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Lipopolysaccharide Compromises Human Sperm Function by Reducing Intracellular cAMP

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A worldwide decline in the quality of human semen is currently occurring. In mammals, sperm are produced from diploid stem-cell spermatogonia by spermatogenesis in testes and become mature in epididymis. Nevertheless, these biological processes can be affected by Gram-negative bacterial infection mediated by lipopolysaccharide (LPS), the major endotoxin of Gram-negative bacteria. It is well known that LPS can disturb spermatogenesis and affect sperm maturation and quality *in vivo*. However, the effect of LPS on the ejaculated mature sperm *in vitro* remains unclear. Thus, this study aimed to assess the *in vitro* toxicity of LPS on human sperm function and to elucidate the underlying mechanism. Human sperm were incubated with LPS (0.1-100 μg/ml) for 1-12 h *in vitro* and, subsequently, sperm viability, motility and capacitation, and the acrosome reaction were examined. LPS dose-dependently inhibited total and progressive motility and the ability to move through a viscous medium of the sperm but did not affect sperm viability, capacitation, and the acrosome reaction. To explore the underlying mechanism of LPS's actions, we examined the effects of LPS on the intracellular concentrations of cyclic adenosine monophosphate (cAMP) and calcium ($[Ca^{2+}]_i$) and protein-tyrosine phosphorylation of human sperm, which are key regulators of human sperm function. LPS decreased intracellular cAMP dose-dependently but had no effect on $[Ca^{2+}]_i$ and protein-tyrosine phosphorylation of human sperm. These findings suggest that LPS inhibits human sperm motility by decreasing intracellular cAMP.

Keywords: $[Ca^{2+}]_i$; cyclic adenosine monophosphate; lipopolysaccharide; protein-tyrosine phosphorylation; sperm motility

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Introduction

A worldwide decline in the quality of human semen is currently occurring. One of the main factors affecting male fertility is male genital tract inflammation associated with infections by pathogenic microorganisms (Schuppe et al. 2008). Such microorganisms may infect the testis, epididymis, prostate, and other accessory sex glands, which in turn can affect spermatogenesis and sperm development and maturation (Spiess et al. 2007). Bacteria are the most common pathogens separated from the semen of infected men. Especially Gram-negative bacteria, including *Escherichia coli* (*E. coli*), *Staphylococcus aureus*, and *Mycobacterium tuberculosis*, reduce the semen quality of infected males (Bartoov et al. 1991). Therefore, it is important to intensively study the relationship between Gram-negative bacterial infection and male infertility.

Bacterial lipopolysaccharide (LPS) is a major component of cell wall of Gram-negative bacteria (Raetz and Whitfield 2002). Infecting bacteria release LPS, which binds to cell membrane receptors, notably Toll-like receptors, and stimulates pathogen-associated molecular patterns (PAMPs) (Akira and Hemmi 2003). Nuclear factor-κB (NF-κB) is subsequently activated to transcribe downstream inflammatory factors (Gioannini and Weiss 2007). Several studies have revealed that LPS has male reproductive toxicity (Kajihara et al. 2006; Brecchia et al. 2010; Metukuri et al. 2010; Winnall et al. 2011; Aly et al. 2012; Zhang et al. 2014). Intraperitoneal administration of 0.1 mg/kg/day LPS to ICR mice for 7 days reduced the epididymal sperm count and motility via apoptosis of spermatogonia (Kajihara et al. 2006). A single administration of 5 mg/kg LPS to rats inhibited the secretion of testicular hormones and disrupted spermatogenesis (Bhushan et al. 2008; Metukuri et al. 2010;

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Aly et al. 2012). In rabbit models, acute exposure to a single dose of 0.05 mg/kg LPS reduced male fertility. The same dose of LPS administrated to rabbits over 56 days resulted in infertility and disturbed sperm membrane integrity (Brecchia et al. 2010). *In vitro* experiments on cultured Sertoli cells showed that 10 μg/ml LPS significantly decreased the expression of self-renewal-related genes for spermatogonial stem cells (Zhang et al. 2014). To date, such studies have mainly focused on the toxicities of LPS for testicular function and spermatogenesis but the effect of LPS on mature sperm *in vitro* is poorly known. Despite mammalian sperm are produced and matured in male reproductive system; they should be ejaculated into the female reproductive tract to finish the fertilization. Therefore, studying the effects of LPS on the function of ejaculated sperm will provide new insights for understanding the male reproductive toxicity of LPS.

In this study, we assessed the *in vitro* toxicity of LPS on the viability, motility, capacitation, and acrosome reaction (AR) of ejaculated human sperm. To explore the underlying mechanisms, the effects of LPS on the intracellular concentrations of cyclic adenosine monophosphate (cAMP) and calcium ($[Ca^{2+}]_i$) and protein-tyrosine phosphorylation of human sperm were also examined.

Materials and Methods

Sample collection and treatments

Semen samples were collected by masturbation from healthy donors who had reproductive history during the recent two years (sperm counts > 40 million/ml, total motilities > 60% and progressive motilities > 45%, 25-35 years old men from different careers and backgrounds, living in Xiangyang, Hubei, China). The collection of the semen samples was approved by the Institutional Ethics Committee on human subjects of Xiangyang Hospital. The sperm were harvested by direct swim-up in human tubal fluid (HTF) medium (capacitating medium containing 25 mM $NaHCO_3$ and 3% bovine serum albumin, BSA, Merck Millipore Corporation, Darmstadt, Germany) or HS solution (uncapacitating medium containing 135 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 20 mM HEPES, 5 mM glucose, 10 mM lactic acid, and 1 mM Na-pyruvate at pH 7.4 with NaOH) as described previously (Luo et al. 2015).

In this study, we used a LPS from *E. coli* (serotype: O127:B8), which was reported to be present in male genital tract and semen of suspected infertile men (Bartoov et al. 1991) and in female reproductive tract of infected women (Cook et al. 2001). Additionally, basing on the dose used in the *in vitro* experiments on cultured Sertoli cells (10 μg/ml) (Zhang et al. 2014), we set a range of concentrations of LPS (0.1, 1, 10, and 100 μg/ml) to examine the effect of LPS on ejaculated human sperm. To simulate the state of ejaculated sperm in female reproductive tract, the sperm were incubated in HTF medium at 37°C. LPS from *E. coli*, serotype (O127:B8) was purchased from Sigma Chemical Company (St. Louis, MO, USA). In this study, 50 μl aliquots of sperm sample of a single donor were mixed with equal volumes of HTF medium or HS solution containing different concentrations of LPS to final doses of 0, 0.1, 1, 10, and 100 μg/ml and incubated at 37°C in a 5% CO_2 incubator for different time according to the experimental protocols.

Sperm viability and motility

Human sperm were incubated by different concentrations of LPS for 1 h and 12 h in HTF medium, respectively. Sperm viability was examined using Royo™ eosin staining kit (Nanchang Royo™ Biotech Co., Ltd, Nanchang, China) according to the user manual. The head of the dead sperm was stained red, while the head of viable sperm was not stained. Sperm motility was analyzed with a computer-assisted sperm analysis (CASA) system (WLJY-9000, WeiLi. Co., Ltd., Beijing, China). Sperm total motility and progressive motility were recorded. A minimum of 200 sperm were counted for each assay.

Penetration of the artificial viscous medium

Sperm penetration assay into a methylcellulose solution mimicing the viscous environment in female reproductive tract was performed as described previously (Luo et al. 2015). Briefly, human sperm were first capacitated in HTF medium for 3 h and then mixed with different concentrations of LPS. The open ends of the 7.5-cm flattened capillary tubes (1.0-mm inner depth; Elite Medical co., Ltd., Nanjing, China), which contain 1% (w/v) methylcellulose made up using HTF medium and sealed with plasticine in one end, were inserted into the sperm samples and incubated (37°C, 5% CO_2) for 1 h. The sperm numbers at 1 and 2 cm from the base of the tube were counted. The cell densities were normalized to values from parallel of untreated controls.

Capacitation and the acrosome reaction

For determining the effects of LPS on the sperm capacitation and AR, human sperm were capacitated in HTF medium containing different concentrations of LPS for 4 h. Chlortetracycline (CTC) staining was performed to evaluate the capacitation (the sum of CTC staining pattern "B" and "AR") and AR (CTC staining pattern "AR") as previously described (Luo et al. 2015).

Sperm intracellular cAMP and $[Ca^{2+}]_i$

To examine the sperm cAMP, human sperm were incubated in HTF containing different concentrations of LPS for 1 h. Then, the cAMP was determined using cAMP-Screen cAMP immunoassay system (Thermo Fisher Scientific, Waltham, MA, USA) and recorded by the EnSpire® Multimode Plate Reader (Perkinelmer, Waltham, MA, USA) according to the user manual. Human sperm were loaded with 5 μM Fluo-4 AM (Molecular Probes, Waltham, MA, USA) and 0.05% pluronic F-127 (Molecular Probes) in HTF medium for 30 min at room temperature in dark and subsequently washed in HTF medium. Five aliquots of 99 μl stained sperm were loaded into an ELISA Plate (Nest Biotechnology Co., Ltd, Rahway, NJ, USA). The Fluo-4 fluorescent (F) was recorded by the EnSpire® Multimode Plate Reader (Perkinelmer) using the 503-nm excitation light and 525-nm emission light at 2-s time interval. After recording for 50 s, five aliquots of 1 μl HTF containing different concentrations of LPS were added into the stained sperm samples to make the final doses of 0, 0.1, 1, 10, and 100 μg/ml and continued recording for 250 s, respectively. Then, 10 μM of the calcium ionophore A23187 was added as a positive control and recorded for another 70 s. The sperm $[Ca^{2+}]_i$ were calculated by the formula of $\Delta F/F_0$ (F_0, the mean fluorescent intensity before adding LPS; $\Delta F=F-F_0$) (Alasmari et al. 2013).

Western blotting procedures

The effect of LPS on protein-tyrosine phosphorylation of

human sperm was analyzed by western blotting assay according to previously published methods (Luo et al. 2015). Briefly, human sperm were capacitated in HTF medium containing different concentrations of LPS at 37°C in a 5%-CO_2 incubator for 4 h. As the uncapacitated control, human sperm were incubated in uncapacitated HS solution for 4 h (Uncap). The sperm proteins were isolated and a phosphatase inhibitor was added to the lysis buffer to eliminate the phosphatase activity. The proteins (50 μg) of each sample were electrophoresed on a 10%-SDS-polyacrylamide gel and transferred onto a polyvinylidenedifluoride (PVDF) membrane (GE Healthcare, Fairfield, CT, USA). Then, the PVDF membrane was blocked with 5% BSA and incubated with the anti-phosphotyrosine monoclonal antibody 4G10 (Merck Millipore Corporation) at a 1:1,000 dilution overnight and HRP-conjugated goat anti-mouse IgG at a 1:5,000 dilution (Thermo Fisher Scientific) for 1 hour. Subsequently, the PVDF membrane was visualized using the ECL detection kit (Thermo Fisher Scientific).

Statistical analyses

Differences between controls and the treated samples were assessed with unpaired *t*-tests. Statistically significant differences were determined at $p < 0.05$ with statistics software GraphPad Prism (version 5.01).

Results

LPS inhibits human sperm mobility

In this study, we determined the toxicity of LPS to ejaculated human sperm. Firstly, the effect of LPS on sperm viability was tested. After treatment with a range of concentrations of LPS for 1 h and 12 h, human sperm were stained with eosin. Sperm viability was not significantly affected by LPS in either the short-term (1 h, Fig. 1A) or the long-term (12 h, Fig. 1B) *in vitro* exposure. Subsequently, the effect of LPS on sperm motility was examined with a computer-assisted sperm analysis system. Total motility and progressive motility were significantly inhibited compared with the controls following exposure to 0.1-100 μg/ml LPS for 1 h and 12 h (Fig. 2). Additionally, the inhibition of sperm motility by LPS presented a dose-dependent manner (Fig. 2).

LPS decreases the ability of sperm to penetrate through a viscous medium

After ejaculation of sperm into the female reproductive tract, their extracellular environment becomes viscous. The ability to penetrate viscous fluids forms a part of a comprehensive evaluation of sperm motility, especially in relation to hyperactivation (Ivic et al. 2002; Alasmari et al. 2013). Here, we observed that LPS inhibited sperm motility, suggesting that the ability of sperm to penetrate through a viscous medium might be affected by LPS. To test this hypothesis, we observed LPS-incubated sperm in 1% methylcellulose, a medium previously used to assess this property of human sperm (Ivic et al. 2002). The cell density at a penetration distance of 1 cm was significantly decreased in the sperm samples exposed to 10 μg/ml and 100 μg/ml LPS relative to control sperm (Fig. 3A). At 2 cm penetration, the cell density was reduced in sperm samples exposed to 1-100 μg/ml LPS (Fig. 3B). These results indicate that LPS lowers the ability of human sperm to penetrate a viscous medium.

LPS has no effect on sperm capacitation and the acrosome reaction

Before sperm are able to fertilize an oocyte, they must undergo a series of physiological and biochemical changes, such as capacitation and the AR (Patrat et al. 2000; De Jonge 2005). Thus, we tested whether LPS affects human sperm capacitation and AR *in vitro*. Human sperm were capacitated in HTF medium, which contains a capacitation activator, $NaHCO_3$ and BSA. The capacitation ratio of sperm induced by HTF medium was approximately 40% (Fig. 4A), whereas, in sperm incubated in non-capacitating HS solution, the value was < 10% (Fig. 4A, Uncap). Up to 100 μg/ml LPS had no significant effect on capacitation in

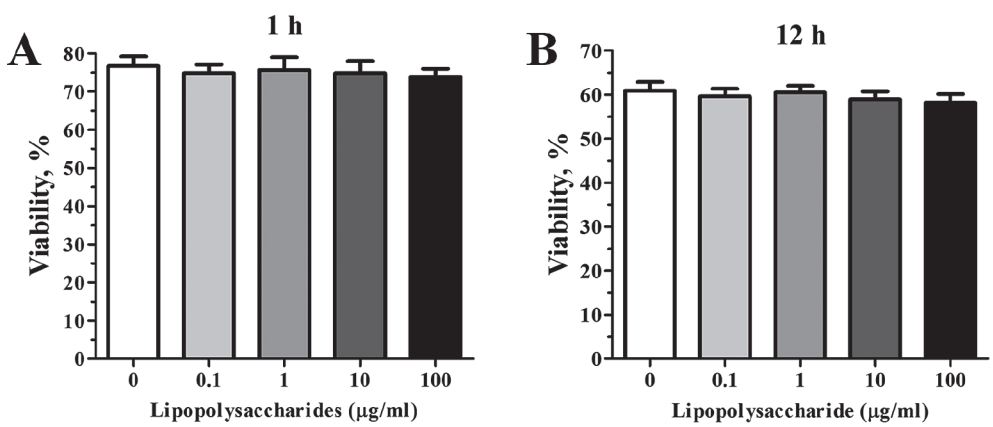

Fig. 1. The effect of LPS on human sperm viability *in vitro*. The ejaculated human sperm were purified and incubated with LPS (0.1-100 μg/ml) in HTF medium at 37°C, 5% CO_2 incubator for 1 (A) and 12 hours (B), respectively. The sperm viability was assessed by eosin staining. A minimum of 200 sperm were counted for each assay. LPS has no significant effect on sperm viability with short-term (A) and long-term (B) incubation *in vitro* ($p > 0.05$). Bar: mean ± SEM (n = 10).

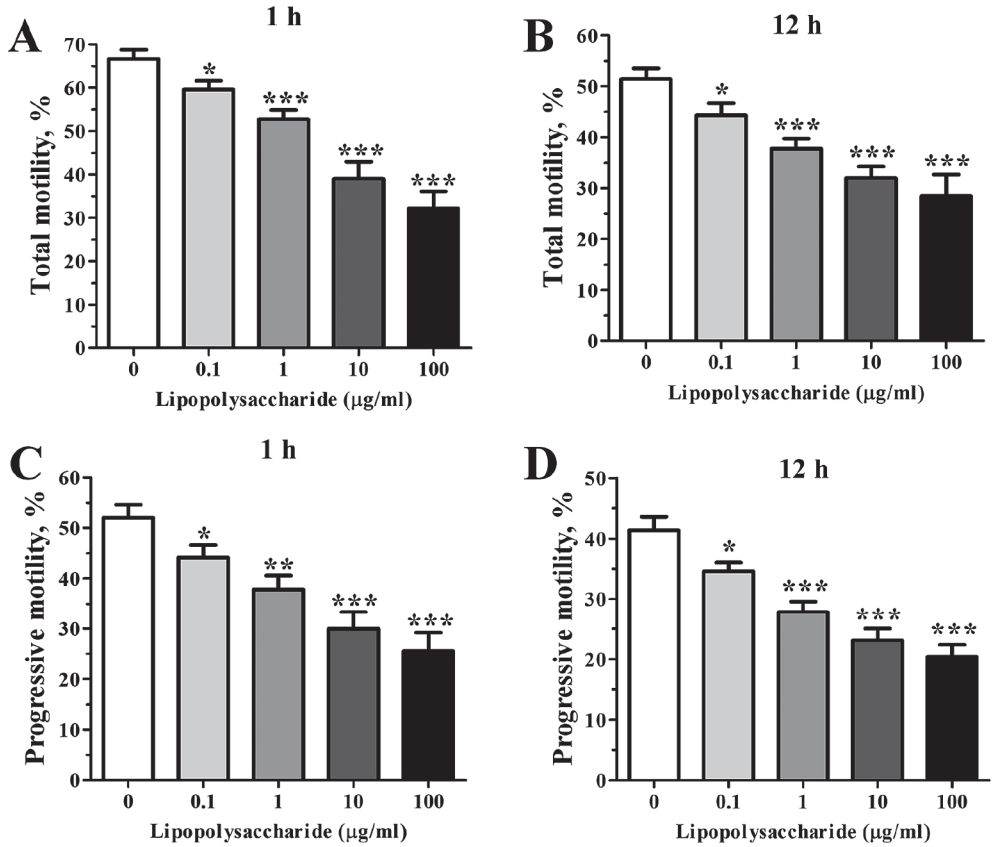

Fig. 2. The effect of LPS on human sperm motility *in vitro*. The ejaculated human sperm were purified and incubated with LPS (0.1-100 μg/ml) in HTF medium at 37°C, 5% CO_2 incubator for 1 (A and C) and 12 hours (B and D), respectively. The motion parameters including total motility (A and B) and progressive motility (C and D) were analyzed by Computer-Assisted Sperm Analysis (CASA). A minimum of 200 sperm were counted for each assay. LPS dose-dependently inhibited sperm total motility (A and B) and progressive motility (C and D) *in vitro*. Bar: mean ± SEM. *p < 0.05, **p < 0.001 and ***p < 0.0001, *t*-test, $n = 10$.

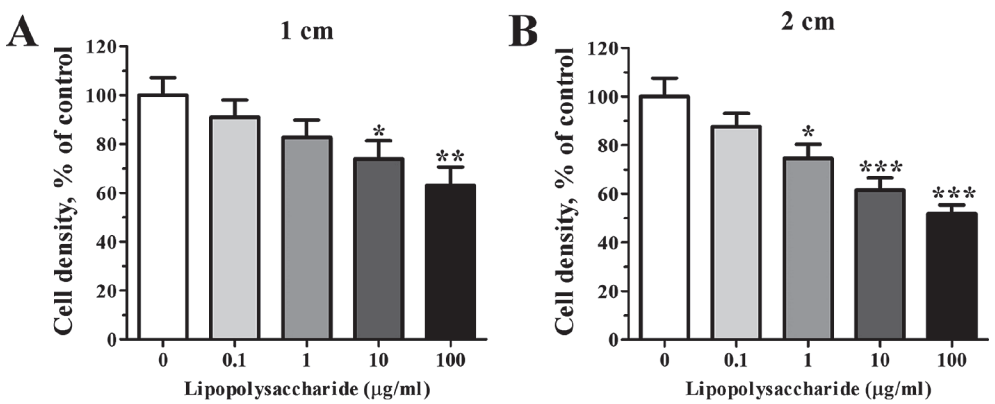

Fig. 3. The effect of LPS on human sperm's ability to penetrate through artificial viscous medium. The ejaculated human sperm were first capacitated in HTF medium for 3 h and then mixed with different concentrations of LPS (0.1-100 μg/ml) at 37°C, 5% CO_2 incubator. The open ends of the 7.5-cm flattened capillary tubes (1.0-mm inner depth), which contain 1% (w/v) methylcellulose made up using HTF medium and sealed with plasticine in one end, were inserted into the sperm samples and incubated (37°C, 5% CO_2) for 1 h. The sperm numbers at 1 (A) and 2 cm (B) from the base of the tube were counted. The cell densities were normalized to values from parallel of untreated controls (0 μg/ml LPS). Bar: mean ± SEM. *p < 0.05, **p < 0.001 and ***p < 0.0001, *t*-test, $n = 12$.

the HTF medium (Fig. 4A). Under normal conditions, human sperm spontaneously undergo AR after capacitation. However, spontaneous AR is not a requirement for fertilization and sometimes even disturbs fertilization. Thus, the proportion of spontaneous AR sperm is less than 20% in normal male individuals (Patrat et al. 2000). Here, we

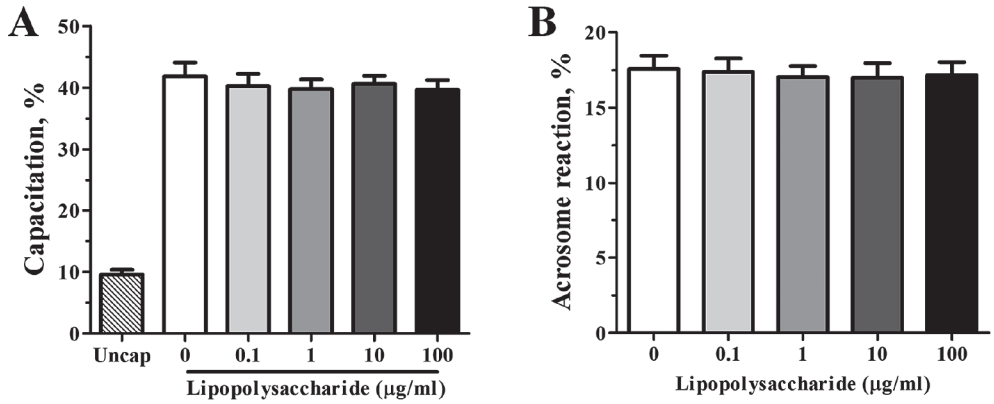

Fig. 4. The effect of LPS on human sperm capacitation and the acrosome reaction *in vitro*. The ejaculated human sperm were purified and capacitated in HTF medium containing the LPS (0.1-100 μg/ml) at 37°C, 5% CO_2 incubator for 4 h. The ratios of capacitation (A) and spontaneous acrosome reaction (B) were assessed by chlortetracycline staining. As the uncapacitated control, human sperm were incubated in uncapacitated HS solution for 4 h (Uncap). Bar: mean ± SEM, n = 10.

found that the proportion of spontaneous AR in all of the LPS-exposed sperm samples was below 20% and was not significantly different from the control (Fig. 4B).

LPS reduces intracellular cAMP but does not affect $[Ca^{2+}]_i$ and protein-tyrosine phosphorylation of human sperm

To explore the inhibitory mechanism of LPS on sperm function, we first examined the effect of LPS on the intracellular cAMP concentration, which has been reported to be a key factor in regulation of sperm motility (Lasko et al. 2012; Dey et al. 2014; Jansen et al. 2015). Sperm were incubated in a range of concentrations of LPS for 1 h and the cAMP concentration was assessed by immunoassay. LPS decreased sperm cAMP dose-dependently with an IC_{50} (50% inhibitory concentration) of 1.09 μg/ml (Fig. 5A, B).

Sperm motility and the ability to penetrate a viscous medium are regarded as $[Ca^{2+}]_i$-dependent processes (Publicover et al. 2007, 2008; Alasmari et al. 2013). Thus, it was reasonable to determine whether LPS affected the $[Ca^{2+}]_i$ of human sperm. We used a cell-permeable Ca^{2+}-sensitive fluorescent dye (Fluo4-AM) to detect sperm $[Ca^{2+}]_i$. In fact, sperm $[Ca^{2+}]_i$ was not significantly changed by a range of concentrations of LPS (Fig. 5C, D). At the end of each experiment, 10 μM of the calcium ionophore A23187 was added as a positive control. A remarkable increases of sperm $[Ca^{2+}]_i$ were caused by adding A23187 (Fig. 5C), which confirmed that the sperm used in the experiments were viable to respond the changes of $[Ca^{2+}]_i$.

In human sperm, protein-tyrosine phosphorylation is regarded to be an essential process for capacitation, the AR and motility (Bajpai et al. 2003; Naz and Rajesh 2004). Therefore, we tested whether LPS could affect the protein-tyrosine phosphorylation of human sperm during capacitation. Human sperm were capacitated in HTF medium containing different concentrations of LPS at 37°C in a 5%-CO_2 incubator for 4 h. The protein-tyrosine phosphorylation of the human sperm was detected by western blotting. Plenty of tyrosine-phosphorylated proteins (Molecular weight: 36-100 kD) were recognized by anti-phosphotyrosine monoclonal antibody in capacitated human sperm (0-100, Fig. 5E), while the uncapacitated control (incubated in HS medium without $NaHCO_3$ and BSA) presented faint signaling (uncap, Fig. 5E). However, the protein-tyrosine phosphorylation showed no obvious differences in human sperm capacitated in HTF medium containing different concentrations of LPS (0-100, Fig. 5E). These results are consistent with our functional results that LPS has no effect on capacitation and the AR of human sperm (Fig. 4).

Discussion

Animal models of LPS infection have been established *in vivo* by administration of bacterial LPS. These studies have demonstrated that LPS can induce temporary infertility and testicular dysfunction *in vivo* (Kajihara et al. 2006; Brecchia et al. 2010; Metukuri et al. 2010; Winnall et al. 2011; Aly et al. 2012; Zhang et al. 2014). However, whether this testicular toxic endotoxin affects mature sperm *in vitro* remained unclear. In this study, we have shown that LPS significantly inhibits the motility of human sperm and its ability to penetrate through a viscous medium (Figs. 2 and 3). These results imply that LPS may cause dysregulation of sperm functions even after sperm production, development, and maturation. The risk of infertility associated with the reproductive toxicity of LPS could be further increased if LPS accumulates in the environment of maturing sperm, e.g., in the seminal plasma, prostatic fluid, reproductive tract fluid, and follicular fluid. In addition, the present study only examined the toxicity of one kind of LPS from *E. coli* (serotype: O127:B8) which was reported to be present in male genital tract and semen of suspected infertile men (Bartoov et al. 1991) and in female reproductive tract of infected women (Cook et al. 2001). Thus, other types of LPS from the Gram-negative bacteria in suspected infertile men or women can also affect sperm function, but whether they will cause different influence to sperm function is required further studied.

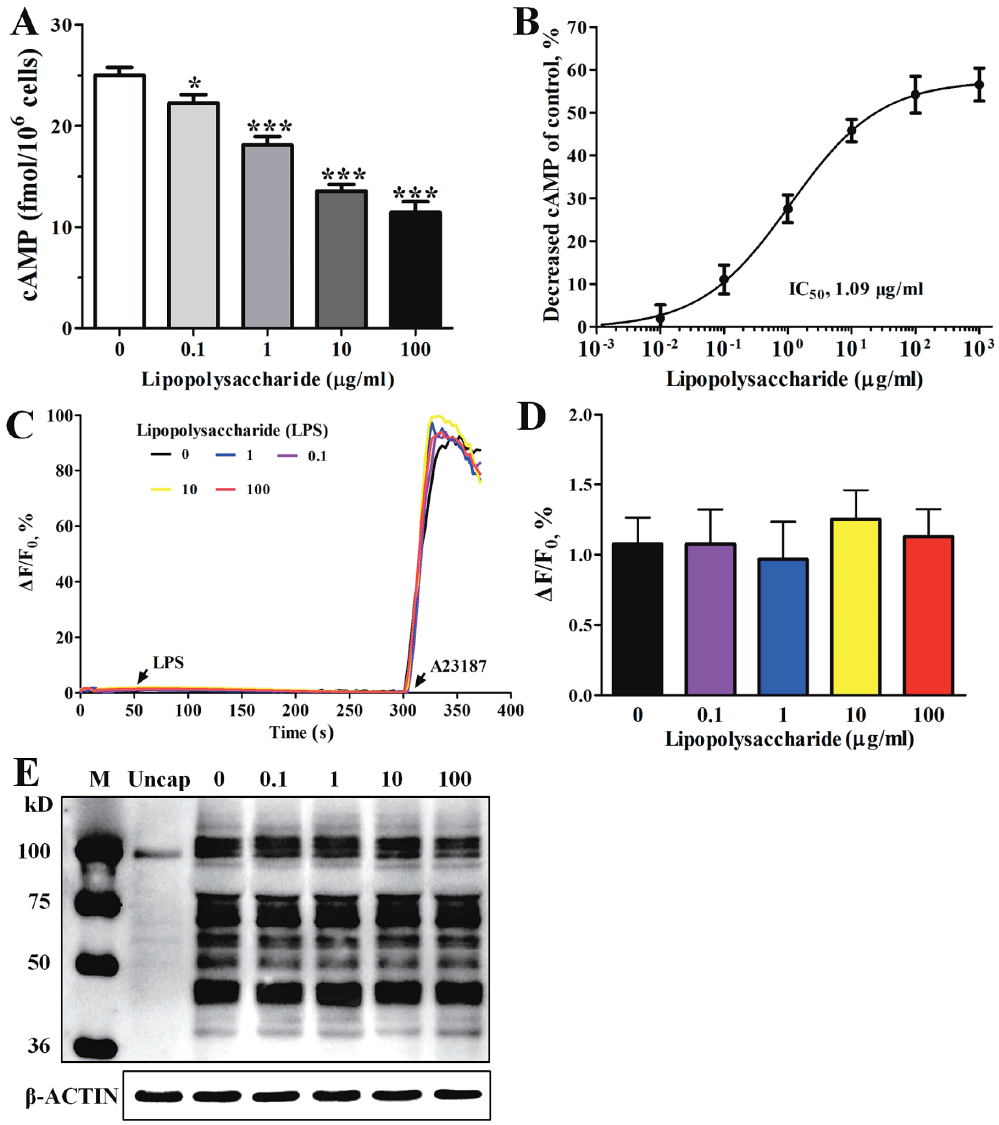

Fig. 5. The effects of LPS on intracellular cAMP, $[Ca^{2+}]_i$ and protein-tyrosine phosphorylation of human sperm *in vitro*. A and B, The changes of intracellular cAMP of human sperm exposed to LPS. The intracellular cAMP of LPS-treated sperm was determined using cAMP-Screen cAMP immunoassay system described in Materials and Methods. The dose-response curve was constructed by 7 doses (0, 0.01, 0.1, 1, 10, 100 and 1,000 μg/ml) and fit by a statistics software GraphPad Prism (version 5.01). Bar: mean ± SEM. *$p < 0.05$ and ***$p < 0.0001$, *t*-test, $n = 10$. C and D, The effect of LPS on human sperm $[Ca^{2+}]_i$. Human sperm $[Ca^{2+}]_i$ was monitored after loading cells with 5 μM Fluo4-AM and the fluorescence intensity of the sperm was detected by the EnSpire® Multimode Plate Reader described in Materials and Methods. A time-course curve showed the real-time changes of sperm $[Ca^{2+}]_i$ (C). Arrows indicated the time of adding drugs. The calcium ionophore A23187 was added as a positive control to monitor the availability of sperm to respond the changes of $[Ca^{2+}]_i$. The statistical analysis of the effects of LPS on sperm $[Ca^{2+}]_i$ was calculated as the mean $\Delta F/F_0$ of the time frames from 50 s to 300 s indicated in Fig. 5C (20 sperm were analyzed). E, The alteration of protein-tyrosine phosphorylation in human sperm exposed to LPS *in vitro* was measured by western blot using anti-phosphotyrosine monoclonal antibody 4G10. *β*-ACTIN is a loading control.
M, marker; Uncap, the uncapacitated control.

Many studies have shown that LPS regulates cell functions by modulating the expression of related genes (Kajihara et al. 2006; Beck et al. 2008; Bhushan et al. 2008; Okazaki et al. 2009; Brecchia et al. 2010; Zhang et al. 2014; Magi et al. 2015). However, unlike other cells, mature sperm are fully differentiated cells with highly condensed nuclei that lack active transcriptional machinery (Alasmari et al. 2013). Thus, gene expression that mediates functional modulation is no longer the key factor for mature sperm. Instead, second messengers, such as cAMP and Ca^{2+}, and protein phosphorylation play important roles in the modulation of a range of activities that are essential to sperm function (Publicover et al. 2007, 2008; Lasko et al. 2012; Alasmari et al. 2013; Dey et al. 2014; Jansen et al. 2015).

In this study, we found that LPS dose-dependently reduced the intracellular cAMP of human sperm (Fig. 5A, B) but it did not affect $[Ca^{2+}]_i$ (Fig. 5C, D) and protein-tyrosine phosphorylation of human sperm (Fig. 5E). The reduced intracellular cAMP may explain the decreased sperm motility and the reduced ability to penetrate a viscous medium. This result is consistent with previous reports claiming that intracellular cAMP is critically involved in LPS-mediated signaling pathways and is significantly decreased by LPS in other cell types (Patrizio et al. 1995; Schlegel et al. 2009). These observations indicated that an LPS-induced decrease of cAMP is a common means of regulating cell functions in different cell types; and especially in human sperm, intracellular cAMP is the main regulator in LPS-mediated reduction of motility.

Previous studies have reported that LPS perturbs intracellular Ca^{2+} homeostasis in microglial cells, cardiomyocytes, and lung microvessels (Choi et al. 2002; Beck et al. 2008; Wang et al. 2009; Kandasamy et al. 2013; Magi et al. 2015). In contrast, the present study showed that human sperm $[Ca^{2+}]_i$ was unaffected by LPS (Fig. 5C, D). This discrepancy may be attributed to two factors: first, LPS changes $[Ca^{2+}]_i$ by regulating the expression of genes encoding Ca^{2+} and Na^+/Ca^{2+} channels in microglial cells, cardiomyocytes, and lung microvessels (Choi et al. 2002; Beck et al. 2008; Wang et al. 2009; Kandasamy et al. 2013; Magi et al. 2015). However, sperm lack active transcriptional machinery so that LPS could not affect intracellular Ca^{2+} homeostasis by regulating gene expression. Second, LPS affects intracellular Ca^{2+} homeostasis by release of Ca^{2+} from endoplasmic reticulum (ER) Ca^{2+} stores (Kandasamy et al. 2013). However, the mature sperm lack ER. In fact, the unchanged $[Ca^{2+}]_i$ is consistent with observations that LPS has no effect on the $[Ca^{2+}]_i$-dependent functions, such as sperm capacitation and AR.

In conclusion, we examined the effects of LPS on human sperm function and conclude that *in vitro* exposure of human sperm to LPS inhibits motility by reducing intracellular cAMP. Our study represents a further significant advance in the understanding of the mechanism of reproductive toxicity of LPS and provides a theoretical basis for interpreting the pathogenesis of male infertility caused by bacterial infections.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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