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The Effect of Isoniazid (INH) on Erythrocyte Sedimentation Rate in the Lizard, *Uromastix hardwickii*

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Abstract: Erythrocyte sedimentation rate of isoniazid treated lizard was worked out. The gradual decrease for day 5 to day 10 and day 15 was found to be statistically significant. This is indicative of increase in erythrocyte membrane permeability with increase in the span of treatment. Newer cells are flooded in the blood stream due to loss of older cells. Newer cell resist sedimentation on day 10 and day 15.

Key words: Erythrocyte sedimentation rate, isoniazid

Introduction

Patients receiving isoniazid (INH) are carefully evaluated at intervals for symptoms of various after effects and clinical workers prefer to determine serum glutamin-oxalacetic transaminase activity at monthly intervals (Byrd ***et al.***, 1977). Since, an elevation greater than 3 times normal is a cause for discontinuation of the drug. Because more untoward effects due to exposure to the drug occur following the start of therapy.

Signs and symptoms of excessive disorders may develop when isoniazid is administered for long duration. INH should be administered with great care (Maddrey and Birtnott, 1973, Stead and Texter, 1973). Continuation of the drug after symptoms have appeared tends to increase the severity of damage.

Very recently, Ahmad ***et al.*** (2003a) have reported neutrophilia, eosinophilia and lymphocytopenia following administration of the drug in ***Uromastix hardwickii***. Therefore, in the present study, INH was administered for longer period of 5, 10 days and 15 days to determine the adverse reactions on erythrocyte sedimentation rate (ESR) in ***Uromastix hardwickii***.

Materials and Methods

Design of experiment

There were altogether 6 groups of 3 control and 3 test, comprise of 5 lizard each. Day 0 individual blood samples from the interior abdominal vein of each lizard was obtained prior to the administration of 0.6 mg INH per day. Blood samples belonging to I and II group were again collected on day 5; of group III and IV on day 10 and blood samples of each lizard of group V and VI on day 15.

Drug information

The compound called isoniazid (INH) is isonicotinic acid hydrazide. It is still considered the primary drug for the chemotherapy of tuberculosis. The discovery of INH was somewhat fortuitous; as nicotinamide possesses tuberculostatic action. Examination of the compounds related to nicotinamide revealed that many pyridine derivatives possess tuberculostatic activity. The isonicotinic acid derivatives are water-soluble while the mechanism of action is unknown.

Drug administration

The drug is rapidly absorbed from the intestine and is distributed through out the body. 0.06 mg day^{-1} of INH in syrup form was given to each lizard of test group II for a period of 5 days. The same dose was administered to lizards of test group IV for 10 days and lizards of test group VI for 15 days.

Collection of blood

For all individual estimations blood samples were in fact essential. For this purpose, required amount of blood, from the anterior abdominal vein of controls and tests was drawn on day 5, day 10 and day 15.

The phenomenon of sedimentation

The phenomenon of ESR has been extensively investigated (Ham and Curtis, 1938; Ruhenstroth, 1961 and Thygesen, 1942). The rate of fall of red cells is influenced by a number of interacting factors.

Basically, it depends upon the difference in specific gravity between the red cells and plasma. The actual rate of fall is influenced very greatly by the extent to which the red cells form rouleaux by adhesion to each other; thus large clumps of cells attached mainly side to side; sediment more rapidly than single cells; such as in the present study. Other factors which influence sedimentation include the ratio of red cells to plasma, i.e., the packed cell volume (PCV), plasma viscosity, the verticality of the sedimentation tube, the bore of the tube, the nature of the anticoagulant used and the dilution, if any, of the blood.

The all important rouleaux formation in human blood is mainly controlled by the concentration of fibrinogen and to a lesser extent of 2 α-globulins in the plasma. Glycoproteins also play a part in rouleaux formation (Weicker and Kuhn, 1967). In anemia, the alteration of the ratio of red cells to plasma encourages rouleaux formation and accelerates the rate of sedimentation.

Estimation of erythrocyte sedimentation rate (ESR)

The estimation of ESR has been widely used in clinical medicine. Many methods for its measurement have been devised (Ham and Curtis, 1938 and Nichols, 1942), differing with respect to the anticoagulant used, the volume of blood employed, the dimension of the tube in which the measurement is carried out, the time allowed for sedimentation to take place and the method of recording the results. Westergren's (1921) method, however, is the most sensitive technique and used almost universally.

Method of westergren

Westergren tube is a straight glass tube 30 cm in length and 2.5 mm diameter; it is calibrated in mm from 0 to 200. Venous blood is diluted with 38 g l^{-1} trisodium citrate in the proportion of one part of citrate to four parts of blood. The test should be carried out within 2 h of collecting the blood. The sample is well mixed and the blood is then drawn up into the Westergren tube to the 200 mm mark. The tube is vertically placed on the stand. The height of the clear plasma above the upper limit of the column of sedimenting red cells is then read to the nearest mm. This figure in mm/h is the ESR. Specially made racks with adjustable leveling screws are available for holding the sedimentation tubes firmly in an exactly vertical position. Alternatively, they can be suspended and allowed to hang down vertically. It is conventional to setup sedimentation rate tests at room temperature. However sedimentation is normally accelerated as the temperature rises (Manley, 1957). Therefore, if a test is to be used as a research tool it is advisable to control the temperature (Manley, 1957). The recommended temperature is 22-27°C.

Results

Table 1 shows that ESR in controls on day 5 was 7.2 ±0.83 mm h^{-1}; it was 8.2±0.83 mm h^{-1} on day 10 and 6.8±0.83 mm h^{-1} on day 15. This indicates that the ESR ranges between 7 to 8 mm h^{-1} normally in the lizard. In INH treated groups the ESR was 10.6±1.52 mm h^{-1} on day 5 to 10.0±1.58 mm h^{-1} on day 10 and 9.0±1.58 mm h^{-1} on day 15. The obtained values for test group were higher than those of controls ($P<0.05$; F-test) indicating that erythrocyte membrane became more permeable in tests than controls.

The significant difference between the mean ESR values was obtained when control groups were compared with their respective test groups ($P<0.05$; t-test).

The values obtained for days 5, 10 and 15 show a gradual decrease of mean ESR, indicating that newer erythrocytes are flooded into the blood stream to replace the dying and deceased cells. These cells show resistance to sedimentation on day 10 and day 15 i.e. 0.6% on day 10 and 1.6% on day 15.

Table 1: The effect of oral administration of 0.06 mg day^{-1} isoniazid on the erythrocyte sedimentation rate (ESR) of *Uromastix hardwickii*

	ESR min/h					
	Day 5		Day 10		Day 15	
No. of animals	C	T	C	T	C	T
1	8	13	8	10	6	8
2	6	10	8	9	7	10
3	7	9	9	11	8	11
4	8	11	7	8	7	9
5	7	10	9	12	6	7
Mean ±SD	7. 2 ±0.83	*10.6 ±1.5 2	8. 2 ±0. 8 3	*10.00 ±1.58	6.8 ±0.83	*9.0 ±1.58

* ($P<0.05$)

C and T indicate values of control and test, respectively

Discussion

Isoniazid is a tuberculostatic bactericidal agent, used for the treatment of tuberculosis, often in conjunction with other drugs like Rifampicin. Though a highly recommended drug for tuberculosis, yet its adverse effects on other functions of the body cannot be ruled out. It is a Vit-D inhibitor (Bengoa ***et al.***, 1984 and Brodie ***et al.***, 1982) producing primary hyperparathyroidism (Kavascs ***et al.***, 1993). It produces drug toxicity by inhibiting the activity of Vit-C which is a very potent antioxidant (Matsuki ***et al.***, 1991). Isoniazid acts as Vit-B_6 antagonist leading to neuropathies and neurotoxicity (Mbala ***et al.***, 1998). Vit-B_6 is an important co-factor in RBC maturation.

Ahmad ***et al.*** (2003b) have reported that administration of INH inhibits the synthesis of phospholipid in erythrocytes. Deprivation of phospholipid from erythrocyte membrane appears to be an additional factor influence ESR. In the present study, the elevation of ESR was far greater than controls.

Teklu and Wahab (1994) have reported the similar results in febrile and afebrile patients with pulmonary tuberculosis, treated Isoniazid. Febrile patients were reported to be more anemic and significantly high ESR as compared to febrile patients.

Xia ***et al.*** (2003) reported of elevated ESR, granuloma and tissue necrosis in patients with tuberculosis of pancreas and peripancreatic lymph nodes in Immunological patients in China.

Hasegama ***et al.*** (2000) reported a clinical case of increased ESR and C-reactive protein with normal WBC count in a patient with tuberculous peritonitis. Present study also observed genetic heterogeneity with regard to the rate of sedimentation in normal lizards. In keeping with the facts, such variability may also be common to controls and tests, both in animals and men.

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