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Effect of P/E-selectin blockage on antisperm antibody development and histopathological alterations in experimental orchitis

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Abstract

Aim: This study aimed to evaluate the effect of P/E-selectin blockage on antisperm antibody (ASA) development and histopathological alterations in experimental orchitis.

Materials and Methods: Thirty-six Wistar albino-type male rats weighing 100–150 g were included in the study. Rats were allocated into six groups ($n = 6$) including control (CG), sham (SG), orchitis (OG), antimicrobial treatment (AG), P/E-selectin blockage (PESG), and both antimicrobial and P/E-selectin treatment (TG) groups. In CG, serum samples were taken from the tail vein prior to the procedure and followed by extraction of both testes. In SG, 1 ml of saline solution was injected in testicular parenchyma. OG was obtained by injecting 0.1 ml 106 cfu/ml *Escherichia coli* (0:6 strain) and 1 ml saline solution into the right testes. AG received ciprofloxacin (50 mg/kg/day) twice a day through gastrogavage 24 hours after generating orchitis. In PESG, P/E-selectin antibody (100 μg) was administered intravenously via the tail vein 24 hours after the induction of orchitis. Finally, both ciprofloxacin and P/E-selectin antibody were administered in TG 24 hours after the induction of orchitis for 14 days. At the end of treatment, 1 ml of serum sample was obtained to evaluate the ASA, P-selectin and E-selectin levels. In order to evaluate spermatogenesis (Johnsen score) and testicular injury (Cosentino score), both testes were extracted at the end of the 14th day.

Results: In orchitis-induced groups (OG, ATG, PSEG, TG), ASA levels were significantly increased at the 14th day when compared to SG ($p < 0.05$). In TG, ASA levels were decreased when compared to AG. However, similar alteration in ASA levels was not detected in PSEG ($p > 0.05$). In OG and AG, P-selectin levels were decreased at the 14th day when compared to levels observed on 0 day ($p < 0.05$). E-selectin levels on 0 day showed that each group had higher levels of E-selectin when compared to CG

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($p > 0.05$). There was no significant difference regarding E-selectin when compared to CG ($p > 0.05$). No significant differences regarding E-selectin levels were detected on the 0th and 14th days between AG and CG ($p > 0.05$). When the Cosentino and Johnsen scores were compared among groups, TG and PSEG has decreased scores of Cosentino than OG on the right testicle ($p < 0.05$). In contrast, an increased Johnsen score was detected in TG and PSEG when compared to OG ($p < 0/05$). No significant difference was detected for both Cosentino and Johnsen scores on the left testicle ($p > 0.05$). There was no difference with regard to the right and left testicular injury in TG. In P/E-blocked groups, decreased histopathological alterations were observed in the contralateral testis.

Conclusion: P/E-selectin blockage may reduce ASA production after orchitis when combined with antimicrobial treatment. P/E-selectin blockage not only has a protective effect on blood–testis barrier but also decreases the histopathological alterations in both the affected and contralateral testis. Histopathological parameters of spermatogenesis may also be prevented by P/E-selectin blockage in experimental orchitis.

Epididymo-orchitis is one of the most frequently encountered pathologies leading to acute scrotum. Although is the most frequently isolated pathogen, mumps and other viral infections, and such noninfectious autoimmune disorders as vasculitis can be held responsible in the etiology of epididymo-orchitis [1].

The testes are quite well-protected organs in the human body, which are characterized by a barrier structure that neither allow any spermatozoal antigen to leak out nor allow any circulating immunoglobulins or immunologically active cells to breach in. This barrier is the blood–testis barrier comprising the cellular junctions both between the Sertoli cells resting upon the basal membrane in a testis and between the Sertoli cells and the neighboring germ cells [2]. Any event resulting in impairment of the blood–testis barrier, such as unilateral epididymo-orchitis, torsion, varicocele, testicular cancer or trauma, and testis biopsy, may lead to the generation of the antisperm antibodies [3]. It has been documented in the various studies that acute epididymo-orchitis gives way to the production of antisperm antibody, which ultimately harm not only the affected site but also the contralateral testis [3,4].

Selectins are carbohydrate ligands binding on the leukocyte and endothelial cells that play a major role in the process of inflammation. They also mediate leukocyte rolling and adherence to endothelium. Each selectin mediates different characters and speed during rolling. Since selectins and their ligands have different kinetics of expression, they function in different phases of inflammation process. [5,6]. P/E-selectins are known be one of the most important mediators of neutrophil infiltration in orchitis, thereby making a great contribution to the impairment in the blood–testis barrier [7–10]. We aimed hereby to demonstrate that testicular damage can practically be prevented though P/E-selectin blockage as an add-on therapy to the antimicrobial treatment directed to the eradication of the responsible pathogen in an orchitis model created experimentally in the light of all aforementioned knowledge.

1. Materials and methods

Thirty-six Wistar albino-type male rats, weighing 100–150 g, were included in the study. Rats were allocated into six subgroups, each containing six rats. The procedures were performed in a clean environment where local aseptic and antiseptic conditions were met. Anesthesia was achieved by intraperitoneal administration of 60 mg/kg ketamine HCI (Ketalar, Eczacıbaşı Lambert, İstanbul, Turkey) prior to the procedures. In the control group (CG), serum samples were taken from tail vein prior to the procedure and followed by extraction of both testes; in the sham group (SG), 1 ml of saline solution was injected in testicular parenchyma. Orchitis group (OG) was obtained by injecting 0.1 ml 10^6 cfu/ml *E. coli* (0:6 strain) and 1 ml saline solution into the right testis. In the antimicrobial therapy group (AG), ciprofloxacin (50 mg/kg/day, Bayer Schering Pharma, Bayer Türk Kimya San. Ltd. Şti., Istanbul, Turkey) was administered twice a day through gastrogavage 24 hours after generating orchitis by the aforementioned method. In the P/E-selectin blockage group (PESG), P/E-selectin antibody (100 μg mixture, BD Pharmingen TM Purified Rat Anti-MouseCD62P, BD Biosciences, USA; anti-U1 snRNP A/B, Acris GmbH, Germany) was administered intravenously 24 hours after the induction of orchitis. Tail vein was used for intravenous route. Finally, both ciprofloxacin (50 mg/kg/day, through gastrogavage) and P/E-selectin antibody (100 μg mixture, iv) were administered to the treatment group (TG) 24 hours after the induction of orchitis. Treatment (antibiotic and P/E-selectin antibody) was continued for 14 days in the groups. At the end of treatment 1 ml of serum sample was obtained to evaluate the antisperm antibody (ASA), P-selectin and E-selectin levels. In order to evaluate spermatogenesis and testicular injury, both testes were extracted at the end of the 14th day.

1.1. Biochemical investigation method

Spectrophotometric records using the ELISA method were obtained from the serum samples on the 0th and 14th