
ORIGINAL ARTICLE

THE EFFECTS OF MEFENAMIC ACID ON HEMATOCRIT OF THE LIZARD, *UROMASTIX HARDWICKII*

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ABSTRACT

Mefenamic acid is an analgesic, antipyretic and anti-inflammatory agent. In addition induces several hematological disturbances. Present study was conducted to determine the alterations in blood PCV of the lizard *Uromastix hardwickii* after the administration of 7.1 mg / ml; 10.5 mg / ml and 14.0 mg / ml mefenamic acid per individual per day for 12 days to 3 test groups. The mean values of PCV were $15.5 \pm 0.81\%$, $14.5 \pm 0.25\%$ and $12.0 \pm 0.25\%$ for 3 test groups respectively in comparison to $23.5 \pm 0.40\%$ for control. Thus a significant dose dependant reduction in mean PCV per cent following the administration of mefenamic acid for 12 days indicates the extra vascular hemolysis due to destructive change in the red cell membrane through autoantibody mechanism.

Keywords: PCV, mefenamic acid, hemolytic anemia.

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INTRODUCTION

Autoimmune hemolytic anemia (AIHA) due to warm-reacting IgG auto-antibodies constitutes a considerable proportion of hemolytic anemias of acquired type. In all types of such anemias the hemolytic mechanism reflects a disorder of the patients physiological processes resulting in the potential destruction intrinsically of his own and also of other normal red cells (Maedel and Sommer, 1993; Engelfriet *et al.*, 1992).

Warm AIHA is also associated with autoimmune disease thus a majority of authors prefer to designate these disorders as auto-immune hemolytic disease (Leddy and Swisher, 1973; Gurpreet *et al.*, 2004). Shortened red cell life-span *in vivo* and the evidence of “auto-immunity” directed against these red cells can be demonstrated by a positive direct antiglobulin test which is the hallmark of autoimmune hemolytic anemia (Jafferries, 1994).

The two major distinctions recognized under each basis of classification require a variety of chemical laboratory findings in addition to demonstration of a variety of serological methods i.e. Coomb's test.

It is known that administration of a number of commonly prescribed drugs produces hemolytic disorders. One of such drugs is mefenamic acid (Schwartz *et al.*, 2000). It is also known that erythrocytes of *Uromastix hardwickii* are more resistant to hypotonic shock than human erythrocytes (Ahmad *et al.*, 2004). Therefore the aim of this study was to investigate mefenamic acid-induced membrane alterations leading to red cell destruction and production of auto-immune hemolytic anemia.

MATERIALS AND METHODS

Choice of animals

The spiny tailed lizard exists in the desert and semi-desert regions of Karachi and Thatta District. These lizards are easily available, cost less and are easily managed. Thus, for the present study animals were obtained from local suppliers. An examination of the literature indicates that reptiles as a whole have been neglected as a research material.

Temperature

One of the important factors in the physical environment is temperature. Since, poikilotherms are incapable of maintaining their body temperature; variation in the ambient temperature will affect their body temperature and alter their physiological state. Therefore, in order to obtain reproducible results the temperature was kept constant at $32 \pm 1^\circ\text{C}$ during the experimental period.

Drug information

Mefenamic acid (Ponstan) marketed by Parke-Davis is available for oral administration in 250 mg tablets. The

usual recommended initial dose is 500 mg to be followed by 250 mg every 8 hour (i.e. 2 g for 24 hrs.). 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 (Donlad, 1992).

Toxicity

Adverse reactions during normal treatment have been mild and infrequent. The more common reactions include drowsiness, nausea and dizziness (2%). Nervousness, ringing of ears, gastrointestinal tract (GIT) discomfort and headache were more rarely encountered. Diarrhea is sometime associated with GIT hemorrhage. Although the latter may occur less readily than after aspirin. Therefore mefenamic acid therapy should be discontinued (Ponstel, 2004).

Further adverse effects reported occasionally are hypertension, tachycardia, dyspnea, pruritis, blurred vision, liver, kidney and blood disorders including hemolytic anemia. The use of this drug for pregnant woman is contraindicated. Patient with renal function, gastric inflammation and asthmatics should be treated with caution (Girdwood, 1979; Alpharetta, 2003; Ahmad *et al.*, 2005a,b).

Design of experiment

40 *Uromastix* almost equal in body weight (approximately 250 g) and size were sorted out from the stock to form four equal groups. One of the groups served as control and other served as tests.

The tablets Ponstan (Parke Davis) each containing 250 mg mefenamic acid purchased from local market (Batch # 0352463 / 2003) were used to make different doses in distilled water for the administration to test animals.

Dosimetry

Although no data is available about the administration of mefenamic acid specifically to reptiles, however the maximum average dose of analgesic other than mefenamic acid in reptiles is 30 mg / kg / 24 hrs. (Bennett, 1998; Lawton, 1999) which is quite similar to the dose of mefenamic acid in the present experiment calculated from the dose for human i.e. 28.57 mg / kg / 24 hrs. (i.e., 7.5 mg / 250 g & 7.1 mg / 250 g respectively).

In the experiment I the suspension was diluted in such a way that 1 ml contained 7.1 mg of drug. Thus each test animal received 1 ml oral suspension per day for 12 days. In the experiments II and III test animals were given 10.5 mg and 14.0 mg mefenamic acid per day respectively for a period of 12 days.

Animals of control group received 1 ml distilled water simultaneously. The test and control animals were fed 1ml

5% glucose solution thrice a week, while blood samples were drawn daily for 12 days and packed cell volume (PCV) was estimated.

Determination of packed cell volume (PCV)

Most accurate determination of the volume of packed red cells / 100 ml of blood 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:

- 1 Macro-method
- 2 Micro-method

The macro-method, which 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% higher as 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 (Ahmad *et al.*, 2003). Therefore, micro-method is more popular now-a-days. Strumia *et al.* (1954) used a centrifuge with 37,000 rev / min at 28,000 g centrifugal force; using micro-tubes 32 mm in length to pack completely without trapped plasma in 1 minute. However, the machines presently in use provide a centrifugal force of 12000 g and need 3-5 minutes centrifugation. According to Garby and Vuille (1961) the mean amount of plasma trapped is 1.3%.

It has been shown that the venous hematocrit overestimates 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% of the volume of packed red cells in venous blood (Chaplin *et al.*, 1953).

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. Over and above the heparinized capillary tubes are inexpensive and disposable.

The measurement of the PCV after centrifugation of blood can be used as simple screening test for pathological condition. Further, in conjunction with accurate estimations of hemoglobin and red cell count, knowledge of the PCV is valuable for the calculation of absolute values.

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 (Ahmad *et al.*, 2005a).

For obtaining hematocrit values heparinized capillaries were filled with the blood sample and their distal ends were sealed with special wax, the plastocin. 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

A consideration of Fig.1 indicates that PCV decreased in test animals following the administration of mefenamic acid, whereas in control, mean value of PCV remained almost constant and statistical analysis shows no significant difference from day 0 to day 12.

Test animals of experiment I given a dose of 7.1 mg / ml mefenamic acid showed a significant ($P < 0.05$) reduction in PCV from day-4, onwards in comparison to control (Fig. 1) and on day-12, the mean PCV of test animals was $15.5 \pm 0.81\%$ which indicates approximately 38% reduction from its initial value of $25.5 \pm 0.12\%$.

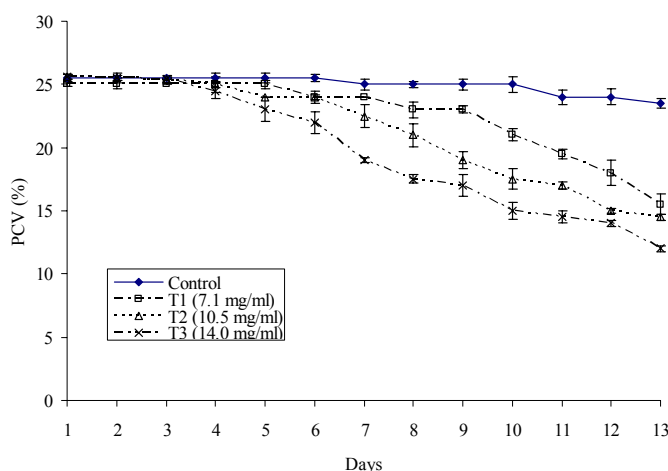


Fig. 1: Comparison of PCV (%) of control and tests following the administration of different doses of mefenamic acid in *Uromastix hardwickii*.

In the experiment II, the dose administered to test animals was 10.5 mg / ml mefenamic acid and PCV begin to reduce significantly ($P < 0.05$) from day-3 (Fig. 1). On day-12, mean PCV was $14.5 \pm 0.25\%$ which showed a 42% reduction in PCV.

The animals of experiment III were given a high dose i.e. 14 mg / ml mefenamic acid. The mean PCV which was $25.5 \pm 0.20\%$ on day-0, begin to decrease significantly ($P < 0.05$) from day-2 in comparison with control (Fig. 1). The reduction in PCV was rapid and on day-12, mean PCV was $12 \pm 0.25\%$ which showed a 50% decrease from its normal mean PCV.

Further statistical analysis (Two-way ANOVA) of data showed that the significant reduction ($P < 0.05$) in mean PCV was depending on dose as well as on the length of treatment i.e. after the administration of doses of 7.1, 10.5 and 14.0 mg / ml mefenamic acid, mean PCV began to decrease from day 4, 3 and 2 respectively.

DISCUSSION

PCV is measured following the centrifugation of blood, is a simple test in the screening of pathological state. In addition simultaneous estimation of Hb and erythrocytes count; the knowledge of PCV is of great importance for the calculation of absolute values.

The mechanism of action of mefenamic acid is not completely understood but may be related to the inhibition of prostaglandins synthetase thus effectively relieving the pain and inflammation (Ponstel, 2004).

Non-steroidal anti-inflammatory drugs (NSAID) are safest when low doses are taken for brief periods, prolonged use of larger doses results in many side effects (Grant, 2005). Adverse side effects of prolonged administration of mefenamic may also be observed on hemic system producing more fragile red blood cells resulting in hemolytic anemia (Ahmad et al., 2005 b). Patients on long-term treatment with mefenamic acid are recommended to check their hematocrit if they exhibit sign or symptoms of anemia (Alpharetta, 2003). Although the exact mechanism is unknown, mefenamic acid, induces extra vascular hemolysis perhaps by altering a red blood cell membrane protein and rendering it antigenic. (Petz, 1993; Ahmad et al., 2005 a,b).

In the present study the comparison of PCV values of control and tests for 12 days show a significant difference as mefenamic acid results in immune mediated hemolytic anemia due to production of antierythrocyte IgG antibodies (Petz, 1993). Prolonged administration of mefenamic acid also results in leucopenia thus reducing the mean PCV about 50% to its normal value at higher doses administered for more than one week.

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Received: 21-3-2005 – Accepted: 7-3-2006