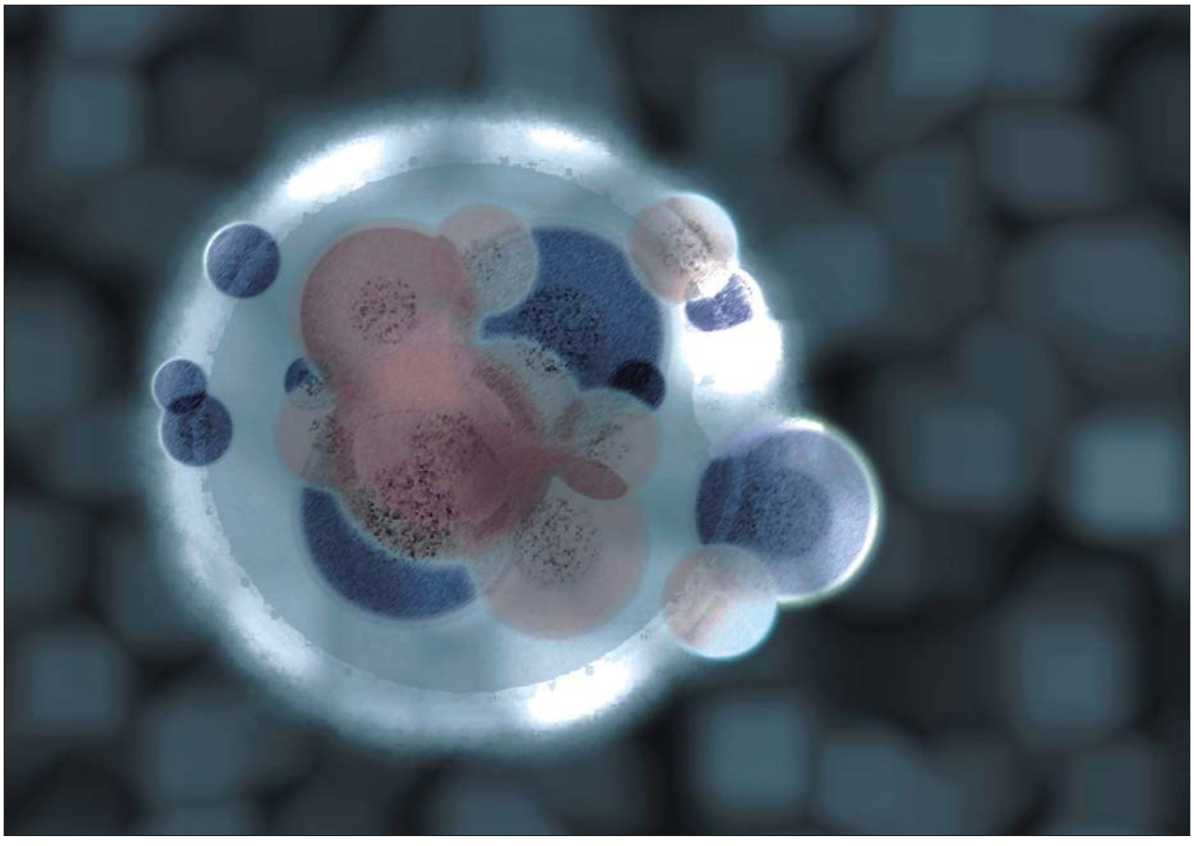

Journal of Biological Sciences

ISSN 1727-3048

science alert

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Journal of Biological Sciences 5 (4): 394-397, 2005
ISSN 1727-3048

The Effect of Mefenamic Acid on the Erythrocyte Sedimentation Rate in the Lizard, *Uromastix hardwickii*

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Abstract: Erythrocyte sedimentation rate of mefenamic acid (Ponstan) treated lizard was studied. There was gradual decrease following 7.1, 10.5 and 14.0 mg mL^{-1} day^{-1} for 12 days in the three respective groups. Thus ESR value increases in relation to the increase in the amount of mefenamic acid.

Key words: Mefenamic acid, ESR, lizard

INTRODUCTION

Mefenamic acid (Ponstan) receiving lacertilian groups treated with specific doses were evaluated daily for Erythrocyte Sedimentation Rate (ESR) during the span of 12 days. Symptomatic excessive disorders develop when the drug is administered[1] and continued administration of the drug, following the development of symptoms results in increased damage seriously.

In a recent study Ahmed and Sohail[2] has reported the development of a serious Autoimmune Haemolytic Anemia (AHA). In the present study the effect of Ponstan on the reptilian ESR is worked out in detail.

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[5,6]. 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.

Design of Experiment: Forty *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 others served as test.

Normally 2 g of mefenamic acid (8 tablets) is a dose prescribed for a human subject for a day. Therefore, normal doses of mefenamic acid for a Uromastix weighing 250 g is 7.1 mg day^{-1}. This dose of mefenamic acid was given orally every day to every 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 every 24 h for 12 days and each control received 1 mL of distilled water simultaneously. The test and control animals were fed 1 mL 5% glucose solution thrice a week. The ESR level was estimated daily for 12 days. along with daily administration of the drug.

In the second experiment test animals were given 10.5 mg of mefenamic acid per day for a period of 12 days and the ESR was estimated in the same way as that 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 ESR was estimated in the same way.

Drug information: Mefenamic acid, N(2,3-Xylyl)-anthralinic acid is a newer analgesic, anti-pyretic and anti-inflammatory agent, first marketed in. J. 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.

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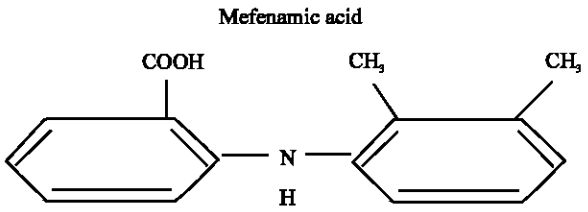
Routes of administration: The drug Ponstan (Parke Davis) is available for oral administration as it is insoluble in any suitable solvent.

Structure:



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

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 h as 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 asthematics 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[7].

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.

Estimation of the Erythrocyte Sedimentation Rate (ESR): The estimation of the Erythrocyte Sedimentation Rate (ESR) has been widely used in clinical medicine. Many methods for its measurement have been devised[8-10] differing with respect due to the anticoagulant used, the volume of the 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.

Westegren's[11] method, however, is almost universally used. The phenomenon of the ESR has been exhaustively investigated[9,12,13]. 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; out the actual rate of fall is influenced very greatly by the extent to which the red cells form rouleaux; since large clumps of cells adhering to each other (mainly side to side) sediment more rapidly than single cells. Other factors which affect the sedimentation include the ratio of red cells to plasma i.e. the PCV, plasma viscosity, the vertically or otherwise 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 is mainly controlled by the concentration of the fibrinogen and to a lesser extent of 2 and globulins in the plasma. Glycoproteins also appear to play a part in rouleaux formation[14]. In anemia, the alteration of the ratio of red cells to plasma encourages rouleaux formation and accelerates the rate of sedimentation.

Method of Westergran: The westergran 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 31.3 g L^{-1} trisodium citrate in the proportion of one part of citrate to 3 parts of blood. The test should be carried out within 2 hours of collecting the blood. The sample is well mixed and then the blood is then drawn up into the Westergran tube to the 200 mm mark. The tube is placed exactly vertical and left undisturbed for 60 minutes. 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/ hour is the ESR. Specially more 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[8]. It is conventional

to set up sedimentation rate test at room temperature, however sedimentation is normally accelerated as the temperature rises[15]. Therefore, if a test is to be used as a research tool it is advisable to control the temperature.

RESULTS

The examination of Table 1-3 indicates that ESR gradually increased in all test groups from day zero to day 12 due to decreased erythrocytes count. While ESR value for controls remained almost stable ranging between 7.00 to±0.0 to 6.5±0.25 mm h^{-1}.

The test animals treated with 7.1 mg mL^{-1} mefenamic acid showed an increased ESR of 12.0±0.77 mm h^{-1} on day 12 in comparison to its initial value of 7.00 to±0.0 mm h^{-1} on day zero (Table 1).

Following the 5th day of administered of drug a marked increase in ESR was observed (Table 1).

The animals of test group following the administration of 10.5 mg mL^{-1} mefenamic acid also showed an increase of ESR values ranging between 6.5±0.35 to 14.0±0.18 mm h^{-1} on day zero and day 12, respectively (Table 2). A marked difference of ESR values between control and test evident from the 4th day of treatment (Table 2).

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

Days	Control ESR (mm h^{-1})	Test ESR (mm h^{-1})
0	7.0±0.00	7.0±0.00
1	7.0±0.18	7.0±0.00
2	7.0±0.00	7.0±0.00
3	7.0±0.00	7.0±0.00
4	7.0±0.00	7.0±0.35
5	7.0±0.00	7.5±0.25
6	7.0±0.00	7.5±0.00
7	7.0±0.00	8.0±0.30
8	7.0±0.18	8.0±0.35
9	6.0±0.00	10.0±0.35
10	7.0±0.00	10.0±0.00
11	6.0±0.35	10.0±0.61
12	6.5±0.25	12.0±0.77

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

Days	Control ESR (mm h^{-1})	Test ESR (mm h^{-1})
0	7.0±0.00	6.5±0.35
1	7.0±0.18	6.5±0.00
2	7.0±0.00	7.0±0.35
3	7.0±0.00	8.0±0.61
4	7.0±0.00	8.0±0.41
5	7.0±0.00	9.0±0.00
6	7.0±0.00	9.0±0.31
7	7.0±0.00	9.5±0.77
8	7.0±0.18	11.0±0.94
9	6.0±0.00	11.0±0.86
10	7.0±0.00	12.0±0.25
11	6.0±0.35	12.5±0.30
12	6.5±0.25	14.0±0.18

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

Days	Control ESR (mm h^{-1})	Test ESR (mm h^{-1})
0	7.0±0.00	6.5±0.22
1	7.0±0.18	6.5±0.00
2	7.0±0.00	7.0±0.25
3	7.0±0.00	9.0±0.61
4	7.0±0.00	9.0±0.30
5	7.0±0.00	10.0±0.18
6	7.0±0.00	11.0±0.00
7	7.0±0.00	13.5±0.61
8	7.0±0.18	13.5±0.77
9	6.0±0.00	14.0±0.18
10	7.0±0.00	15.0±0.88
11	6.0±0.35	16.0±0.61
12	6.5±0.25	16.0±0.94

Each figure is the mean of 10 samples with ±SD

The test group administered 14 mg mL^{-1} mefenamic acid showed similar results i.e. an increased ESR from day zero to day 12 (Table 3). The increase in ESR on day 12 was 9.5 mm h^{-1} more in contrast to 6.5±0.22 mm h^{-1} on day zero. ESR values from day 3 onward remained high in comparison to control (Table 3).

In test groups the increased values of ESR was a dose dependent. The lower dose resulted in less increase in ESR, whereas the higher dose increased the ESR to greater extent. Statistical analysis by t-test indicated a significant increase ($p<0.05$) of ESR in all test groups in comparison to control.

DISCUSSION

Present data indicated that mefenamic acid affects ESR in all the 3 test groups of *Uromastix hardwickii*. With the increase in the amount of dose, a rise in ESR value was observed.

Several investigation have shown that the number of red cells is influenced by a variety of factors[9,16]. Basically it depends upon the difference in the specific gravity of plasma and erythrocytes. It has long been known that anaemic with reduced red cell number and reduction in the intrinsic sedimentation ability of the cells fails to maintain against the acceleration of effect of an increased proportion of plasma[17].

A number of changes have been described in the red cells it grows old. The sedimentation of the older cells due low dose starts from day 5; whereas the sedimentation following the higher doses started from day 3. It must be particularly mentioned that a dose of 14 mg/day resulted into a 50% more increase in ESR as compared to the other two lower doses. However, earlier studies lack any mention in this respect.

It must be remembered that relationship between viscosity and ESR; which increases in parallel. This can be observed in control and test groups during first 3 days. But the fall of red cells become fast when the viscosity is not great.

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