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# Effects of platelet-rich plasma against experimental ischemia/reperfusion injury in rat testis

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## Summary

### Background
Testicular torsion is a common problem and, to date, there is no agent to preserve testicular function following detorsion. Platelet-rich plasma (PRP), with its rich growth factor composition, has proven beneficial in regenerative therapy. It is believed that PRP has not been studied in testis for ischemia/reperfusion (I/R) injury.

### Objective
This study investigated the effect of PRP in an I/R rat model 1 month after detorsion.

### Study design
Of 24 adult male Sprague–Dawley rats, 18 were randomly assigned into three groups, with six in each: control, I/R and I/R + PRP. The PRP was prepared from the remaining six. Each group underwent right orchiectomy. Ischemia was performed by rotating the left testis 720° and fixing with a nylon suture for 4 h. Reperfusion occurred 4 h later by removing the suture, and PRP was administered at a dose of 10 μl ($2000 \times 10^9$/l) into the left testis via the intraparenchymal route. Animals were sacrificed at the fourth week, and testes were taken for malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), myeloperoxidase (MPO), transforming growth factor β (TGF-β), and caspase-3 measurements.

### Results
Ischemia/reperfusion caused a significant increase in MDA, MPO and caspase-3 activity, and significant decrease in GSH levels and SOD activity. The PRP treatment helped correct the alterations in SOD, caspase-3, and MPO activities and MDA levels. However, the mean MDA level and MPO activity were not totally restored compared with the controls. Serum testosterone levels of the I/R group were significantly lower compared with the control and I/R + PRP groups. TGF-β and caspase-3 protein expressions were significantly higher in the I/R group compared with the control group and were low with PRP administration compared with I/R groups (summary Table).

### Discussion
The findings of the present study suggest that PRP, by inhibiting neutrophil infiltration and oxidative stress and increasing antioxidant defense, exerts protective effects on testicular tissues against I/R. This study had some limitations: a scoring system was not used in the assessment of spermatogenesis in the histopathological findings and specific testis cell types were not histologically assessed.

### Conclusions
In light of the biochemical, histological and, especially, hormonal findings, intraparenchymal PRP injection may have a protective effect in testicular tissue against I/R injury.

**Summary table** Measurements in the three groups.

| | I/R compared with control group | | I/R + PRP compared with control group | | I/R + PRP compared with I/R group | |
|---|---|---|---|---|---|---|
| MPO (U/mg protein) | ↑ | *** | ↑ | * | ↓ | ++ |
| Caspase-3 activity (nmol pNA/min/mg protein) | ↑ | *** | ↔ | | ↓ | +++ |
| SOD (U/mg protein) | ↓ | ** | ↔ | | ↑ | ++ |
| MDA (mmol/mg protein) | ↑ | *** | ↑ | *** | ↓ | ++ |
| GSH (nmol/mg protein) | ↓ | ** | ↔ | | ↔ | |
| Testosterone (nmol/l) | ↓ | *** | ↔ | | ↑ | ++ |
| FSH (nmol/l) | ↑ | *** | ↑ | * | ↔ | |
| LH (nmol/l) | ↑ | ** | ↔ | | ↓ | ++ |

Symbols that represent statistically significant difference compared with control group:
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
Symbols that represent statistically significant difference compared with I/R group:
$^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$.
↑ symbol: statistically significant increase.
↓ symbol: statistically significant decrease.
↔ symbol: no significant difference is present ($P > 0.05$).
MPO, myeloperoxidase; SOD, superoxide dismutase; MDA, malondialdehyd; GSH, gluthatione; FSH, follicle stimulating hormone; LH, luteinizing hormone; I/R, ischemia/reperfusion; PRP, platelet-rich plasma.

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## Introduction

Testicular torsion is a urologic emergency that can lead to testicular dysfunction and fertility loss due to ischemia. The annual incidence of testicular torsion is reported as 3.8 in 100,000 by the age of 18 years [1]. The rate of orchiectomy in epidemiological studies has been reported as 41.9% in boys undergoing surgery for testicular torsion, which has not changed significantly since 2000 [1]. Long-term follow-up is warranted to assess the true incidence of subsequent atrophy after acute testicular torsion. Sessions et al. reported the incidence of testicular atrophy as 27% over 20 years [2]. As a similar degree and duration of torsion can result in testicular infarction or salvage, a third component must contribute to severity of vascular compromise at any given degree of torsion [2]. While degree of rotation has been implicated in clinical outcomes, time delay is the most important factor in predicting outcomes after appropriate critical care [3—5].

Detorsion is critical for providing normal blood flow to the testis. Interestingly, detorsion of a testis in a short time is not always helpful and may still result in problems; this is due to reperfusion injury [6]. Although detorsion is necessary to return blood flow, reperfusion to the ischemic tissues may exaggerate the injury. Therefore, several studies have examined ischemia/reperfusion injury and efficiency of treatment with potential therapeutic agents in torsion-detorsion with free radical scavengers (edaravone), antioxidant drugs (vitamin E, raxofelast, taurine, alpha-lipoic acid, simvastatin, resveratrol, edaravone, melatonin, and apocynin), anti-inflammatory drugs (melatonin, apocynin, taurine, resveratrol, simvastatin, ebselen, alpha-lipoic acid, vitamin E, ascorbic acid), nonsteroidal anti-inflammatory drugs (NSAIDS) (like ibuprofen, dexketoprofen), nitric oxide (NO) donors (L-arginine), neutrophil elastase inhibitors (sivelestat), phosphodiesterase type 5 (PDE5) inhibitors (sildenafil, tadalafil), and glibenclamide [7]. It is believed that, to date, no clinical study of therapeutic agents for the treatment of testicular torsion in humans has been performed. The pharmaceutical approaches to diminish I/R-induced oxidative stress suggest several potential pharmaceutical agents. Ideally, the administration of these agents just before intervention and/or immediately after surgery could result in better control of the reperfusion insult to both testes. Unfortunately, studies provide different time intervals for both detorsion and treatment [7]. A proper agent after surgical or manual detorsion of testicular torsion is still needed to restore normal testicular functions.

Platelet-rich plasma (PRP) is a unique autologous agent derived from the human and animal blood that is rich in growth factors, cytokines, and hormones; it has been reported to reduce oxidative stress and reactive oxygen species generation and upregulate the expression of various antioxidant enzymes [8]. Platelet-rich plasma is used in a variety of clinical applications in cell therapy, but it is believed that, to date, it has not been studied in testicular torsion.

Platelet-rich plasma treatment has been found to be beneficial in in-vivo ischemia/reperfusion organ models of kidney, heart, and limb muscle [9—12]. These studies evaluated PRP in ischemia/reperfusion organ models because growth factors like epidermal growth factor (EGF), insulin-like growth factor (IGF), transforming growth factor beta 1 (TGF-β1), and vascular endothelial growth factor (VEGF) are generally released during ischemia/reperfusion injury for various protective purposes.

Several studies have evaluated I/R injury in rat testis but almost all of them have evaluated hormonal changes in animals or humans with normal testes, confounding interpretation of hormonal status. The present study evaluated the protective effects of PRP against I/R injury in solitary rat testis.

## Materials and methods

The resource equation method was used to determine the number of rats for the present study. This method revealed that each group was to have an animal number between five and seven [13]. Animal research ethics suggests that an investigator reduce the number of animals as much as possible. It was therefore decided to use one animal more than the minimum per group (six) [14].

A total of 24 male Sprague—Dawley rats (8 weeks old, 180—200 g), supplied by Marmara University Animal Center (DEHAMER), were housed in an air-conditioned room with 12-h light—dark cycles; temperature (22 ± 2 °C) and relative humidity (65—70%) were kept constant. Marmara University School of Medicine Animal Care and Use Committee (no. 35.2015.mar) approved all experimental protocols.

### Experimental protocol

Rats were divided into three groups, with six in each: sham operated control (C) group, I/R group, and I/R + PRP group. The remaining six rats were used for the preparation of PRP. The PRP was administered at a dose of 10 μl ($2000 \times 10^9$/l) into the left testis upon detorsion.

To induce ischemia, rats were anesthetized with xylazine (20 mg/kg) and ketamine (50 mg/kg). A right subinguinal scrotal incision was made and right orchiectomy was performed in each group to prevent right testis contribution to hormone production, enabling unbiased hormone level measurement. Afterwards, through a subinguinal incision, the left testis was brought out and rotated 720° clockwise, reinserted, and fixed to the scrotal wall with a 4/0 nylon suture through the tunica albuginea and subcutaneous tissue. The incision was closed with a 4/0 nylon suture. After 4 h, using the same incision line, the testis was counter-rotated to its natural position and intratesticular PRP was injected into the left testis parenchyma. The testis was fixed to the scrotal wall with a 4/0 nylon suture to prevent spontaneous torsion and was left for 4 weeks to evaluate the long-term results of I/R injury. In the sham-operated control group, rats underwent similar surgical procedures without torsion and detorsion.

After the fourth week, the rats were decapitated. Blood was collected for hormone measurements. The testicular tissues were cut in half through the vertical axis in a standardized fashion; half was preserved for biochemical analyses and Western blotting, and the other half was sent