

LOW DOSES OF PRISTANE IN COMPARISON WITH HIGH DOSES IN INDUCTION OF ARTHRITIS IN FEMALE SPRAGUE DAWLEY RATS

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ABSTRACT

OBJECTIVE: To compare the severity of arthritis induced by low and high doses of pristane in female Sprague-Dawley rats.

METHODS: The study was conducted in Postgraduate Medical Institute, Lahore. Twenty-four female Sprague-Dawley rats were randomly divided into three equal groups each consisting of 8 rats (n=8); group A served as healthy control, group B was given 150µl pristane and group C was given 500µl pristane intra-dermally in order to induce arthritis. The severity of arthritis was evaluated by total leukocyte count (TLC), neutrophil count, lymphocyte count, clinical score of arthritis and body weight. TLC, neutrophil and lymphocyte count was taken at day 0 and 15 while clinical score of arthritis and body weight were taken at day 0 and then on every alternate day till day 30.

RESULTS: Development of arthritis in both groups B and C was accompanied by rise in TLC, neutrophil count and clinical score of inflammation while lymphocyte count and body weight was decreased in both groups as compared to group A (p<0.001). In group A the clinical score of inflammation was zero throughout the study period. The score of group B and C, which was initially zero at day 0, increased to 12±1 and 16±1 respectively at day 15. The difference was found to be significant at day 15 when group B and C were compared with group A (p<0.001). The difference between group B and C was also significant (p<0.001).

CONCLUSION: Evaluation of results supported severe induction of arthritis with 500µl pristane than 150µl.

KEY WORDS: Arthritis, Rheumatoid (MeSH); Pristane (MeSH); Leukocyte Count (MeSH); Neutrophil Count (Non-MeSH); Lymphocyte Count (Non-MeSH); Clinical score of inflammation (Non-MeSH); Body Weight (MeSH).

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INTRODUCTION

Pristane is a natural saturated terpenoid alkane primarily obtained from shark liver oil, from which its name is derived (Latin *pristis*, "shark"). It is also found in mineral oil and some foods and in the stomach oil of birds in the order Procellariiformes.¹ Biosynthetically, pristane is derived from phytol and it also occurs in trace amounts in marine algae.² Its chemical name is 2,6,10,14-Tetramethylpentadecane and molecular weight is 268.52 g/mol.³

Pristane is used in research for induction of apoptosis, alveolar hemorrhages, lupus nephritis, tumors and microsomal enzyme cytochrome P4501A.⁴ It also causes macrophage activation and oxidative stress, expression of genes, but mostly it is used for the pathogenesis of autoimmune diseases such as arthritis and lupus.⁵

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Pristane-induced arthritis (PIA) in the rats has been described as an animal model of inflammatory arthritis which exhibits features similar to rheumatoid arthritis (RA) in humans. It resembles the human condition by its chronic inflammatory nature, destructive and symmetrical involvement of peripheral joints.⁶ Like RA the main histological features of PIA are inflammatory cell infiltrate, synovial hyperplasia, bone erosion and pannus formation.⁷ It induces arthritis in rats with interesting genetic and immunological features.⁸

There are different methods for induction of arthritis in rats. Mycobacterial adjuvant can be used for induction of arthritis in rats. Severe arthritis is induced but the disease is not restricted to joints and arthritis does not progress.⁹ Arthritis can also be induced by immunization to specific collagen proteins; collagen-II, collagen-II antibody and collagen XI.¹⁰ The type II collagen induced arthritis is useful in studying the major histocompatibility complex association and autoimmune recognition of autoantigens. However, the role of collagen proteins as autoantigens in RA is not proven to be critically important.¹¹ Arthritis can be achieved by injecting Freud's adjuvant, proteoglycan, cartilage oligomeric matrix protein, bacteria; borrelia, Staphylococci.¹² Nowadays the use of pristane to induce arthritis is becoming more frequent.¹³

Pristane induced arthritis (PIA) is usually preferred because of its earlier onset than other arthritis inducing agents. It mainly affects the large joints like wrist/ankle and metacarpophalangeal/metatarsophalangeal joints, having more severe inflammation and hardly involves other organs.¹²

Rizwan Faisal and colleagues performed a study and induced arthritis in

rats by injecting 500 μ l pristane at the base of tail intra-dermally. Within two weeks arthritis was developed.¹⁴ Teresina Laragione and co-workers induced arthritis in rats by single intra-dermal injection of 150 μ l pristane at the base of tail. Arthritis in this case too developed within two weeks time.¹⁵

The present study was conducted to find out that which dose (150 μ l or 500 μ l) of pristane is effective enough to produce significant arthritis in rats. This will help the researcher to decide straight away the dose of pristane rather than waiting for the results of one dose and then again trying with another dose if failed in inducing arthritis with first dose. This will not only help to prevent the wastage of time but also the wastage of money because pristane is too expensive (current price of 2500 μ l pristane is 500US\$; dated 21-06-2016).

METHODS

Animals

Adult healthy female Sprague Dawley rats were kept in animal house at Post Graduate Medical Institute Lahore in iron cages under hygienic conditions. The room temperature was maintained at 25 \pm 2 $^{\circ}$ C and was fed rat chow and water *ad libitum*. Rats were habituated to the holding room for a minimum of one week before undergoing experimental procedures with a 12-h light, 12-h dark cycle. The protocols of the experiment of this study were reviewed and approved by the ethical committee of Post Graduate Medical Institute, Lahore.

All the rats were randomly divided in to three groups by lottery method; as A, B and C. Each group consists of eight adult healthy female Sprague Dawley rats. The 24 sample size was estimated by using power and precision 3.0 software. It was estimated at 5% level of significance and 90% power of test.

Induction and evaluation of arthritis

The rats in the three groups received rat chow and drinking water for 15 days. On day 0, Group A was given 500 μ l of distilled water intra-dermally at the base of tail. Group B was given 150 μ l pristane (Sigma-Aldrich, USA) while group C was given 500 μ l pristane (Sigma-Aldrich, USA) by a single intra-dermal injection at the base of tail. As expected arthritis in both groups was induced within two weeks.

Parameters evaluated were total leukocyte count (TLC), differential leukocyte count (neutrophil count, lymphocyte count), clinical scoring of arthritis and body weight.

Blood Sample:

Total leukocyte count

At day 0 and 15, 1ml blood was collected by cardiac puncture under light anaesthesia (inhalation of chloroform). Blood was put in the test tubes containing ethylenediaminetetraacetic acid (EDTA) and checked for TLC.¹⁶

Estimation of TLC was done by Neubauer chamber. Blood was diluted 1:20 by mixing 0.02ml of blood with 0.38ml of diluting fluid containing 2% glacial acetic acid to lyse red blood cells. Diluting fluid was prepared by adding 2ml of glacial acetic acid and one drop of gentian violet to 98ml of distilled water. Blood was then transferred to Neubauer chamber under cover slip. The chamber was charged with a capillary and was then examined under low power microscope. Cells were counted in 4 corner squares. WBC count was calculated as below.

WBC per cu mm = Number of cells counted \times 50

Neutrophil and Lymphocyte count

At day 0 and 15, neutrophil and lymphocyte count was checked.¹² 1ml blood was collected by the same procedure as described for TLC.

Procedure: A blood drop was taken on one end of slide and was spread with the aid of a slide corner by a steady flow of hand movement to make a thin smear. It was air dried and fixed in methanol. A 1:9 dilution of Giemsa stain was prepared (1 part of Giemsa stain and 9 parts of distilled water) and smear was then stained with it for 20-30 minutes. It was then washed with tap water, air dried and examined with oil immersion lens under microscope. Hundred (100) WBCs were counted and percentage of WBCs was calculated. Neutrophil to lymphocyte ratio was also calculated.

Clinical score of inflammation

Clinical scoring of arthritis for all the rats were performed at day 0 and then on every alternate day. Arthritis score for one limb ranged from 0 to 4. 0 = no swelling, no redness, no joint involvement; 1 = swelling and redness of one joint; 2 = two joints involved; 3 = more than two joints involved and 4 = severe

arthritis in the entire paw (maximum score per limb was 4). The four limbs score was added to yield a total score for each rat (maximum total score per rat was 16).¹²

Body weight

Body weight was measured on electronic balance at day 0 and then on every alternate day.¹² Scores at day 0 and 15 were analyzed statistically.

Statistical Analysis

After collection data was entered and analyzed by using SPSS 20.0 software. The mean and standard deviation was determined for total leukocyte count, neutrophil and lymphocyte count, clinical score of inflammation and body weight. Data following normal distribution and having homogeneity of variances were compared among groups by using one-way analysis of variance (ANOVA) and Tukey's test was used for post hoc analysis. P-value \leq 0.05 was considered statistically significant.

RESULTS

Total leukocyte count:

The 24 rats in the study were randomly divided by lottery method into three groups of 8 each. At day 0 mean TLC was 5025 \pm 509/cmm in group A, 5280 \pm 492/cmm in group B, 5263 \pm 941/cmm in group C. In group A, TLC remained unchanged over time. At day 15 in group B and C TLC was 8453 \pm 690 and 12563 \pm 795/cmm respectively. Significant difference was found at day 15 when group B and C were compared with group A with p-value <0.001 each. Group C was also significantly different from group B with p-value <0.001 (Table I).

Neutrophil count:

Neutrophil percentage was 30.1 \pm 2.8 in group A, 31.5 \pm 1.5 in group B and 32.0 \pm 2.3 in group C at day 0. It remained unchanged over time in group A. In group B and C, the neutrophil percentage at day 15 was 32.6 \pm 2.1 and 35.1 \pm 2.5 respectively which was significantly higher than day 0. When group B and C were compared with group A, the p-value was found to be <0.001 for each group. Difference between group C and B was also significant with p-value <0.001 (Table I).

Lymphocyte count:

In group A, lymphocyte count re-

TABLE I: COMPARISON OF TOTAL LEUKOCYTE COUNT, NEUTROPHIL COUNT, LYMPHOCYTE COUNT, CLINICAL SCORE OF INFLAMMATION & BODY WEIGHT AT TWO READING TIMES (N=8)

Parameters	Day 0 Mean±SD			Day 15 Mean±SD		
	Group A*	Group B	Group C*	Group A*	Group B	Group C*
Total leukocyte count	5025±509	5280±492	5263±941	5175±542	8453±690 [§]	12563±795 [§]
Neutrophil count	30.1±2.8	31.5±1.5	32.0±2.3	29.0±1.7	32.6±2.1 [§]	35.1±2.5 [§]
Lymphocyte count	67.9±3.3	66.2±2.5	66.4±3.2	69.1±1.6	64.3±1.3	61.5±2.7 [§]
Clinical score of inflammation	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	12±1.0 [#]	16±1.0 [§]
Body weight (% of baseline)	100±0.0	100±0.0	100±0.0	123.88±5.25	98.33±4.73 [#]	93.13±4.19 [§]

A* & C* (data previously published) 13, 15; +p ≤ 0.05; #p ≤ 0.01; §p ≤ 0.001

mained same throughout the study. The lymphocyte percentage was 67.9±3.3 in group A, 66.2±2.5 in group B and 66.4±3.2 in group C at day 0. At day 15 Group B had insignificant difference with group A with p-value >0.05 while significant difference existed between group C and A with p-value <0.001. Significant difference was also found between group C and B with p-value <0.001 (Table I).

In group A the clinical score of inflammation was zero throughout the study period. The score of group B and C, which was initially zero at day 0, increased to 12±1 and 16±1 respectively at day 15. The difference was found to be significant at day 15 when group B and C were compared with group A with p-value <0.001 each and the difference between group B and C was also found to be significant with p-value <0.001 (Table I).

Body weight:

The weight of the animals was not similar at day 0 after random allocation so it was converted into percentage and was considered as 100%. Then the trend of change in weight was studied in percentages. In group A the weight continued to increase and weight reached to 123.88±5.25% of the base line at day 15. In group B and C the weight reduced to 98.33±4.73 and 93.13±4.19 respectively at day 15.

DISCUSSION

The current study represents the first study of comparing the severity of induction of arthritis with different doses of pristane. The purpose of this study was to investigate for the accurate dose of pristane which can induce arthritis significantly in rats. All the parameters including TLC, neutrophil count, lymphocyte count, clinical score of inflammation and

body weight were significantly elevated in both group B (150µl pristane) and group C (500µl pristane) as compared to group A (healthy control). Arthritis found to be significantly high in group C when it was compared with group B.

After a single injection of pristane a chronic relapsing arthritis develops in rats. Pristane induced arthritis (PIA) is characterized by a sudden onset of disease; two weeks after induction. In PIA the main pathological features are edema accompanied by an acute phase response, infiltration of mononuclear and polymorphonuclear cells, pannus formation and erosion of cartilage and bone. It mainly affects large joints like wrist/ankle and metacarpophalangeal/metatarsophalangeal.¹⁷

A study was performed by Olofsson in which he used 150µl pristane to induce arthritis in rats. He monitored arthritis development in all the four paws by macroscopic scoring system. Fifteen was the maximum score per paw, so the maximum score for the four limbs became 60. He found the induction of arthritis in all the rats (100%) with mean score 32±0.7.^{17,18} It is evident from the mean score that severity of arthritis was only 53%.

Jian Ping and colleagues compared the effects of pristane with other arthritic agents in rats. He induced arthritis in rats by injecting 150µl pristane. The development of arthritis was monitored by changes in body weight and clinical score of inflammation; the maximum score for the four paws was 16 as it was 4 for each paw.¹⁹ The results of the study showed that severity grade of arthritis was 51% with mean score 8.1±2.9. There was mild change in body weight.

In China a study was performed in which 150µl of pristane was given to

produce arthritis in rats. The severity of arthritis was monitored by plasma nitric oxide levels and macroscopic scoring system; maximum score for one paw was graded as 15 and 60 for four paws. When results were evaluated it was found that severity of arthritis was only 40% in the diseased group.²⁰

In another study 500µl of pristane was used for induction of arthritis in rats. Arthritis was developed within two weeks and its severity was evaluated by TLC, neutrophil count, lymphocyte count, clinical score of arthritis and body weight. It was found that arthritis was induced in all the rats (100%). There were significant changes in all the parameters when they were evaluated at day 15. The TLC, neutrophil count and clinical score of arthritis were increased while lymphocyte count and body weight were decreased as compared to day 0. The TLC was almost twice of day 0 reading and all the paws were maximally inflamed and swelled with mean score 16±1; indicating severe arthritis.¹²

Holmberg and coworkers investigated the effect of 500µl pristane in inducing arthritis in rats. The severity of arthritis was evaluated by interferon gamma, tumor necrosis factor alpha and macroscopic scoring system. The maximum score per limb and rat was 15 and 60 respectively. All the parameters were found to be significantly high. The severity of arthritis according to macroscopic scoring system was 57%.²¹

A study was performed in Spain in which 500µl of pristane was used to produce inflammation. TLC along with other parameters was evaluated. After 10 days of injecting pristane, TLC was observed to be 5 times more in diseased rats as compared to control rats; indicating severe inflammation.²²

In all the above mentioned studies rats were used as experimental animals and arthritis was induced by different doses (150µl and 500µl) of pristane. In these studies mainly TLC, clinical score of inflammation and body weight along with other inflammatory mediators and markers were used as parameters, which showed induction of mild arthritis with 150µl of pristane and severe with 500µl. In the present study TLC, neutrophil count, lymphocyte count, clinical score of inflammation and body weight were kept as parameters. The changes were mild in all these parameters with 150µl of pristane and marked with 500µl; showing severe induction of arthritis with later dose.

CONCLUSION

The potent increase in the count of leukocytes, neutrophils and clinical score of inflammation, and marked reduction in lymphocyte count and body weight suggested induction of severe arthritis with 500µl of pristane than 150µl.

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CONFLICT OF INTEREST

Authors declared no conflict of interest

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AUTHORS' CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

RF: Conception and design; Acquisition, Analysis and interpretation of data, Drafting the manuscript; final approval of the version to be published

MG: Analysis and interpretation of data, Drafting the manuscript, Final approval of the version to be published

AA: Critical revision, Drafting the manuscript, Final approval of the version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.