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THE EFFECT OF POLYMORPHONUCLEAR LEUKOCYTES ON HUMAN SPERM

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ABSTRACT

Previous work from our laboratory has shown that secretory products of polymorphonuclear leukocytes (PMNs) decreased sperm motility and caused sperm aggregation in the mouse. In this study we report the same effect of PMN secretory products, superoxide and hydrogen peroxide, on sperm aggregation. The aggregation can be abolished in the presence of seminal plasma but not in the presence of vaginal lavage. Aggregation of sperm can reduce the number of free sperm in the medium which in turn reduces the probability of fertilization. Therefore, sperm aggregation caused by oxygen radicals produced by PMNs can be implicated as a possible cause of infertility in men or women who have gential tract inflammation and high levels of PMNs in their reproductive tissues.

INTRODUCTION

Numerous viable leukocytes are present in human semen, and recent studies indicate that semen from a significant percentage of infertile men contain dramatically increased numbers and unusual profiles of white blood cell.[1, 2] The presence of excess numbers of leukocytes in semen has been correlated with reduced sperm fertility as determined by hamster egg[3] and human oocyte[4] penetration assays. An association of increased number of seminal leukocytes and decreased sperm motility has also been reported.[3,5,6] Diverse effects of polymorphonuclear leukocytes (PMNs) on fertility have been documented. PMNs are known to be sperm phagocytic cells. Intrauterine injection of PMNs and their extracts markedly reduced fertility in many species of animals.[7-9] Using an immunohistological technique, PMNs were identified and found to be the major leukocyte subpopulation found in both normal and infertile semen.[1] Semen from infertile patients contained significanlty more PMNs than semen from fertile donors.[1-2] Recently, we reported the effects of oxygen radicals produced by PMNs on mouse sperm motility and aggregation.[10] In the present study, we demonstrated the effect of PMN secretory products, namely superoxide (SO) and hydrogen peroxide (HPO) on human sperm aggregation.

MATERIALS AND METHODS

Sperm from healthy normospermic donors were separated from other cell types by Percoll discontinuous gradient (47%, 90%).[11] Motile sperm in the 90% Percoll pellet were adjusted to 10×10^6 sperm/ml in culture medium (CM, Whitten's medium, supplemented with 0.4% bovine serum albumin). Seminal plasma obtained from the top fraction was centrifuged (600 xg, 10 min), passed through 0.45 μm filter and kept at -70°C until used.

PMNs, lymphocytes and red blood cells (RBC) were prepared from venous blood. Lymphocytes were separated by Ficoll hypaque. PMNs were prepared from the buffy coat above the RBC pellet after Ficoll hypaque. PMNs were prepared from the buffy coat above the RBC pellet after Ficoll hypaque separation. PMNs and RBC were separated by dextran sedimentation.[12] Vaginal secretions were obtained from volunteers at the periovulatory stage of the menstrual cycle by lavage with 10 ml of Hanks balanced salt solution and collected into a modified syringe.

Effects of secretory products of viable PMNs on sperm were studied using a chemotaxis chamber with 0.45 μm pore size filter (Transwell #3413, Costar, Cambridge, MA). Aliquots of 100 μl containing 10^6 human sperm in Whitten's/BSA were placed in the upper compartment and were co-incubated with 1 ml of intact PMNs in the lower compartment. RBC, lymphocytes or medium alone placed in the lower compartment were used as controls, The chambers were incubated at 37°C in a 5% CO_2 incubator. At various time points, aliquots of sperm suspension were removed and analyzed for sperm motility and sperm aggregation. Aggregated sperm was observed under a light microscope at 100×and 400×magnifications. A volume of 10 μl of sperm suspension was placed on a slide, covered with a cover slip supported at four corners with Nivea cream drops, squashed lightly until the suspension spreaded over the cover. The aggregations of sperm which contained more than 5 sperm were scored.

Sperm motility were analyzed using Hamilton-Thorn Research HTM-2030 Motility Analyzer with Mackler chamber. The analyzer was set up as follows: minimum contrast and size were 9 and 8; low and high size gate were 0.4 and 1.8; low and high intensity gate were 0.6 and 1.8, respectively. Hypoosmotic swelling test (HOS) were performed according to Van der Ven *et al.*[13]

RESULTS

Marked sperm aggregation was observed in sperm preparation that has been co-incubated with PMNs, but not in the samples co-incubated with RBC, lymphocytes or medium (Table 1). The number of aggregation was reduced with lower numbers of PMNs in the lower compartment. All the sperm found in the aggregations were tail to tail aggregated (Fig. 1). The numbers of sperm in the aggregation complex were increased with time of incubation and numbers of PMNs present in the lower chamber (Fig. 1). Sperm motility and percentage of swollen sperm as determined by HOS test were not significantly different from the controls during 6 h of incubation. Activated PMNs by adding lipopolysaccharide (LPS, 15 μg/ml) or N-forml-L-methionly-L-leucyl-L-phenylalanine (FMLP, 10^{-6}M) to PMN cultures did not enhance the effects of PMNs on sperm aggregation or motility.

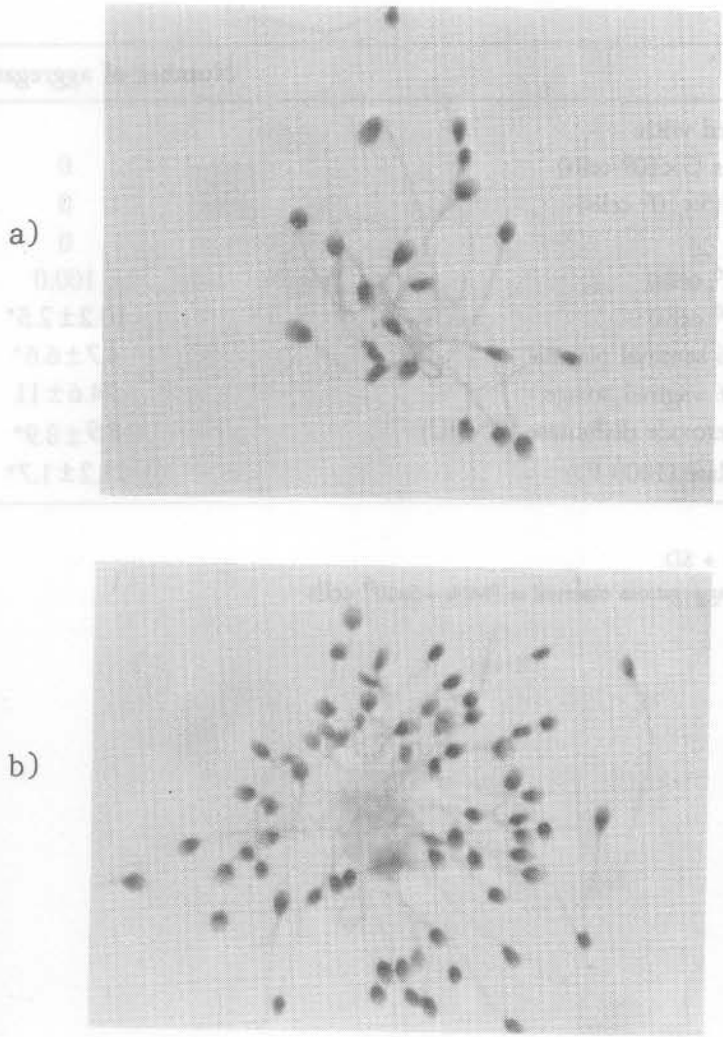

Fig. 1. Aggregated sperm induced by oxygen radicals from PMNs. Aggregated sperm were stained with Hemacolor. The numbers of sperm in the aggregation complex were increased with time of incubation: a) 1 hour; b) 2 hour.

TABLE 1 Effect of PMNs on sperm aggregation

Condition	Number of aggregations (%)
Sperm co-incubated with:	
1. red blood cells (5×10^6 cells)	0
lymphocytes (5×10^6 cells)	0
medium	0
2. PMNs (5×10^6 cells)	100.0
PMNs (1×10^6 cells)	10.2 ± 2.5*
3.[a] PMNs + 5% seminal plasma	4.7 ± 6.6*
PMNs + 10% vaginal lavage	74.6 ± 11
4.[a] PMNs + superoxide dismutase (500 IU)	8.9 ± 8.9*
PMNs + catalase (1000 IU)	21.2 ± 1.7*

Data represent means + SD

a: Number of sperm aggregations observed at PMNs = 5×10^6 cells

*: $p < 0.01$

The number of sperm aggregations was reduced dramatically in the presence of 5% seminal plasma, but not in the presence of 10% vaginal lavage. Addition of superoxide dismutase (500 IU) or catalase (1000 IU) to PMN cultures, effectively abolished the effect of PMNs on sperm aggregation (Table 1).

DISCUSSION

It has been demonstrated in that PMNs can reduce sperm motility and caused sperm aggregation[10]. The current study demonstrated that secretory products of PMNs caused sperm aggregation but did not affect sperm motility in humans.

Addition of superoxide dismutase and catalase which are SO and HPO scavengers, diminished the formation of aggregations, indicating that both SO and HPO were the cause of aggregation. The same observations were also demonstrated in the mouse study.[10] The toxicity of SO and HPO toward mammalian spermatozoa is well documanted.[14-17] Under aerobic condition, spermatozoa do undergo spontaneous lipid peroxidation, produce toxic oxygen radicals and concurrently lose their motilities. Superoxide dismutase had been shown to be the principal enzyme to defend against peroxidation in human sperm.[15] The effective counteraction of human seminal plasma on the toxic effects of the peroxidation process of human sperm has been demonstrated.[17, 18] The sperm-aggregation effect of PMNs as demonstrated in this study, was also reduced in the presence of seminal plasma. The protective effect of seminal plasma may be due to the presence of superoxide dismutase in the seminal plasma.[17] On the other hand, sperm aggregation still occurred when vaginal lavage was added to PMN cultures. Therefore, sperm aggregation induced by PMNs' secretory products may occur in the upper female genital tract where seminal plasma is present in minute amounts. In normal semen, which has a low number of PMNs and high concentration of superoxide dismutase in the seminal plasma, the number of free sperm may or may not be significantly altered by PMNs. On the other hand, these effects may be pronounced in samples which contain a high number of leukocytes, such as infertile or inflammatory semen. Hence, beside the direct effect of PMNs on sperm phagocytosis, the aggregation of sperm induced by PMN products can also play a major role in decreasing the percentage of motile sperm by reduction in the numbers of free sperm.

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บทคัดย่อ

คณะผู้วิจัยได้เคยรายงานผลของเม็ดเลือดขาวชนิดพอลิมอโฟนิวเคลียต่อตัวอสุจิของหนูถีบจักร ผลผลิตจากเม็ดเลือดขาวชนิดนี้สามารถลดการเคลื่อนที่ของตัวอสุจิ และทำให้ตัวอสุจิเกาะกลุ่มกัน (aggregation) ในรายงานนี้ คณะผู้วิจัยได้ศึกษาผลของเม็ดเลือดขาวชนิดดังกล่าวต่อตัวอสุจิของมนุษย์ พบว่าซุปเปอร์ออกไซด์ และไฮโดรเจนเปอร์ออกไซด์ซึ่งเป็นผลิตผลของเม็ดเลือดขาวชนิดนี้ สามารถทำให้ตัวอสุจิของมนุษย์เกาะกลุ่มกันได้เช่นกัน การเติมน้ำกาม (seminal plasma) ลงในหลอดขณะทดสอบ สามารถยับยั้งการเกาะกลุ่มของตัวอสุจิที่เหนี่ยวนำโดยเม็ดเลือดขาวได้ ในขณะที่น้ำล้างช่องคลอดสตรีไม่มีผลดังกล่าว การเกาะกลุ่มของตัวอสุจิเนื่องจากอนุมูลออกซิเจน (oxygen radical) ที่ผลิตโดยเม็ดเลือดขาวพอลิมอโฟนิวเคลียนี้ อาจเป็นปัจจัยที่สำคัญในการลดปริมาณของตัวอสุจิที่สามารถเคลื่อนที่และไปปฏิสนธิกับไข่ได้ โดยเฉพาะอย่างยิ่งในชายหรือหญิงที่มีการอักเสบของอวัยวะในระบบสืบพันธุ์และมีเม็ดเลือดขาวชนิดนี้ในปริมาณสูง