

Expression of FAS ligand in the ipsilateral and contralateral testicles of rats subjected to the torsion of the unilateral testicular cord¹

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ABSTRACT

PURPOSE: To investigate the expression of FAS ligand (FASL) in ipsilateral and contralateral testicles of rats submitted to ischemia/reperfusion.

METHODS: Wistar rats (n=21) distributed into groups control (GC), n=5, testicular exposure; ischemia (GI), (n=8), Torsion in the left testicular Cord (TCT) for three hours followed by orchietomy without distortion and orchietomy of the contralateral testicle after 24 hours; and reperfusion (GR), (n=8), left TCT for 3 hours and distortion and repositioning on the scrotum and bilateral orchietomy after 24 hours. Quantification of the FASL expression by immune-histochemistry.

RESULTS: Statistical analysis showed similarity between GC and GI ($p>0.05$), differences detected are concentrated on the GR ($p<0.05$), increase in immunoexpression of FASL in the subgroups Right GR (406.8+-61.5) and Left GR (135.3 +-28.9) with significant predominance in the GR subgroup.

CONCLUSION: Ischemia/reperfusion increased the FASL expression significantly in contralateral testicles in GR, in rats.

Key words: Fas Ligand Protein. Spermatic Cord Torsion. Immunohistochemistry. Ischemia. Reperfusion Injury. Oxidative Stress. Rats.

Introduction

Testicular Cord Torsion (TCT) results from the inadequate rotation of the testicle around its longitudinal axis. It is a pathological condition that makes the testicles ischemic, and surgical intervention is usually necessary to re-establish blood flow¹.

The treatment consists of surgical distortion and bilateral orchidopexia by transscrotal access, with orchiotomy indicated only for cases that clearly unviable that did not reperfund after surgical distortion, or with necrosis².

In histological terms, the torsion seems to induce in the contralateral testicle a focal lesion in the seminiferous tubules, characterized by apoptosis and shedding of germinative cells, possibly mediated by cellular and humoral immune response³.

The contralateral testicular lesion from the torsion of the testicular cord is controversial. Some authors show that the unilateral testicular torsion causes contralateral testicular lesion⁴, while others show that there is no contralateral lesion⁵⁻⁷.

Several theories have been proposed in the attempt to explain the possible damage to the contralateral testicle, including autoimmune reaction, subclinical episodes of contralateral testicular torsion, release of acrosomic enzymes, vasomotor reflex, neuroendocrine response, subjacent defect in the spermatogenesis and intrinsic gonadal abnormality^{8,9}.

The FAS ligand is a glucoprotein isoalted in 1994¹⁰. The FAS ligand protein (FASL) is an extrinsic activator of apoptosis that can be quantified by immuno-histochemistry. To determine the behavior of FASL, in experimental model, facing oxidative stress is important in the comprehension of the activation pathway of the apoptosis in the testicles submitted to the ischemia / reperfusion phenomenon.

In the testicle, the FAS system plays an important role in the apoptosis of germinative cells, after ischemia / reperfusion, stress and exposure of the testicle to toxic agents^{11,12}.

In a study involving FAS and FAS ligand in the apoptosis of testicular germinative cells by zearalenone (ZEA), in rats, the authors concluded that the acute exposure of a non-steroidal estrogenic mycotoxin (ZEA) induced the apoptosis in the germinative cells of male rats. This toxicity is partially mediated through the modulation of the FAS and FASL system. However, while a maximum number of apoptotic cells and a peak in FAS expression were seen at 12 hours, the levels in FASL expression increased in a time dependant form¹³.

In a study to quantify the FASL expression, using azoxymethane which induces oxidative stress in the gastric

mucosa of Wistar rats, the FASL protein was analyzed using the immuno-histochemical method, showing increase in its expression three hours after azoxymethane application¹⁴.

To determine the behavior of the FASL facing the oxidative stress in an experimental model is important in the comprehension of the activation pathway of the apoptosis, and it could explain what occurs in the contralateral testicle during TCT. The present study had the objective of evaluating the immuno-histochemical expression of the apoptosis activating protein, FASL in ipsilateral and contralateral testicles of rats submitted to the ischemia/reperfusion phenomenon, by torsion of the unilateral testicular cord.

Methods

The research project was approved by the Ethics Committee in animal use of the Federal University of Mato Grosso do Sul (UFMS), under protocol number 317/2011.

A total of 21 rats (*Rattus norvegicus albinus*), male, of the Wistar lineage, having mean weight of 280 ± 20 grams, 70 days old, from the Animal Colony, Mato Grosso do Sul Federal University. The animals were kept in the experiment bioterium for seven days prior the procedures. Randomly, the animals were distributed into three groups: control (GC), n=5, only testicular exposure; ischemia (GI), (n=8), torsion of the left testicular cord at 720° for three hours followed by immediate orchietomy of this testicle without distortion and orchietomy of the contralateral testicle after 24 hours; and reperfusion (GR), (n=8), torsion of the left testicular cord at 720° for three hours followed by distortion and repositioning of the testicle in the scrotum and bilateral orchietomy after 24 hours, subdividing each group into two subgroups: E (left-ipsilateral) and D (right-contralateral).

Immunohistochemistry

The immuno-histochemical processing (*avidin-biotin complex*, ABC technique) was made using the anti-FAS ligand primary antibody (termination epitope – N20, Santa Cruz®, product SC834, lot A1812, dilution 1:100), secondary antibody Dako LSAB® (product K0609, lot 10055833) and revealer of color DAB (diamino + benzidine) Dako® (product K3468, lot 10029914, dilution 1:10), with immunopositivity of the anti-FAS ligand biomarker expression, in brown coloring.

For the immune-histochemical quantification, 40 seminiferous tubules were selected, previously marked with software to avoid double counting. We counted the Sertoli

cells with a 40x lens, identifying those with strongly positive immunoexpression for FAS ligand.

Statistical analysis

The software GraphPad Prism 5.0 was used for the statistical analyses. The sample distribution of the data was determined by the Kolomogorov-Smirnov normality test. The multiple comparisons were made using one-way ANOVA followed by the bicaudal “t” Student post-test for the comparison of the left and right sides of each experimental group and Tukey post-test for the differences among the groups.

All of the statistical tests were made at a significance level of 5%.

Results

The results obtained are presented in Table 1.

TABLE 1 – Mean count of Sertoli cells that expressed FAS ligand in the control, ischemia and reperfusion groups – 2012.

Groups	Testicles		p
	Ipsilateral	Contralateral	
Control	60.2 ± 21.4 ^a	63.8 ± 34.6 ^c	0.880
Ischemia	88.8 ± 33.5	68.8 ± 35.3	0.305
Reperfusion	135.3 ± 28.9 ^b	406.8 ± 61.5 ^d	<0.0001*

p related to the comparison of the FASL expression between the ipsilateral and contralateral testicles

a p>0.05 in relation to the ischemia group

b p<0.05 in relation to the control and ischemia groups

c p>0.05 in relation to the ischemia group

d p<0.0001 in relation to the control and ischemia groups

The control group presented statistical equality (p=0.880) among the count in the left (60.2 ± 21.4) and right (63.8 ± 34.6) sides. The same was seen for the group which the tissue ischemia was made (p=0.305), with the left and right sides statistically similar (88.8 ± 33.5 and 68.8 ± 35.3, respectively).

In the reperfusion group, however, there was significant difference between the sides (p<0.0001), with a lower number of Sertoli cells expressing the FAS ligand on the left side (135.3 ± 28.9) than the right (406.8 ± 61.5) (Figure 1).

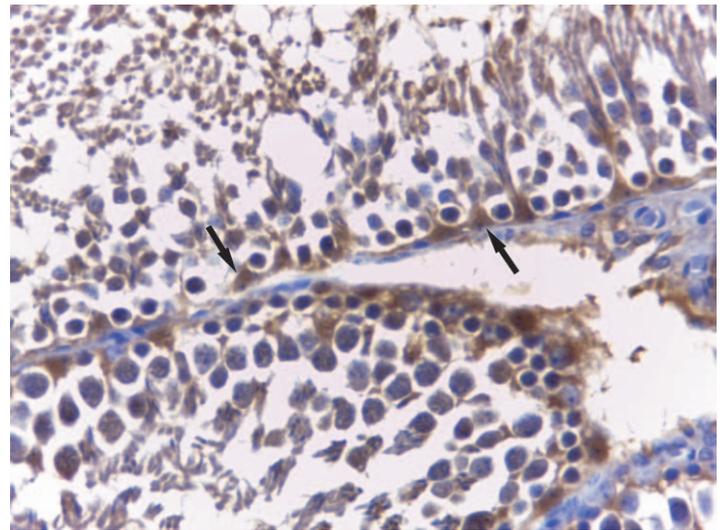


FIGURE 1 – Photomicrography of seminiferous tubules, x400 increase, showing the Sertoli cells with strongly positive expression of FASL, in brown.

In the statistical analysis of the results from the ipsilateral testicles between the studied groups, we observed a presence of significant differences among the tested procedures (p=0.0008). The “control” and “ischemia” groups were similar (p>0.05), been that the differences detected were found in the “reperfusion” group (p<0.05) according to the representation in Figure 2.

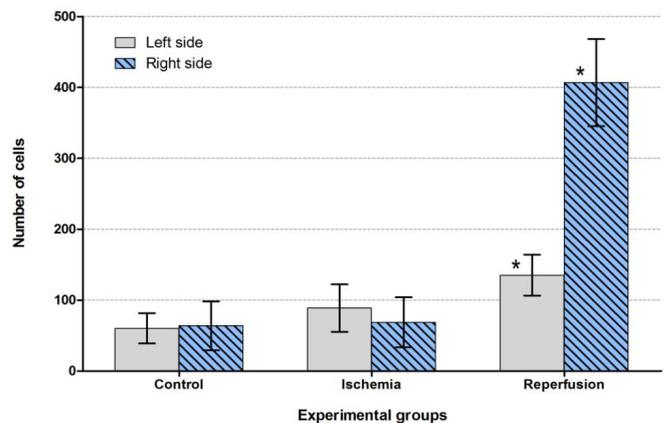


FIGURE 2 – Mean count of Sertoli cells that expressed FAS ligand, according to each side and experimental group.

In the contralateral testicles statistically significant differences were seen among the procedures (p<0.0001). Although, the “control” and “ischemia” groups were similar (p>0.05), and the differences detected were concentrated in the “reperfusion” group (p<0.0001).

Discussion

The topic of torsion of the testicular cord has been widely researched under several perspectives like the effect of ischemia on the testicular parenchyma, the phenomenon of ischemia/reperfusion, activation of apoptosis as potential influence in the extension of the tissue lesion, involvement of the FAS system in the induction of apoptosis in germinative cells, the damage on the contralateral testicle and the means to avoid or decrease the effects of the torsion. The diverse methodology in the studies has as consequence varied and diverging results¹⁵⁻¹⁸.

In relation to the degree of TCT that can lead to testicular lesion, there are studies that show after a torsion of 720°, there was residual blood flow, varying between 0%¹⁵, 8.8%¹⁹ and 38.3%²⁰ and reaching after distortion, statistically similar values to the pre-torsion blood flow, varying from 24 hours¹⁵, and after 30 days^{19,20}, this fact can try to explain the lesion in the contralateral testicle.

Most of the revised studies in literature that have studied the FASL expression in rats used toxic substances like zearalenone¹³ and azoxymethane¹⁴, differently from this study, where we studied the FASL expression through the induction of the ischemia/reperfusion phenomenon by torsion / distortion of the testicular cord in rats. We observed that the oxidative stress caused by toxic substances as well as the method used in this study induced the increase in FASL expression.

The FASL expression in the testicles of rats, by points in time (three, six, 12, 24 and 48 hours), after application of zearalenone, showed stable increase until 48 hours¹³. In another study with rats using clamping of testicular arteries and veins using vascular clamps for 60 minutes and reperfusion by points in time (zero, 12, 24 and 48 hours), Sertoli cells were identified positive for FASL in any point in time, with slight elevation at 24 hours of reperfusion¹⁷.

There expected was that there was a higher number of Sertoli cells expressing FASL, in the ipsilateral testicles of GR; however, this experimental study showed important increase in Sertoli cells expressing FASL in the contralateral testicles of GR, warning on the possibility of reperfusion along with the increase of FASL expression be the cause of damage in these testicles, further research is necessary to confirm and justify these results. Confirming these results in other studies, this could be another theory to explain the lesion on contralateral testicle.

Further research is needed to elucidate when is the right moment to intervene, keeping or not the testicle that suffered torsion in the cord, therefore protecting the spermatogenesis of the

patients, and thus their reproductive capacity.

Conclusion

The expression of FAS ligand increased in the ipsilateral and contralateral testicles of the reperfusion group, after three hours of ischemia and 24 hours of reperfusion, more significantly so in the contralateral testicles.

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