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THE EFFECTS OF ISONIAZID ON THE OSMOTIC RESISTANCE OF LACERTILIAN ERYTHROCYTES

MAHMOOD AHMAD, TAZEEN NAIM, RUQAIYA HASAN AND MANSOOR AHMAD*
Department of Physiology, University of Karachi
Karachi-75270, Pakistan
**Department of Physiology, New York Medical College*
Valhalla, New York, U.S.A.

ABSTRACT:

Osmotic fragility of erythrocytes is increased by a number of commonly used analgesics, antimicrobial and antimalarial drugs, resulting in drug-induced hemolytic anemia. Study of erythrocyte osmotic fragility of control and test blood was determined after 5,10 and 15 days of 0.06 mg/ml/day Isoniazid (INH) administration to test subjects. Isoniazid treated subjects exhibited greater RBC fragility than control in 0.1 to 0.3 hypotonic solutions. Control subjects exhibited only 3 per cent destruction in 0.4 per cent saline; whereas test subjects exhibited 12 and 11 per cent destruction on day 5, and 15 in 0.4 per cent concentration of saline, while 10,13 and 15 per cent destruction in 0.5 per cent saline. The study indicated that following prolonged administration of Isoniazid a great number of osmotically fragile RBCs enter the blood stream.

INTRODUCTION

Erythrocytes are especially vulnerable to shortened survival secondary to enzyme deficiencies. Unlike other tissues, the mature erythrocyte cannot increase protein synthesis to maintain enzyme activity. Enzyme deficiencies occur when amino acid substitutions result in a poorly functioning enzyme instability, therefore enzyme activity decreases rapidly as the erythrocyte ages, indicating the first two abnormalities.

Two major metabolic pathways exist in the erythrocyte. Glucose is utilized to generate ATP, NADH, and NADPH. ATP is used to meet the energy requirements and NADH to reduce methemoglobin. The NADPH is utilized to reduce oxidized glutathione, which in turn, is required to maintain protein sulfhydryl groups in a reduced state and to detoxify hydrogen peroxide. Numerous enzyme deficiencies have been described that interrupt either pathway and may further result in hemolytic anemia.

Drug-induced hemolytic anemia occurs in patients with Glucose-6-phosphate (G6PD) deficiency following exposure to certain oxidant drugs. Drugs that cause hemolysis in G6PD deficiency include analgesics, sulfonamides, sulfones, antimalarials and nonsulfonamide antibiotics. Isoniazid (INH) may cause hemolytic anemia in individuals with Glucose-6-phosphate (G6PD) deficiency (Rang *et al.,* 1996). Deficiency of G6PD results in sensitivity of erythrocytes to oxidant-induced hemolysis. Exposure of deficient erythrocytes to oxidants results in Heinz body formation by precipitation of oxidized hemoglobin. The presence of Heinz bodies cause decreased deformability of the erythrocytes with trapping and destruction in the spleen, reticuloendothelial system, and in small blood vessels. In A-G6PD deficiency, the enzyme is unstable, but the enzyme activity is normal in young erythrocytes. In the Mediterranean variant, the enzyme is not only unstable but also shows decreased activity, in young cells.

Other factors that cause hemolysis are viral infections, especially influenza and hepatitis, which may cause hemolysis in G6PD deficiency. INH has been associated with hepatotoxicity, abnormal liver function test (LFT), clinical jaundice and multilobular necrosis. About 1 per cent of INH using patients develop clinical hepatitis and up to 10 per cent sub-clinical abnormalities (Katzung, 1989).

The osmotic fragility of the erythrocyte membrane to hypotonic solutions is investigated theoretically. The fragility curves exhibit a strong transmittance rise. This variation is assumed to result from changes in the scattering properties of erythrocytes under dialysis resulting from swelling and hemolysis. Hemolysis alone causes the abrupt sigmoidal increase of the collimated transmittance with the time (Mazeron *et. al.,* 2000).

It is suggested that INH inhibits phospholipid synthesis and as well as damages the cell membrane. It chelates divalent metals and through chelating, copper, iron, and cobalt becomes more effective *in vitro* than the parent drugs. Moreover, any modification of the molecule, which prevents chelation, produces an inactive compound. It is possible that the drug becomes active only after it has chelated a metal either inside or outside the cell. As the drug is a hydrazide, it can react with aldehydes such as pyridoxal phosphate, the coenzyme for decarboxylases and transminases. It is unlikely that inhibition of these enzymes in the *Tubercle bacillus* could do more than contribute to the bactericidal effect (Mollison, 1957).

MATERIALS AND METHODS

i) Design of experiment:

There were altogether 6 groups of 5 lizards each. 0-day individual blood sample from the anterior abdominal vein of each lizard of each group was obtained prior to administration of 0.06 mg/ml/day INH. Blood samples from the anterior abdominal vein of the lizards belonging to groups I and II were again collected on day5 to determine the osmotic fragility of erythrocytes.

ii) Drug Administration:

The drug is rapidly absorbed from the intestine and is distributed throughout the body (Ahmad *et al.,* 2003). 0.06 mg/ml/day of INH as syrup was given to each lizard of test group for a period of 5 days.

iii) Collection of Blood:

For determination of osmotic fragility; required amount of blood from the anterior abdominal vein of each individual of control and test group, was drawn on day 5 (Ahmad *et al.,* 2001a, b).

iv) Osmotic Fragility Test:

The method was designed by Parpart and Co-workers (1947). For this purpose hypotonic saline is buffered to pH 7.4, and the blood is added to a range of hypotonic solutions from 0.1 to 1.0 per cent concentrations. The test is carried out at room temperature and hemolysis is read photometrically.

A stock solution of buffered sodium chloride, osmotically equivalent to 10 per cent NaCl is prepared by dissolving 90g NaCl; 13.65 g disodium hydrogen phosphate (Na_2HPO_4, anhydrous); 2.43 g sodium dihydrogen phosphate (NaH_2PO_4, $2H_2O$) in distilled water and the final volume is adjusted to 1 liter. This solution keeps for months without deterioration in a well stoppered bottle. In preparing hypotonic solution for use; it is convenient to make first, a 1 per cent solution from the 10 per cent stock solution. This is again diluted with distilled water to 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 and 0.1 per cent. Each of these dilutions are made up to a volume of 50 ml. This amount of solution keeps well at 4^{o}Cfor some weeks; but should be discarded if moulds develop.

Procedure:

A series of hypotonic solutions ranging from 0.1 per cent NaCl to 1.0 per cent NaCl was prepared. 0.05 ml freshly collected, heparinized blood was added to 5 ml of each of the hypotonic solutions and immediately mixed thoroughly by inverting the tubes several times until bright red color is obtained. The tubes are allowed to stand at room temperature for 30 minutes and re-mixed and centrifuged for 5 minutes at 1,200 to 1,500 rpm. The amount of hemolysis in each tube was measured and read as per cent of transmittance in a Beckman spectrophotometer at 540 nm. The supernatant from 0.9 per cent NaCl tube is used as the blank. All tests were carried out at room temperature. The recommended temperature is, however, 25-27^{o}C (Keele *et. al.,* 1983).

When a range of hypotonic solutions has been used a “fragility curve” is drawn by plotting a graph of the percentage of hemolysis in each tube against the corresponding concentration of NaCl solution. In normal subjects an almost symmetrical sigmoid curve results.

RESULTS

A consideration of table 1 indicates that erythrocyte membrane became more fragile with INH treatment. A maximum fragility is obtained in 0.1 to 0.3 per cent saline, while newly synthesized erythrocytes fragilited in 0.4 to 0.5 per cent hypotonicities. Fragility of control and test red cells is related to the concentration of saline as well as the age of RBCs ($P<0.005$). When the fragility of red cells belonging to control groups is compared with test group cells, both the groups showed a significantly different behavior at similar concentrations of saline on the same interval of day i.e., day 5, 10 and 15, respectively ($P<0.05$-χ^2 test).

DISCUSSION

Consideration of Table-1 indicates that RBCs of control group on day 5; day 10 and day 15 showed maximum fragility in 0.1 to 0.3 per cent hypotonic solutions. Only 3 to 5 per cent control red cells were destroyed in 0.4 per cent concentration. On the contrary, test red cell fragility was maximum in 0.1 to 0.3 per cent hypotonic solution. There was 16-11 per cent fragility in 0.4 per cent solution and 10 to 15 per cent fragility in 0.5 per cent hypotonicity. This indicates that drug fragilates the older erythrocytes in hypotonicity ranging from 0.1 to 0.3 per cent; whereas the newer RBCs introduced into the blood stream as replacement were fragilited in 0.4 to 0.5 per cent hypotonicities (Figs. 1, 2 and 3).

Another interesting phenomenon observed was that the fragility curves for control obtained on day 5, 10 and 15 followed more or less the same pattern. For the test groups not only fragilities occurred even at higher concentration of saline, but also there is a deviation from its normal path. This part of fragility curve exhibits the entrance of newly introduced RBCs in the blood stream, which were comparatively more fragile. This specific pattern was more prominent on day 10 and day 15 after the administration of INH (Figs. 1, 2 and 3).

One of the rare disorders that causes non-spherocytic hemolytic anemia is due to abnormalities of the Embden-Meyerhof (EM) Pathway. Hemolysis probably results from the decreased generation of adenosine triphosphate (ATP). Pyruvate kinase deficiency is most common in this group, but is still rare. Splenectomy may give a partial remission. Hexokinase deficiency involves the first enzyme of either the Embden-Meyerhof or pentose pathway. This deficiency causes severe hemolytic anemia. In the end, it may be reminded that INH does not affect erythropoiesis but inhibits the synthesis of phospholipids during erythropoiesis and there by increases fragility of RBC membrane, that is exhibited by the lower, more steeper parts of the curves.

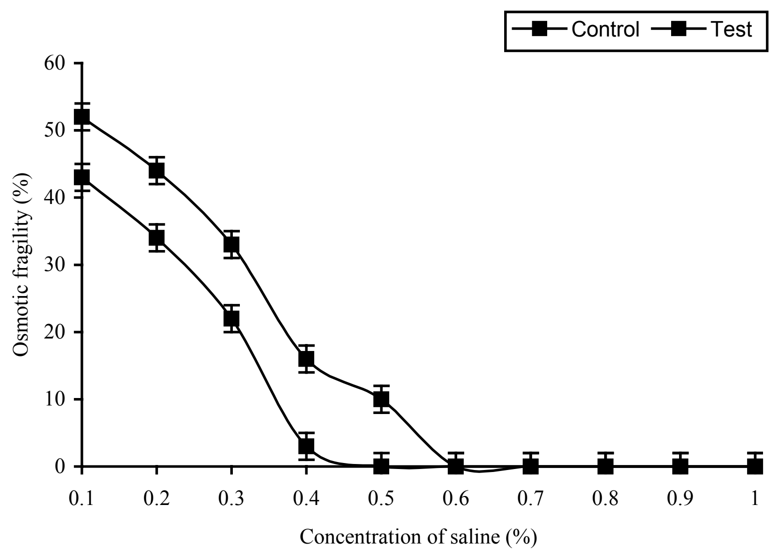

Fig. 1: Osmotic fragility of erythrocytes after administration of 0.06 mg INH/day for 5 days.

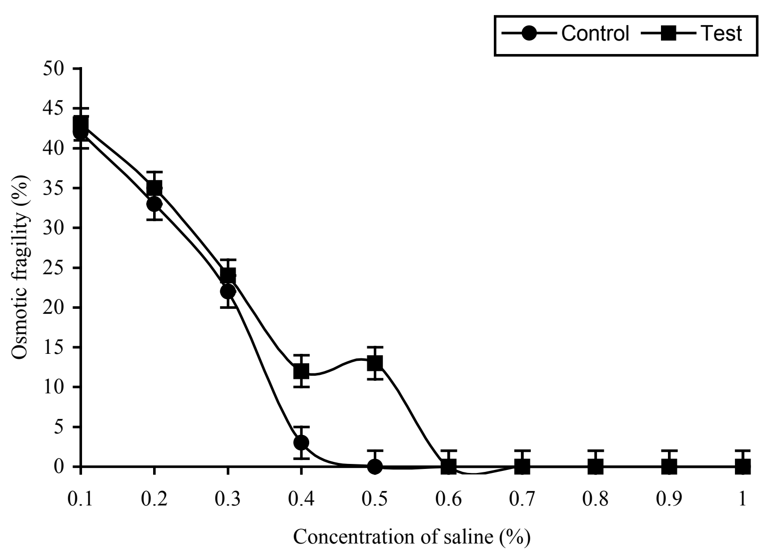

Fig. 2: Osmotic fragility of erythrocytes after administration of 0.06 mg INH/day for 10 days.

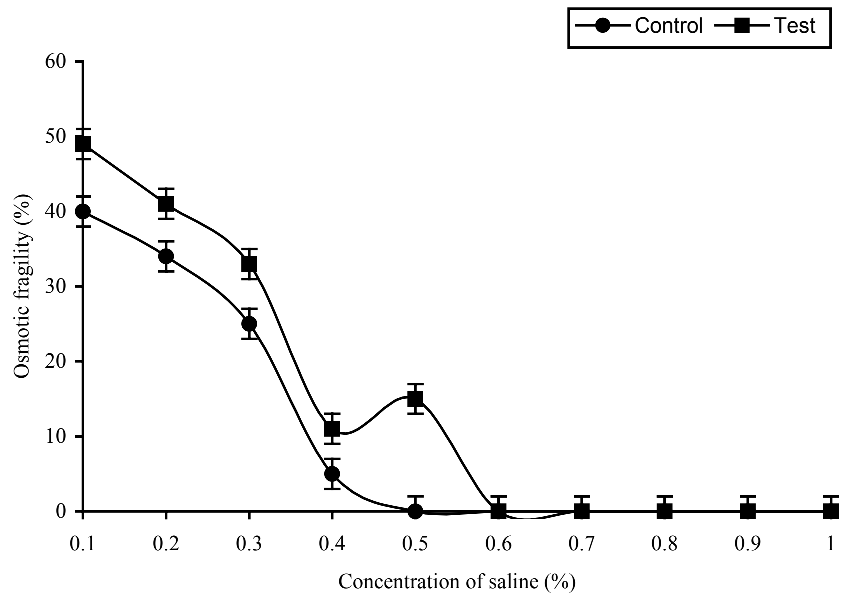

Fig. 3: Osmotic fragility of erythrocytes after administration of 0.06 mg INH/day for 15 days.

Table-1
Osmotic fragility (% of hemolysis) of erythrocytes after 5,10 and 15 days following a daily dose of 0.06 mg Isoniazid

Conc. % Hypotonic solution	Days after administration of drug					
	5		10		15	
	C	T	C	T	C	T
0.1	43	52	42	43	40	49
0.2	34	44	33	35	34	41
0.3	22	33	22	24	25	33
0.4	3	16	3	12	5	11
0.5	0	10	0	13	0	15
0.6	0	0	0	0	0	0
0.7	0	0	0	0	0	0
0.8	0	0	0	0	0	0
0.9	0	0	0	0	0	0
1.0	0	0	0	0	0	0

C and T indicate the values for control and test, respectively.

REFERENCES

Ahmad, M., Mahmood, I., Hasan, R., Javaid, A., Naim, T., Fatima, H. and Ahmad, M. (2001a). *In vitro* effects of ACTH on pigeon crop-sac epithelium. *Pak. J. Pharm. Sci*. **14**(1): 19-23.

Ahmad, M., Mahmood, I., Hasan, R., Fatima, H. and Ahmad, M. (2001b). Effect of dexamethasone on pituitary prolactin in the lizard *Uromastix hardwickii. Pak. J. Pharm. Sci*. **14**(1): 43-46.

Ahmad, M., Hasan, R., Ahmad, M. and Hanif, T. (2003). Isoniazid associated uric acid retention in the lizard *Uromastix hardwickii*. (In press).

Katzung, B.G. (1989). Antimycobacterial Drugs. Basic and Clinical Pharmacology. Prentice-Hell International Inc. **4**: 578-585.

Keele, C.A., Neil, E. and Joels, N. (1983). The Internal Environment; The Red Blood Corpuscles. Samson Wright's Applied Physiology. Oxford University Press. **13**: 32-35.

Mazeron, P., Didelon, J., Muller, S. and Stoltz, J.F. (2000). A theoretical approach of the measurement of osmotic fragility of erythrocytes by optical transmission. *Photochemistry and Photobiology*. **72**(2): 172-183.

Mollison, P.L. (1957). Measurement of survival and destruction of red cell in hemolytic syndromes. *Brit: Med. Bull*. **15**: 59-70.

Parpart, A.K., Lorenz, P.B., Parpart, E.R., Greg. J.R. and Chase, A.H. (1947). The osmotic resistance (fragility) of human red cells. *J. Clin. Invest.* **26**: 636-646.

Rang, H.P., Dale, M.M. and Ritter, J.M. (1996). Chemotherapy; Antibacterial agents. Pharmacology. Churchill Livingstone. **3**: 718-743.