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Intravaginal Administration of Talc Alters Red Cell Fragility and Lipid Peroxidation in Rats

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ABSTRACT: The study was carried out to investigate the effects of fourteen days administration of intravaginal talc solution on erythrocyte indices, osmotic fragility and lipid peroxidation in rats. Intravaginal talc solution was administered for 14 days, following which erythrocyte indices such as red blood cell (RBC) count, haemoglobin concentration (Hb), haematocrit or Packed Cell Volume (PCV), reticulocyte count and erythrocyte osmofragility were determined in addition to plasma malondialdehyde (MDA). The RBC count, Hb, PCV and reticulocyte count were reduced significantly ($p<0.05$) following 14 days administration of intravaginal talc solution, while erythrocyte osmofragility was significantly elevated ($p<0.05$). There was also significant increase ($p<0.05$) in the plasma MDA of talc-treated rats. The study therefore showed that administration of intravaginal talc solution for 14 days may increase red cell osmofragility and lipid peroxidation as well as exhibit anti-erythropoietic properties. In conclusion, it is advisable that caution should be exercised in the administration of talc powder around the perineum of females because of its potential to traverse the female reproductive system and cause systemic damage.

KEYWORDS: Talc, Erythrocyte, Osmofragility, Erythrocyte indices, Malondialdehyde

1. Introduction

Talc, $Mg_3H_2(SiO_3)_4$ is a mineral composed of hydrated magnesium trisilicate that is widely used in the form of talcum powder (IARC, 2010). The purer forms (approximately 90% mineral talc) are used for cosmetic and hygiene products. Perineal use of cosmetic talc is a common practice in different parts of the world (Langseth *et al*., 2007).

Studies have shown that particles and fibres that enter the body can migrate to distant organs (Heller, 1996). For instance, asbestos fibres have been found in ovaries of women exposed to asbestos (Langseth *et al*., 2007). The effects of talc in human have been reported following accidental inhalation (Abraham and Brambilla, 1980), contact with surgical gloves (Sheikh *et al*., 1984), intravenous injection in heroin addicts (Crouch and Churg, 1983) and ingestion of crushed tablets (Farber *et al*., 1982). According to the International Agency for Research on Cancer (IARC), an agency under the World Health Organisation (WHO), the perineal (genital) use of talc-based body powder is "possible carcinogenic to humans" based on evidence from human studies (IARC, 2010).

The objective of this study was to investigate whether multiple vaginal topical administration of talc solution in rats will result into changes in red blood cells indices and lipid peroxidation.

2. Materials and methods

2.1 Talc

Talc was a powder preparation produced by PZ Industries PLC, Lagos, Nigeria,

2.2 Animals

Eighteen female rats averaging four months in age and weighing between 160 to 190g were used for the study. The rats were obtained from the Animal House, Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats were housed in plastic cages with stainless steel

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mesh cover and maintained under standard laboratory conditions (light period 6:30 am to 7:00 pm; 25 ± 2^0C; relative humidity 55%), with food and water freely available. The animals received humane care. The rats were randomly divided into three groups of six per group. Group 1 served as control and received intravaginally 0.1 ml of normal saline. Animals in Groups 2 and 3 received 2 and 4 mg/kg of talc solution respectively prepared in normal saline respectively. The talc solution was administered for 14 days.

2.3 Determination of erythrocyte osmotic fragility

The procedure employed was that described by Olaleye *et al* (1999). Eight centrifuge tubes (numbered 1-8) each containing 5 ml of graded saline concentration (0.1%-0.8%) was used. The ninth tube contained distilled water. A known volume (0.02 ml) of blood was collected from each rat (via a cut from tail pre-soaked in xylocaine) into each centrifuge tube. The contents were thoroughly mixed and incubated for 30 minutes at 29-30^{o}C. The tubes were then centrifuged at 10,000 *g* for 5 minutes. The supernatants were carefully decanted and the optical absorbance (used as a measure of the degree of haemolysis) determined with a spectronic 20 Spectrophotometer. The percentage haemolysis was plotted against the sodium chloride concentration for each animal and the saline.

2.4 Determination of haematological parameters

All haematological parameters were determine by an automated haematological analyser, SYSMEX KY-21 (SYSMEX Corporation, Japan), using whole blood sample.

2.5 Determination of plasma malondialdehyde

The absorbance of the lipid peroxidation products which reacts with thiobarbituric acid forming a pink coloured adduct on boiling was read at 548 nm (Kumar *et al*., 1995).

2.6 Statistical analysis

The data were expressed as mean ± standard deviation (SD) and analyzed using analysis of variance (ANOVA). Student's *t*-test was used to test for differences among means for which ANOVA indicated a significant ($p<0.05$) F ratio.

3. Results

The results of the red blood indices and plasma MDA are shown in Table 1. There was a significant decrease ($p<0.05$) in RBC count, haemoglobin concentration, packed cell volume and reticulocyte count in groups 2 and 3 (treated with 2 and 4 mg/kg of talc solution, respectively) compared to the control group (administered intravaginal normal saline only).

The plasma MDA was significantly higher ($p<0.05$) in the experimental groups (2 and 3) when compared with control group (Figure 1). The osmotic fragility of erythrocytes from rats that received intravaginal talc solution for 2 weeks was significantly greater ($p<0.05$) when compared with the control that had intravaginal NaCl solution.

Administration of intravaginal talc solution for two weeks significantly shifted the fragiligram to the right due to greater hemolysis. The median mean corpuscular fragility (MCF_{50}) in the control animals was 0.47±0.03%. This value was significantly lower ($p<0.05$) than the trend in the animals that received intravaginal talc solutions (0.57±0.04% and 0.62±0.02% for 2mg/kg and 4mg/kg respectively, $p<0.05$ vs control, n=6), however, there was no statistical significant difference between the MCF_{50} of the rats that received intravaginal talc solution (groups 2 and 3) (0.57±0.04% vs 0.62±0.02%, $p>0.05$, n=6). Hemolysis of RBCs of talc-treated rats started at about 0.90 NaCl concentration whereas it was at about 0.80 NaCl concentration for control rats.

Table 1: Effect of intravaginal administration of talc on some red blood cell indices and plasma malondialdehyde concentration of rats

		Talc (mg/kg body weight)	
Parameters	**Control**	**2**	**4**
Red Blood Cells ($x10^6/\mu L$)	7.41 ± 0.3^a	6.34 ± 0.4^b	6.12 ± 0.5^b
Haemoglobin (g/dL)	14.3 ± 1.5^a	12.1 ± 1.3^b	11.6 ± 0.5^b
Packed Cell Volume (%)	45.8 ± 2.9^a	37.6 ± 1.3^b	35.4 ± 2.6^b
Reticulocytes (%)	2.89 ± 0.4^a	2.23 ± 0.2^b	2.12 ± 0.1^b
Malondialdehyde (mmol/L)	1.52 ± 0.06^a	1.68 ± 0.11^b	1.69 ± 0.13^b

Values are expressed as mean of six determinations ± SD;
[ab]Test values with different superscripts along the same row are significantly different ($P < 0.05$)

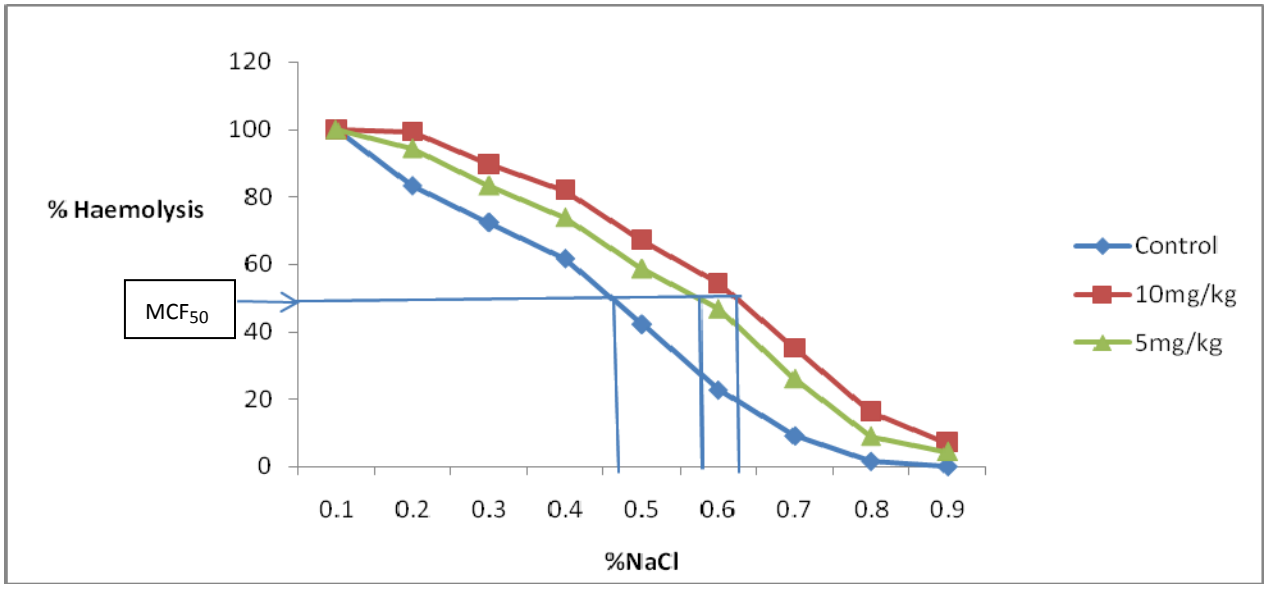

Figure 1: Erythrocyte osmofragiligram of rats following 2 weeks administration of intravaginal talc solution

4. Discussion

The results obtained from this study showed that total red blood cell count, haemoglobin concentration, packed cell volume and reticulocyte count were all reduced while there was an increase in malondialdehyde following application of intravaginal talc solution for 14 days.

Following perineal application, talc particle can migrate from the vaginal to the peritoneal cavity and ovaries (Venter *et al*., 1981). A majority of women experience retrograde menstruation (Olive, 1993); this suggest a mechanism by which talc particles can travel through the female reproductive tract to the ovaries. Furthermore, epidemiological studies have shown decreased risks of ovarian cancer after tubal ligation and/or hysterectomy, suggesting that removing a pathway by which carcinogenic substances can reach the ovaries reduces the risk of ovarian cancer (Rosenblatt and Thomas, 1996; Tortoleru-Luma and Mitchell, 1995).

In the study by Radić *et al* (1988), granulomatosis resulting from subcutaneous talc powder-suspension injections induced strong immunosuppression in rats. The disturbance induced reduction of mononuclear white blood cell count in the peripheral blood, atrophy of the thymic cortex, spleen enlargement, increase in the number of lymph node germinal centres and a significant delay of the first-set and second-set allograft rejection. Neither phagocytic function of the reticuloendothelial system nor erythrocyte count and humoral immune response were found to be altered.

The present study also revealed that there was a significant reduction in the total red blood cell count, haemoglobin concentration, packed cell volume and reticulocyte count following intravaginal talc application. The reduced reticulocyte could be an indication of suppression of bone marrow production of red blood cells. Patients with bone marrow ablative disorders may show a normal or decreased reticulocyte count in spite of severe anaemia. Such patients include those with iron, folate or vitamin B12 deficiency anaemia, pernicious anaemia, immunologic or drug-induced red cell aplasia, leukaemia or metastatic carcinoma and other disorders (Riley *et al*., 2001). This could be one of the mechanisms involved in the reduction of red blood cell indices by talc.

Osmotic fragility tests are used to establish the role of factors affecting the physical integrity of the erythrocytes (Parpart, 1947). Increased haemolysis could therefore be one of the ways by which talc caused reduction of RBC parameters, because of the increased osmotic fragility observed in the talc groups.

Malondialdehyde (MDA) level is commonly known as a marker of oxidative stress and the antioxidant status (Gawel *et al*., 2004). The increased MDA in our study could be due to increased destruction of RBCs which might have generated increased lipid peroxide. This could be one of the mechanisms by which RBC indices are reduced.

From these results, it can be concluded that intravaginal talc has anti-erythropoietic effects. The result also showed that talc increased lipid peroxidation. In view of the systemic effects of topical application of talc solution on the vagina, it may be advised that alternative product such as cornstarch-based cosmetic products which have not been known to have any link with any form of cancer should be used instead of talc-based cosmetic products.

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