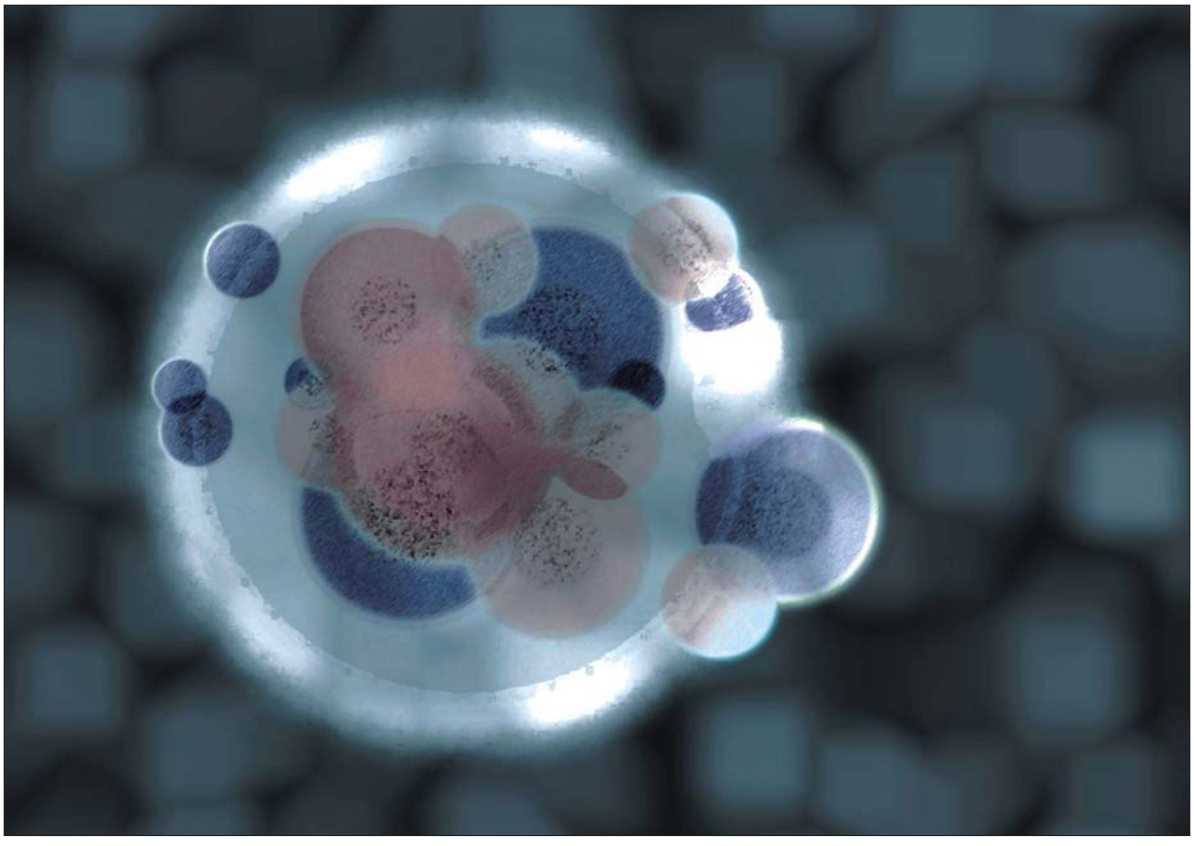

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The Effects of Mefenamic Acid on the Erythrocytes of the Lizard, *Uromastix hardwickii*

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Abstract: Present study deals with the effects of 7.1, 10.5 and 14.0 mg/day doses of mefenamic acid administered daily for 12 days to three groups of *Uromastix*, respectively. Individual blood samples were obtained from the anterior abdominal vein and RBC count determined. The severity of autoimmune disease was checked through decreased no. of erythrocytes. Data indicates significant reduction ($p<0.05$) in number of RBCs observed from day 5 in low doses, at high dose reduction in RBC count observed in day 4.

Key words: Mefenamic acid, lacertilian erythrocytes, drug induced hemolysis

INTRODUCTION

Autoimmune hemolytic disease is characterized by an increased rate of destruction of the patients own erythrocytes. The accelerated hemolysis is mediated by one or more mechanisms involving components of the immune system. The hallmark of this group of diseases are shortened red cell life span *in vivo* and evidence of auto immunity directed against the cells is usually demonstrable by a positive direct antiglobulin of the patients red cells; or a red cell auto antibody in the patients serum. In addition, increased blood production in response to the hemolytic process in-patients.

It is shown that normal red cells do not survive normally in patient with acquired haemolytic disorders[1], Autoimmune Hemolytic Anemias (AHA) are caused by extracorpuscular mechanism. There are number of theories explaining appearance of red cell directed antibodies. One of these postulates are antigenic change in the red cell membrane initiating the immune reaction. Another suggests the loss of self-recognition by the immune system. Best documented is perhaps the manner by which certain drugs induce AHA.

Administration of mefenamic acid induces many alterations in erythrocytes. Observations suggest[2] that mefenamic acid causes functional impairment of red cell membrane. A study to this effect has been made on the lacertilian blood.

MATERIALS AND METHODS

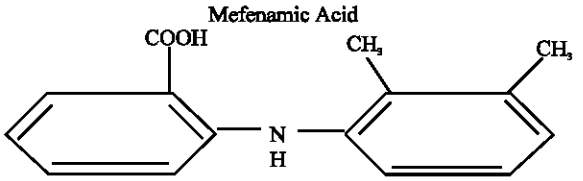
Choice of animals: Large populations of the spiny tailed lizard exist in the desert and semi-desert regions of Karachi and Thatta District[3,4]. The animals are easily available, cost less and are easily managed. Thus, for the present study they were obtained from local suppliers. An examination of the literature indicates that reptiles as a whole have been mostly neglected as a research material.

Temperature: One of the important factors in the physical environment is temperature[5-8]. Poikilotherms are incapable of maintaining their body temperatures and variation in the ambient temperature affects their body and alter their physiological state. Therefore, in order to obtain reproducible results the temperature was kept constant at 32±1°C during the experimental period.

Drug information: Mefenamic acid, N(2,3-Xylyl)-anthralinic acid is a newer analgesic, anti-pyretic and anti-inflammatory agent, first marketed in the United States. In addition, a related derivative, N-(α - α - α-trifluorum-tolyl) anthranilic or flufenamic acid is also marketed in Pakistan. Till now effect of mefenamic acid on organ systems in man or animals have not been observed; except only, following extremely high doses. Renal and hepatic damage was evidenced microscopically and minor effects on reproductive system have been observed in lower animals following large amounts of the drug administration. However no adverse effects were noted in the case of dog.

Routes of administration: The drug Ponstan (Parke Davis) is available for oral administration as it is insoluble in any suitable solvent.

Structure :



N - (2,3- Xylyl) - anthranilic acid

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Absorption, distribution and fate: Mefenamic acid is absorbed slowly in the intestine and reaches maximum blood concentration between 2-4 h. It is strongly bound to plasma protein and is distributed unevenly in various tissues. In the monkey, mefenamic acid has been shown to enter the placenta. In man it is oxidized but the details are not available. In most species it is excreted in the urine. It also makes its presence in the bile through enterocephalic circulation and is recovered in the feces.

Tolerance and addiction: Evidence for tolerance or addiction has not been reported. The drug has no value in the treatment of abstinence symptom in monkey, addicted to narcotics.

Preparation and dose: Mefenamic acid is available in 250 mg tablets. The usual recommended initial dose is 500 mg to be followed by 250 mg every 6 has needed. The drug is indicated for short term administration not exceeding 1 week of therapy. Children under 14 years should not receive the drug until the therapeutic dose has been established.

The use of this drug for pregnant women is contraindicated. Patient with abnormal renal function, gastric inflammation and asthmatics should be treated with caution.

Toxicity: Adverse reaction during normal treatment have been mild and infrequent. The more common reactions include drowsiness, nausea and dizziness (2%). Side effects that involve gastrointestinal system take the form of dyspepsia or upper gastrointestinal discomfort and diarrhea which may be severe and associated with steatorrhea and inflammation of the bowel is relatively common. Therefore fenamates are contraindicated in patients with a history of gastrointestinal disease[9].

Weekly hematopoietic, renal and hepatic studies are recommended during chronic administration, until additional experience is gained with the drug.

Therapeutic uses: Mefenamic acid may be used in place of other non- narcotic analgesics and may be substituted for codeine during postextraction pain.

Design of experiment: 40 *Uromastix* almost equal in body weight and size were sorted out from the stock to form four equal groups. One of the groups served as control and the other as test.

Drug administration: Normally 2 gm of mefenamic acid (8 tablets) is a dose prescribed for a human subject for 24 h. Therefore, normal dose of mefenamic acid for a *Uromastix* weighing 250 g is 7.1 mg per day. Infact this dose of mefenamic acid was given orally everyday to each test animal. The suspension was prepared in distilled water and diluted in such a way that 1 mL contained 7.1 mg of the drug. Thus, each test animal received 1 mL suspension per day for 12 days and each control received 1 mL of distilled water simultaneously. The test and control animals were fed with 1 mL of 5% glucose solution thrice a week. The RBC counts of controls and tests were done on the 6th and the 12th day.

In the second experiment test animals were given 10.5 mg of mefenamic acid per day for a period of 12 days and the blood values estimated in the same way as those of the first experiment.

In the third experiment test animals were given 14 mg of mefenamic acid per day for a period of 12 days and the blood values were estimated in the same way.

Collection of blood: For the estimation of RBCs, blood required was collected from the anterior vein[10,11] of each individual of control and test group on day 6 and day 12.

Erythrocyte counting

Red cell diluting fluid: Hayem's solution was used as a red cell diluent[12]. For this 1 g sodium chloride, 5 g crystalline sodium sulphate and 0.5 g of mercuric chloride was dissolved in 200 mL of double distilled water and the solution was filtered several times to obtain a clear filtrate. Thus all chemicals used in the solution belonged to the reagent grade (E. Merck).

Care: The chief sources of error are improper measurements in making the dilutions and unequal distribution of cells on the haemocytometer. The first was prevented by making the solution in bulk to suffice the requirement throughout the experimental period. The later was prevented to a great extent by thoroughly mixing the blood suspension before it was put on the slide, by allowing the blood to rush under the cover glass with one sweep instead of little waves, by using great care to prevent jarring of the slide or movement of the cover glass and by having the cover glass and slide absolutely grease free. The pipette with the diluted blood was never allowed to stand; since, standing for sometime and reshaking results in inaccurate count.

Therefore, counts were made when a freshly made suspension was available. Every time, the pipette was cleaned at once, using water to wash out the blood, by using alcohol and finally ether. The alcohol and ether was sucked only through a hand bulb suction apparatus. The slide and cover glass was washed with water and the ruled area was carefully dried using a soft lens paper.

Red cell counting: The main object of red cell counting is to investigate the possibility of anemia; as haematological tests are essential for a thorough critical attention.

Therefore, routine erythrocyte examination served as a basic screening procedure.

Moreover, knowledge of the normal range helps in the ordering of tests and the interpreting of the results[13]. It is well known that choice of technique influences the range. Moreover, to maintain the limits of accuracy within the same order, adoption of a single technique is desirable throughout the investigation. The choice of test for routine work should be based on the merit of accuracy.

Procedure: 1 : Two hundred dilution of blood is made in Hayem's solution for red blood cell enumeration. By using a red cell pipette the cell suspension is introduced into Neubaur Counting Chamber, prior to routine calculation[14].

It must be remembered that the most commonly used ruling is that of this improved Neubaur Counting Chamber and the method was adopted simply in favour of standardized pattern of counting[13].

Whole data is analysed statistically by Student's t-test.

RESULTS AND DISCUSSION

Results show number of RBC in controls on day 5 is 1.75±0.03 m/mm^3, it was 1.75±0.02 m/mm^3 on day 10 and 1.71±0.02 m/mm^3, which indicates that RBC count remain stable through out study carried in control group (Table 1-3).

While in test group administered 7.1 mg/day mefenamic acid, number of RBC was 1.68±0.01 m/mm^3 on day 5 to 1.25±0.02 m/mm^3 on day 10 and 1.02±0.02 m/mm^3 on day 12 (Table 1).

The values for RBC in test group given 10.5 mg/day is 1.60±0.02 m/mm^3 on day 5, 1.11±0.02 m/mm^3 on day 10 and 1.00±0.01 m/mm^3 on day 12 (Table 2).

The values for RBC in test group given 14.0 mg/day is 1.55±0.01 m/mm^3 on day 5, 0.95±0.08 m/mm^3 on day 10 and 0.80±0.03 m/mm^3 on day 12 (Table 3).

Present data suggests that in comparison to control no. of RBCs in all test group is reduced significantly ($p<0.05$, t-test).

Mefenamic acid has been used as an analgesic[15], as well as, in the treatment of primary spasmodic dysmenorrhea[16].

Thus the present study deals only with the induction of auto-immune anemia in reptiles. Lysis of 8-10 % human erythrocytes is observed at 0.4% concentration following hypotonic shock.

Normal RBC of *Uromastix* is lysed at 0.4% concentration of hypotonic solution. However, the RBC resistance deteriorated following 7.1 mg/day drug treatment and showed a reduction in RBC count on 5th day and gradual reduction observed till day 12. (Fig. 1).

Table 1: RBC values of control and test following oral administration of 7.1 mg mL^{-1} mefenamic acid per individual/day at 32±1°C

Days	Control RBC (m/mm^3)	Test (RBC m/mm^3)
0	1.77±0.01	1.72±0.02
1	1.77±0.02	1.72±0.01
2	1.79±0.03	1.71±0.01
3	1.72±0.02	1.71±0.01
4	1.77±0.02	1.70±0.01
5	1.75±0.03	1.68±0.01
6	1.80±0.04	1.65±0.02
7	1.72±0.02	1.62±0.01
8	1.70±0.02	1.60±0.02
9	1.70±0.01	1.40±0.03
10	1.75±0.02	1.25±0.02
11	1.68±0.02	1.11±0.03
12	1.71±0.02	1.02±0.02

Table 2: RBC values of control and test following oral administration of 10.5 mg mL^{-1} mefenamic acid per individual/day at 32±1°C

Days	Control RBC (m/mm^3)	Test RBC (m/mm^3)
0	1.77±0.01	1.80±0.03
1	1.77±0.02	1.78±0.01
2	1.79±0.03	1.78±0.01
3	1.72±0.02	1.71±0.02
4	1.77±0.02	1.64±0.02
5	1.75±0.03	1.60±0.02
6	1.80±0.04	1.60±0.03
7	1.72±0.02	1.52±0.01
8	1.70±0.02	1.43±0.04
9	1.70±0.01	1.24±0.03
10	1.75±0.02	1.11±0.02
11	1.68±0.02	1.00±0.01
12	1.71±0.02	1.00±0.01

Table 3: RBC values of control and test following oral administration of 14.0 mg mL^{-1} mefenamic acid per individual/day at 32±1°C

Days	Control RBC (m/mm^3)	Test RBC (m/mm^3)
0	1.77±0.01	1.80±0.01
1	1.77±0.02	1.80±0.01
2	1.79±0.03	1.74±0.04
3	1.72±0.02	1.70±0.02
4	1.77±0.02	1.62±0.02
5	1.75±0.03	1.55±0.01
6	1.80±0.04	1.43±0.06
7	1.72±0.02	1.22±0.02
8	1.70±0.02	1.20±0.04
9	1.70±0.01	1.06±0.04
10	1.75±0.02	0.95±0.08
11	1.68±0.02	0.88±0.01
12	1.71±0.02	0.80±0.03

Each figure is the mean of 10 samples with±SD

In animals treatment with 10.5 mg/day and 14.0 mg/day of mefenamic acid, RBC count was further decreased. Thus indicated the higher the dose, the more the reduction in RBC count (Fig. 2 and 3). Reduction in number of RBC count is dose related. RBC membrane become more fragile by the end of day 12.

Hemolysis results when certain drugs are administered, deficiency in Glucose 6 Phosphate Dehydrogenase enzyme lead to shortening of red cell life span[17].

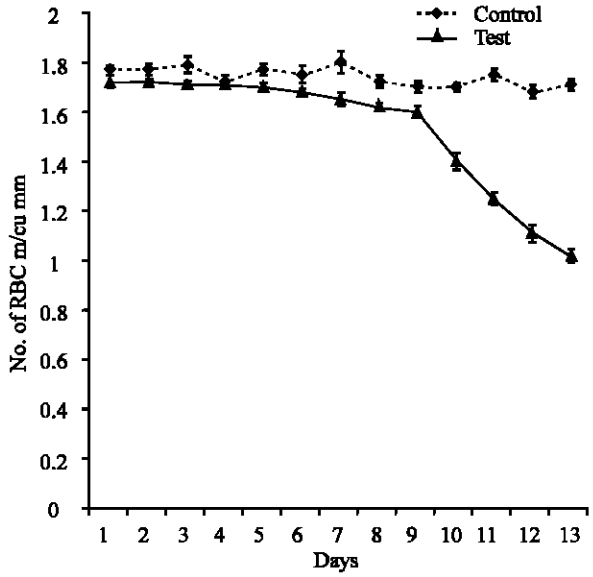

Fig. 1: No. of RBC following oral administration of 7.1 mg mL^{-1} mefenamic acid per individual/day at 32±1°C

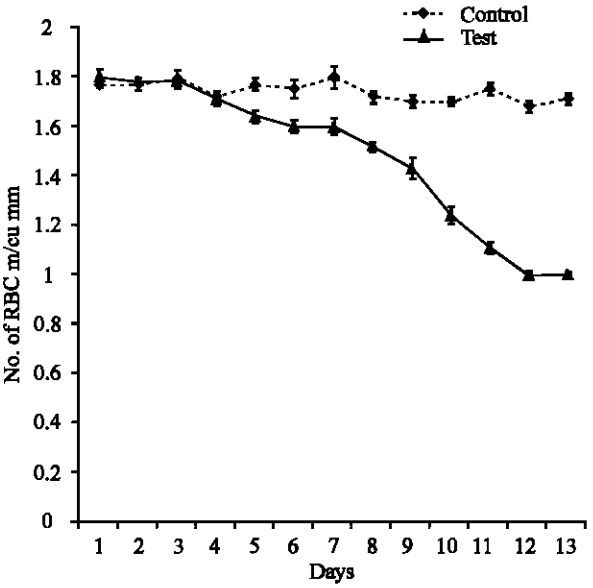

Fig. 2: No. of RBC following oral administration of 10.5 mg mL^{-1} mefenamic acid per individual/day at 32±1°C

The mechanism involved with mefenamic acid appears to induce formation of true auto antibodies capable of reacting with red cells[18].

Cellular injury appears to be mediated by complement activation at the cell surface, Drug ant-drug complexes formed first and then became secondarily bound to target blood cells as "innocent bystanders", either non

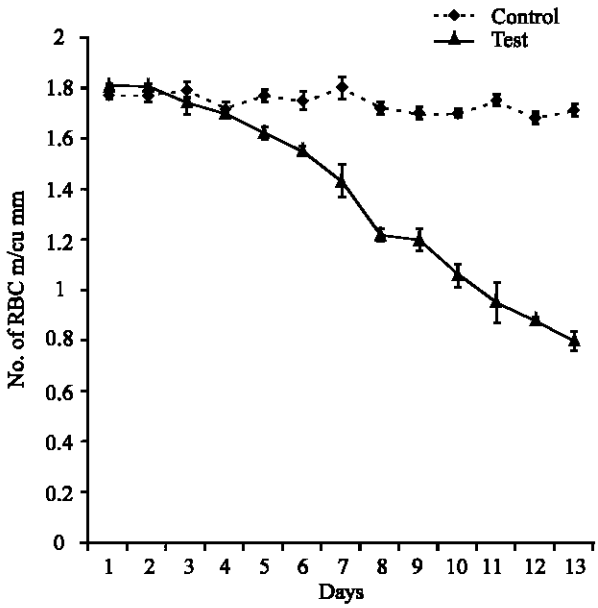

Fig. 3: No. of RBC following oral administration of 14.0 mg mL^{-1} mefenamic acid per individual/day at 32±1°C

specifically or possibly via membrane receptors with the potential for subsequent activation of complement by bound complexes[19,20].

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