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THE EFFECTS OF ISONIAZID (INH) ON THE HEMATOCRIT OF THE LIZARD, *UROMASTIX HARDWICKII*

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In recent years isoniazid has been used as an antituberculous chemophylactic agent. Severe adverse reactions have been reported following its extensive treatment. In addition to hepatic and neurologic disturbances, hematologic alterations have also been reported. Present study was conducted to determine the effect of 0.06 mg isoniazid on the lacertilian packed cell volume. It was 14.0 per cent on day 5, 18.0 and 19.8 per cent on day 10 and day 15 respectively, whereas, it was 24.0, 24.2 and 24.6 per cent in controls on day 5, 10 and day 15 respectively.

INTRODUCTION

Miscellaneous reactions associated with isoniazid (INH) therapy are neutrophilia, eosinophilia, lymphocytopenia (Ahmad *et al*., 2004a), methemoglobinemia and urinary retention. In those with the predisposition to pyridoxine deficiency anemia, the administration of INH may result in its appearance in full-blown form. Treatment with large doses of the vitamin gradually returns the blood picture to normal in such cases (Goldman and Braman, 1972). Overdose of INH, results in coma, seizures and metabolic acidosis.

INH should be administered with cane to minimize all adverse reactions, since it has direct or indirect impact on patient. The purpose of this study is to establish effects of INH on the packed red cell volume (PCV) of the lizard, *Uromastix hardwickii.*

MATERIAL AND METHODS

Design of experiment

Experimental animals receiving INH should be carefully evaluated at intervals for symptoms of various after effects (Byrd *et al*., 1977). Since an evaluation of hematological values greater than 3 times normal is a cause for discontinuation of the drug. As more untoward effects due to INH exposure occur following the start of INH administration (Ahmad *et al*., 2003). Signs and symptoms of excessive disorder may develop when INH is administrated for long duration (Maddrey and Birtnott 1973; Stead and Texter, 1973). Since continuation of INH after symptoms have appeared tends to increase the severity of damage, therefore, the animals of experimental groups were treated for 5, 10 and 15 days, respectively.

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 INH/day. Blood samples from the anterior abdominal vein of the lizards belonging to II groups were again collected on day 5. Blood samples of group IV on day 10 and blood samples of group VI on day 15, and packed cell volume were worked out.

Drug information

INH diffuses readily into all body fluids and cells. The drug is detectable in significant quantities in pleural and ascetic fluids; concentrations in the cerebrospinal fluid are about 20 per cent of those in the plasma. INH penetrates well into gaseous material. The concentration of the agent is initially higher in the plasma and muscle than in the infected tissue, but the later retains the drug for a long time in quantities well above those required for bacteriostasis (Robson and Sullivan, 1963).

Drug administration

The drug is rapidly absorbed from the intestine and is distributed throughout the body. 0.06 mg/day of INH in syrup form was given to each lizard of group II for a period of 5 days. The same dose was administered to lizards of group IV for 10 days and lizards of group VI for 15 days (Ahmad *et al*., 2004a,b).

Collection of blood

For all estimations, individual blood samples were in fact necessary. Therefore required amount of blood from the anterior abdominal vein of each animal of control and test group was drawn separately on day 5, 10 and day 15.

DETERMINATION OF PACKED CELL VOLUME (PCV)

Method

Most accurate determination of packed red cells volume /100 ml of blood (PCV) by the hematocrit technique is the simplest method for determining the increase or decrease of erythrocytes. There are two methods of PCV measurement in current use: (a) Macro-method (b) Micro-method.

The macro-method, uses Wintrobe tubes and is handicapped with delay in tests, inadequacy in mixing of blood sample, incomplete filling, faulty reading and excessive EDTA leading to cell shrinkage. All the more, the degree of oxygenation of blood makes it 1 per cent higher than the venous blood. Variation in the bore of tubes and progression in speed of rotation during 30 minutes of centrifugation are also some of the significant disadvantages (Humera, 1977). Therefore micro-method is more popular nowadays. Strumia *et al*. (1954), used a centrifuge with 37,000 rev/min at 28000g centrifugal force; using micro-tubes 32 mm in length to pack completely without trapped plasma in 1 minute. Presently the machines in use provide a centrifugal force of 12000g and needs 3-5 minutes centrifugation. According to Garby and Vuille (1961), the mean amount of plasma trapped is 1.3 per cent.

There are several advantages of this micro-method. It requires minimum quantities of blood; offers a short centrifuging time and a better packing of erythrocytes (Humera, 1977). Over and above the heparinized capillary tubes are inexpensive and disposable.

Procedure

A high speed micro-hematocrit, Unipan (Polish) 12000 rpm, Type 316, centrifuge with notches in the flat head permitting simultaneous handling of 20-micro hematocrit determinations along automatic electric timer and micro-capillary reader was used in this study.

For obtaining hematocrit values heparinized capillaries were filled with the blood sample and their distal ends were sealed with a special wax, plasticin. By setting the automatic timer at 3-minutes the motor was switched on. Blood volume was read directly against the linear scale with the help of a magnifying glass.

RESULTS

An examination of tables 1-3 indicate that PCV was 24.0 per cent on day 5; 24.2 per cent on day 10 and 24.6 per cent on day 15 in controls. On the contrary, it was 14.0 per cent on day 5, 18.0 per cent on day 10 and 19.8 per cent on day 15 in tests.

The mean PCV of control groups when statistically compared with their respective test groups showed a significant difference ($p<0.05$, t-test) i.e. PCV decreased considerably following INH administration. A comparison of mean PCV values of controls on day 5, 10 and 15 showed a close similarity. Whereas test mean PCV per cent on day 5, 10 and day 15 showed an increase from 14.0 per cent on day 5 to 18.0 per cent on day 10 and 19.8 per cent on day 15. Statistical analysis by Two-way ANOVA showed a significant difference between mean PCV of control and test groups ($p<0.05$).

DISCUSSION

INH is a tuberculostatic bactericidal drug extensively used for the treatment of tuberculosis very often in conjunction with drugs like Rifampicin (Ahmad *et al*., 2003). INH is a highly recommended drug for tuberculosis; but its other effects on the physiology of body systems can not be ruled out. It inhibits vitamin D (Bengoa *et al*., 1984 and Brodie *et al*., 1982) producing primary hyperparathyroidism (Kavascs *et al*., 1993). It produces toxicity by inhibiting vitamin C action as antioxidants (Matsuky *et al*., 1991). INH acts as Vitamin B-6 antagonist producing neurotoxicity leading to neuropathies (Mbala *et al*., 1998).

Table 1
The effects of oral administration of 0.06 mg INH / day on PCV of *Uromastix* for 5 days

No. of animals	PCV per cent	
	C	T
1	24	13
2	26	15
3	25	14
4	22	15
5	23	13
Mean ± SD	24.0 ± 1.58 (5)	14.0 ± 1.00 (5)

C and T indicate values for controls and tests respectively.

Table 2
The effects of oral administration of 0.06 mg INH /day on PCV of *Uromastix* for 10 days

No. of animals	PCV per cent	
	C	T
1	23	14
2	24	18
3	27	21
4	26	20
5	21	17
Mean ± SD	24.2 ± 2.39 (5)	18.0 ± 2.74 (5)

C and T indicate values for controls and tests respectively.

It must also be recommended that vitamin B-6 is a very important co-factor in RBC maturation. Therefore, the measurement of PCV after centrifugation of blood can be used as a simple screening test for any pathological condition. Further, in conjunction with accurate estimations of hemoglobin and red cell count, knowledge of PCV is valuable for calculation of absolute values.

Table 3
The effects of oral administration of 0.06 mg INH/day on PCV of *Uromastix* for 15 days

No. of animals	PCV per cent	
	C	T
1	23	19
2	22	17
3	26	22
4	25	18
5	27	23
Mean ± SD	24.6 ± 2.07 (5)	19.8 ± 2.59 (5)

C and T indicate values for controls and tests respectively.

It has been shown that the venous hematocrit over estimates the proportion of red cell in the circulating blood as a whole. The volume of the packed red cells in the capillary bed is substantially lower than the venous blood; so that the body hematocrit, the average value for the body as a whole is approximately 91 per cent of the volume of packed red cells in venous blood (Chaplin *et al*., 1953).

Although ESR and excessive erythrocyte fragility is known to be an untoward effect of INH therapy; it was not until apparent that great erythrocyte injuries leading to severe anemia may occur in lizard receiving this drug. Studies (Ahmad *et al*., 2003) on *Uromastix* have confirmed this observation. Continuation of exposure to the drug after symptoms of dysfunction have appeared tends to increase the severity of damage.

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