of spleen was increased that create the splenomegaly**.** PHZ induced anemia activate immune response which triggers phagocytosis in the spleen and liver. Apart from this administration of PHZ interfere the binding of erythropoietin (EPO) with erythropoietin receptors (EPOR) so that JAK-STAT would be affected.PHZ also showed genotoxic effect by creating single strand DNA damage.

Keywords: Phenylhydrazine, Haematotoxicity, Haemolytic anemia, EPO receptors.

INTRODUCTION

Phenylhydrazine (Hydrazino-benzene) is the chemical compound characterized by Hermann Emil Fischer in 1895. The chemical structure and physicochemical characteristics are given below:-

Physicochemical Characteristics:-
Chemical formula-C6H8N2
Density- 1.10 g/cm³
Molar mass- 108.14g/mol
Boiling point- 243.5 °C
Melting point- 19.5 °C
Colour- Yellow to pale brown oily liquid

It is mainly used as a chemical intermediate in the pharmaceutical, agrochemical, and chemical industries. PHZ derivatives were primarily used as antipyretics but due to its toxic action on red blood cells made their use dangerous. Phenylhydrazine was mainly used for experimental induction of anemia in animals. PHZ acts as a potent drug against polycythemia Vera[2] a disorder[15], which is characterized by increase in the total number of erythrocytes in the body**.** PHZ decreases Haemoglobin levels, RBC (Red Blood Cell) count and PCV (Packed Cell Volume) whereas increases the MCV (Mean Cell Volume), MCH (Mean Cell Hemoglobin) ,

MCHC (Mean Corpuscular Hemoglobin Concentration) and extramedular haematopoiesis in the spleen and liver[16].

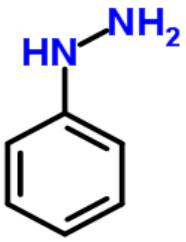

Figure1: Chemical structure of Phenylhydrazine

Mechanism of Phenylhydrazine Induced Toxicity

Haemolytic anemia

Phenylhydrazine (PHZ) –induces hemolytic anemia to study erythropoietin regenerative response through clinical, pathological, and morphological studies. PHZ is absorbed by the inhalation, oral and dermal routs hemotoxicant PHZ causes oxidative stress within erythrocytes resulting oxidation of oxyhemoglobin leading to the formation of methemoglobin which is subsequently converted into irreversible hemichromes that lead to the precipitation of hemoglobin in the form of Heinz bodies[13,14]. PHZ causes damage in skeletal protein, lipid peroxidation, ATP depletion, cation Imbalances, and reduced membrane deformability. All these symptoms show hemolytic anemia[7].

Alteration of iron metabolism

In mammals Iron metabolism is altered by haemolytic anemia induced by Phenylhydrazine. Phenylhydrazine (PHZ)-induced anemia increases the iron absorption[5,12] that induces the expression of iron transport genes (Dcytb, DMT1-IRE and Ireg) in the duodenum so the expression of Dcytb, DMT1-IRE and Ireg1 mRNA was enhanced in the duodenum of PHZ-treated mice. The patterns of gene expression in the Duodenum can be seen by RT-PCR analyses. Dcytb and Ireg1 genes are also involved in iron metabolism in spleen and liver of the PHZ-treated mice. During a period of acute haemolysis the catabolic and anabolic pathways of haemoglobin in the spleen must be regulated to maintain a balance in systemic iron homeostasis by measuring the expression of transferrin receptor (TFR1) and haem oxygenase (HO1). HO1 is an important inducible enzyme involved in haem degradation[3] and also causes iron efflux from cell[9]. The expression of Ireg1, TFR1 and HO1will increased in the spleen of the PHZ-treated mice. In PHZ-treated mice the level of Dcytb in spleen will also be increased .finally local demand and supply of Fe will increases erythropoeitic activity of the spleen so the size of spleen will increase it will cause the splenomegaly. Liver also plays an important role in maintaining body iron homeostasis. We can examine the hepatic expression of several relevant genes following PHZ-induced haemolysis. The expression of TFR1 in liver was significantly increased in PHZ-treated mice, while hepcidin expression will decrease.

Effect of PHZ on immune system

PHZ-induced anemia is also responsible for immune activation[10]. In this respect, PHZ can cross red blood cells and binds with circulating autologous antibodies[8]. This antigen- antibody complex is recognized by macrophage receptors which triggers phagocytosis in the spleen and liver. This indicates that damaged cells are removed intact by the spleen. Apart from blood storage and immune competence, the spleen also acts as the main erythrophagocytic organ in rodents and rabbits which are suffering from PHZ induced haemolytic anemia[4].

Effect of PHZ on JAK-STAT pathway

PHZ also affects the EPO receptors of JAK-STAT pathway which is responsible for the maturation of red blood cells. After Phenylhydrazine-induced anemia, EpoR-HM mice failed to respond with efficient splenic stress erythropoisis[6]. The erythropoietin receptor which is a member of the cytokine receptor family, upon erythropoietin binding, this receptor activates Jak2 tyrosine kinase which activates different intracellular pathways including: Ras/MAP kinase, phosphatidylinositol 3-kinase and STAT transcription factors. The stimulated erythropoietin receptor appears to have a role in erythroid cell survival. Defects in the erythropoietin receptor may produce erythroleukemia and familial erythrocytosis. Disregulation of this cytokine may affect the growth of certain tumors.

Genotoxic effect of phenylhydrazine

PHZ create single strand DNA damage from lung tissue extracts and mouse liver through alkaline elution rate method[11]. In an experiment, the liver DNA from PHZ treated rats was analyzed by electrophoresis and found to be markedly fragmented[1].

DISCUSSION

PHZ is absorbed by the inhalation, oral and dermal routs. After absorption it causes oxidative stress in RBCs and generates reactive oxygen species (ROS) in the RBCs this ROS reacts with haemoglobin and changes the oxyhaemoglobin in to methaemoglobin, hemichromes and other haemoglobin breakdown products such as Heinz bodies .This compound seems to be very useful in models studying mechanism of hemolytic anaemia. PHZ induces a reactive oxygen species formation which results in Peroxidation of lipid and oxidative degradation of spectrin in the membrane Skelton. This chemical has potential for skin and eye irritation in human and animals. After that PHZ translocates the phosphatidylserine from inner to outer of the plasma membrane and causes the membrane lipid peroxidation due to lipid peroxidation RBCs enter in the spleen and uptake by the macrophages. It is signal for Phagocytosis of cell under programmed death by macrophages. It will cause haemolytic anemia. Apart from haemolytic anemia PHZ also alters the iron metabolism by increasing the expression of ferrous transporter (DMT1) in the spleen, duodenum and liver. DMTI transporter promotes the expression of genes related to iron metabolism such as ferric reductase DCytb, Ireg1 and DMT1 in human and mice. Expression level is checked by Northern blot, RT-PCR and immunocytochemistry. Increased mRNA expression of DCytb, DMT1, Ireg1 and IFR1 in spleen and liver will increase the iron demand resulting stimulation of erythropoisis so the size of spleen will increase it will causes the splenomegaly. PHZ also affects the EPO receptors of JAK-STAT pathway which is responsible for the maturation of red blood cells. Phenylhydrazine-induced anemia, EpoR-HM mice failed to respond with efficient splenic stress erythropoisis.

CONCLUSION

From the above result it may be concluded that phenylhydrazine create toxicity at various level PHZ create haemolytic anemia, alter iron metabolism that lead to the splenomegaly and activate immune response. Apart from this PHZ also creates genotoxicity and interfere JAK-STAT pathway.

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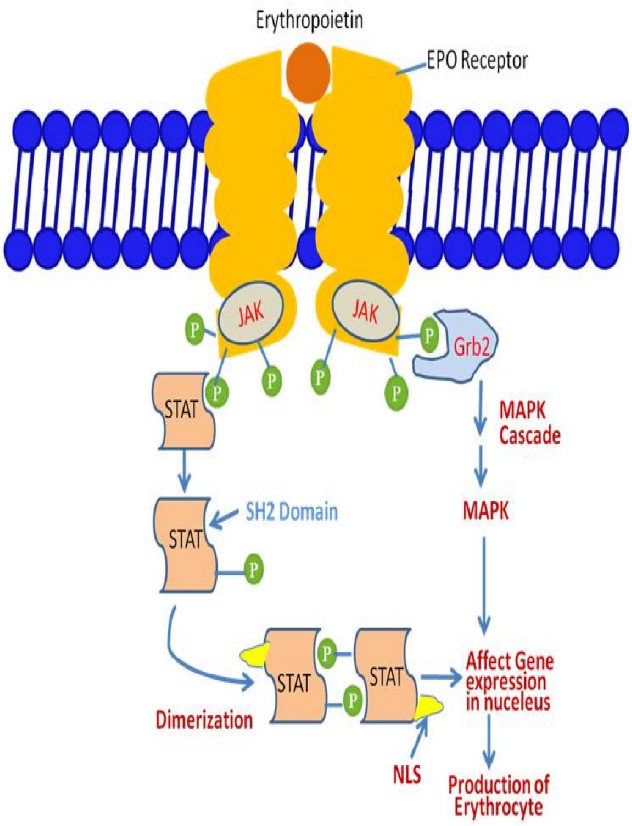

Figure 2(a). Showing the normal functioning of JAK STAT Pathway.

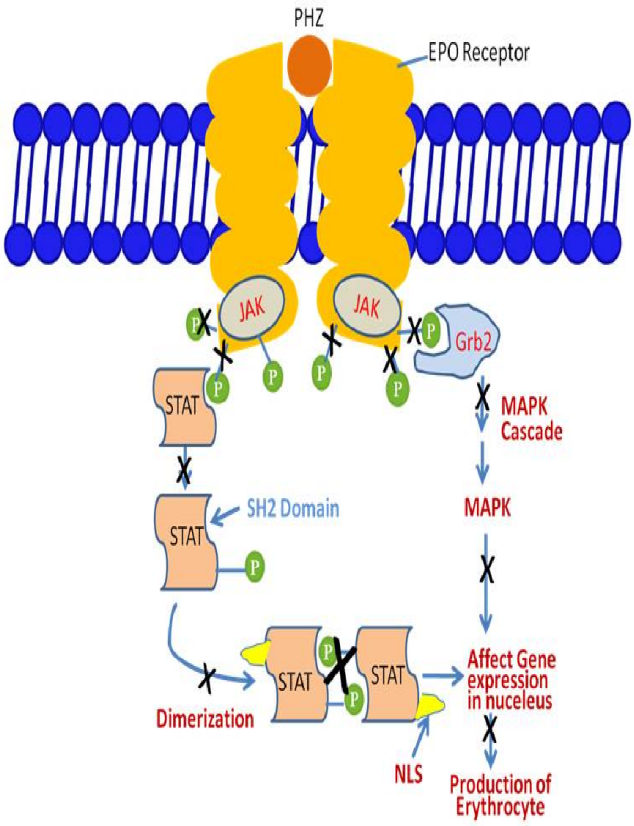

Figure 2(b). Showing the abnormal functioning of JAK STAT Pathway.