

Histopathologic Analysis of Contralateral Testes in Wistar Rats given Exogen Gluthathion after Testicular Torsion-Detorsion

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ABSTRACT

Introduction: Testicular torsion will lead an ischemic process, that will reduce germinal cells, Sertoli cells and eventually Leydig cells. The disfunctional Sertoli cells will destruct the testicular blood barrier and will also cause a derangement in contralateral testes blood supply and its cell structure histologically. Anti-oxidants has been used in some studies to prevent further destruction.

Objectives: To analyze the histopathologic changes in contralateral testes in Wistar rats performed testicular torsion detorsion with and without exogen gluthation as an anti-oxidant.

Material and Method: Fifteen Wistar albino rats, 2-4 months old, weighing 200 – 350 grams, were divided into 2 groups. Group I consists of 7 rats, performed testicular torsion detorsion only. Group II consists of 7 rats performed testicular torsion detorsion and given Tationil Gluthation 25 mg iv, 5 minutes prior to detorsion. Three hours after detorsion, orchidectomy of the contralateral testes were performed in all subjects, and sent for histopathologic examination. Grading of seminiferus tubules damage were evaluated using Cosentino classification and analyzed using Shapiro Wilk test and Mann Whitney test.

Results: Mean of Cosentino grade was 2.71 in group I and 1,57 in group II, with p value 0.01 (p <0.05), proving significant grade difference in both group.

Conclusion: Tationil Gluthation decrease the degree of destruction of seminiferus tubules in contralateral testes of Wistar rats underwent torsion detorsion.

Keywords: *Testicular torsion detorsion, tationil gluthation, histopathology of contralateral seminiferus tubules.*

INTRODUCTION

Testicular torsion is a situation where the funiculus spermaticus twists which causing occlusion and strangulation of blood vessels in the testis and on the epididymis¹. It can cause infertility in men². The ratio of testicular torsion incident is 1: 4000, with the greatest percentage in the age of puberty around 13-16 years. However, it can also found in a fetus in the uterus or a newborn baby could experience undiagnosed testicular torsion thereby the baby may lose unilateral or bilateral testes^{1,3}.

John et al., studied 771 childrens that experienced acute scrotum from age of neonates up to the age of 16 had found that 58% of the population samples have suffered torsion of the appendix testis and 29% of them

were having testicular torsion¹. Agil et al., reported that an overall number of incident of testicular torsion at the age of childhood until the age of consent under 20 in RSHS Bandung approximately 58%⁴.

The ipsilateral testicular torsion can cause depleted fertility, despite theoretically, fertility can still be maintained with only one testis. The influence of ipsilateral testicular torsion on the structure and function of contralateral testes is yet unknown. Existing research still shows controversy³. Several studies have found that testicular torsion affects fertility by influencing the contralateral testis. Linda et al. reported that in the ipsilateral testicular torsion there was damage to the seminiferous tubules of the testes histopathologically in both testes. Testicular torsion causes ischemia, which initially causes germinal reduction, then lowered

the sertoli cells, and subsequently the leydig cells. Furthermore, there will be an impaired sensitivity and responsibility of LH hormone stimulation, leading to failure of spermatogenesis and testicular atrophy^{5,6}

The effect of antisperm-antibodies on sertoli cells has not been widely studied. Hypothetically, other function of sertoli cells are associated with germ cells and immune system. Therefore, antisperm-antibodies are very likely attacking sertoli cells¹. The treatment of testicular torsion never changes. The present worldwide treatment protocol of testicular torsion is to explore as soon as the acute scrotum is suspected as testicular torsion⁷. In addition, testicular viability must be observed after detorsion. If it turns out that the testicles are still workable, then proceed with the orchidopexy by sewing the tunica albugenia and testicles with tunica dartos using non absorbable threads to prevent recurring torsion. If it turns out that the testicles are necrotic, then performed orchidectomy¹. The contralateral testis is always performed orchidopexy, because it is assumed that this contralateral testis has the same predisposing factor for torsion^{1,3,7,8}.

Mehmet Kanter et al., reported the provision of *Ginkgo biloba* (EGb 761) in a torsion-detorsion testis trial showing histopathological changes in the testes, which EGB 761 significantly reduced endothelial nitric oxide synthase (eNOS) activity in testicular ischemic reperfusion. It is proven to be effective in protecting mitochondrial degenerative and widening of intercellular spaces in sertoli and spermatid cells⁹. Correspondingly, the melatonin administration in torsion-detorsion testes in mice has shown to reduce the activity of TUNEL, increasing the expression of proliferating cell nuclear antigen (PCNA) and testosterone from testes tissue which suffered ischemic reperfusion¹⁰.

Dikmen et al., reported that the anti-inflammatory and anti-oxidant effects of Ibuprofen in the torsion-detorsion testes of rats could improve the histopathological features of seminiferous tubules. Ibuprofen has been shown to prevent lipid peroxidation, reduce the accumulation of Malondialdehyde (MDA) in the testes and eNOS immunoreactivity in seminiferous tubular germ cells, and increase testicular tissue¹¹.

Glutathione is a tripeptide (three proteins in one molecule) consists of cysteine, glutamine acid, and glycine. Glutathione is naturally present and produced in

the body system. Tationil® is an exogenous glutathione, one of the exogenous anti-oxidants that has the function of being a good anti-oxidant for the body, as a detoxification of xenobiotic substances in the liver, and it can also enhance the immune system^{12,13}. Tationil® can directly act as a scavenger of free radical and it can stabilize the membrane structure by eliminating or minimizing the formation of acyl peroxide in lipid peroxidation reactions^{13,14}. Bilommi et al., reported Tationil® as exogenous glutathione in wistar rats which were tested by torsion-detorsion testis, giving a lower MDA effect and better histopathological features of seminiferous tubules¹⁴. Rahman reports Glutathione (GSH) is a thiol tripeptide, as an intra and extra-cellular antioxidant protection in the lungs against levels of oxidative stress when an inflammatory process happened¹⁵.

Research purposes

The aim of this study was to determine the histopathological damage of contralateral seminiferous tubules in Wistar rats towards group of given exogenous glutathione and those without exogenous glutathione in testicular torsion-detorsion test.

Research methods

This study used an experimental design with a single-blind randomized test to determine differences in histopathological damage to seminiferous tubules in contralateral testicular tissue of samples given 25 mg iv exogenous glutathione (Tationil®) (conversion dose on wistar) and group of samples with no exogenous glutathione (Tationil®) 25 mg iv in torsion-detorsion experiments in Wistar rats^{16,17}. The sample inclusion criteria were: male mice age 2 to 4 months, the weight around 200 - 350 grams, wistar rats in healthy condition with the characteristics of well-built body, not thin, does not secrete mucus, pus, or blood from the eyes or ears, should not be too much saliva, not having diarrhea, and the respiratory system were good, it has thick slippery clean shining furry, and tame, the testicular anatomy looks normal. The sample exclusion criteria were ill / dead rats before receiving treatment. The sample drop-out criteria were missing rats, sick, or dead during treatment.

Data Analysis

Seminiferous tubule preparations were assessed histopathologically based on the degree of damage in

Cosentino classification:

Degree 1: Observation of the arrangement of germ cells which are irregularly arranged and the seminiferous tubules are more closely aligned

Degree 2: Observation of the arrangement of germ cells which are irregularly arranged and the seminiferous tubules are more closely aligned

Degree 3: Irregular germ cells appear with a shrinking picnotic nucleus and a less clear boundary in the seminiferous tubules

Degree 4: The seminiferous tubules looked pile up and there is coagulophyte necrosis in germ cells.

Statistical analysis using The *Shapiro-Wilk* test if the data distribution is normal and *Mann Whitney* test if it turns out that the data distribution is not normal. The conclusion for the statistical analysis test was based on the significance value of p, if the value of p < 0.05, the hypothesis is accepted. We used the SPSS program version 13 for Windows.

RESULTS

After conducting experiments at the Pharmacology Laboratory of Padjadjaran University, an analysis of histopathological examination was carried out at the Department of Pathology Anatomy, Padjadjaran University by an Anatomical Pathologist who did not know the treatment we gave to the testing animals. There were 2 experimental groups, each group consisted of 7 rats, and 1 rat as a control, so that the total number of animals was 15 rats.

In group I, there was a distribution of histopathological features of damage to the seminiferous tubules of the contralateral testes after the torsion-detorsion testes; we obtained as many as 3 samples (42.85%) are in degree 2; 3 samples (42.85%) are in degree 3; and 1 sample (14.28%) are in degree 4. Whereas in group II the distribution of histopathological features of seminiferous tubular damage to the contralateral testis after torsion-detorsion testis trial which was accompanied by the injection of exogenous Glutathione (Tationil®) 25 mg iv 5 minutes before detorsion, we obtained as much as 3 samples (42.85%) are in degree 1; and 4 samples (57.14%) are degree 2.

Table. 1 Result distribution of Histopathology degree of Seminiferus Tubules Contralateral Testes

	Group 1 Contralateral testis (torsio- detsorsi)	Group II Contralateral testis (torsi- detsorsi+Tationil ®25 mg)
Histopathological Overview of Semiferus Tubules According to the Cosentino Classification	2	2
	4	1
	2	2
	2	2
	3	2
	3	1
	3	1

Control: normal histopathology overview is degree 1.

Histopathological Results of Semiferus Tubular Damage

On the results of the histopathological damage of testicular preparation controls, there were normal histopathology in degree 1, from the two groups given experimental treatments, we found the heaviest degree of damage in degree 4 of group I as much as 1 sample (on the contralateral testes conducted by torsion-detsorsi testis without administration Tationil®).

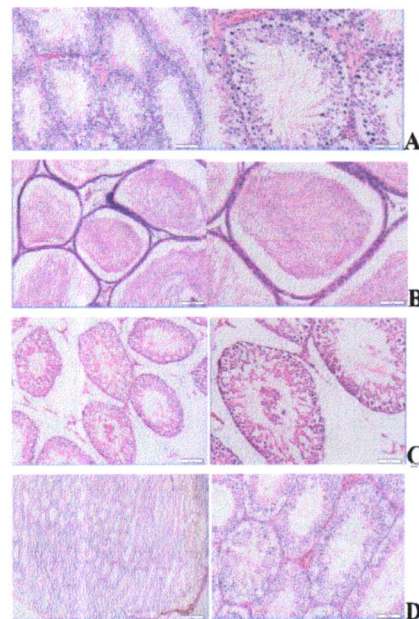


Figure 1. A. Control (Normal Histopatologic Testis) Degree 1; B. Group I (torsion-detorsion contralateral testis) Degree 4; C. Group I (torsion-detorsion contralateral testis) Degree 3; D. Group II (torsion-detorsion contralateral testis + Tationil glutathione) Degree 2

Whereas the lowest degree seen in group II consist of 3 samples of Wistar rats given an injection of exogenous Glutathione (Tationil®) at a dose of 25 mg iv as seen in degree 1 of histopathological figure, which is the normal degree equals to the degree of control. Degree 2 is the closest degree to the normal histopathological degree, which was also seen in this group consist of 4 samples.

Table 2. The Normality test of Group I and Group II Degree Data

Tests of Normality			
	Shapiro-Wilk		
	Statistic	df	Sig.
Kelompok I	.833	7	.086
Kelompok II	.664	7	.001

a. Lilliefors Significance Correction

The different result that we found from group of treatment 1 without exogenous Glutathione injection (Tationil®) were none of the samples showed indication of degree 1, and normal degree as in the control. Degree 2, which is the degree closest to the normal histopathological degrees, is still seen in this group, but the degree of severe damage that is 3-4 degrees is clearly seen in this group, which indicates that the figure of the histopathological damage degree in group I tends to be higher compared to group II.

Table 3. Differentiation Tests of Average Degrees in Groups I and Group II

Descriptive Statistics			
	N	Mean	Std. Deviation
Kelompok I	7	2.71	.756
Kelompok II	7	1.57	.535
Valid N (listwise)	7		

Test Statistics^a

	Derajat
Mann-Whitney U	6.000
Wilcoxon W	34.000
Z	-2.550
Asymp. Sig. (2-tailed)	.011
Exact Sig. [2*(1-tailed Sig.)]	.017 ^a

a. Not corrected for ties.

b. Grouping Variable: Group

The normality test is done using the *Shapiro-Wilk* method.

From the data analysis, we obtained the value of Asymp.Sig (2-tailed) for 0.086 for data degrees in Group I and for 0.001 for data degrees in Group II. Because there is one value that is smaller than alpha (Asymp. Sig. <0.05), which is data degree in Group II, so it can be concluded that the data degrees in Group II are not normally distributed.

The table above shows the average degree in Group I is 2.71 and the average degree in Group II is 1.57 with the p-value is 0.011. If the value compared with alpha, this value is smaller (0.011 < 0.05) which states that there are significant differences between the degrees in Group I and Group II.

DISCUSSION

In addition to the contralateral testes, the damage that occurs in sertoli cells could affect impairment to the blood testicular barrier system, which separates the germinal epithelium including spermatozoa with immune cells of the body. Proteins derived from the germinal and spermatozoa epithelium, enter the blood circulation and are known by immuno-competent as immunogens, which then triggers the immune response to produce antibodies to germinal and spermatozoa epithelial proteins, called antibodies towards sperm, thus damaging the contralateral testes. When the blood testis barrier is corrupted and the sperm enter the bloodstream, the immune system responds to the presence of sperm. The data of this study support this theoretically, where in the testes torsi-detortion causing damage to the contralateral testes. This can be seen from group I, where the histopathological figure on the contralateral testis does not show any normal degree (degree 1), and even shows an increase in the degree of histopathological damage to seminiferous tubules based on Cosentino classification which are degrees 2, 3 and 4.

In line with this study, according to Schanaider et al., the autoimmune mechanism of IgG in the basal membrane of the seminiferous tubules appears to be involved in contralateral testicular damage. From his research, immunofluorescence tests carried out by injecting blue nitro tetrazolium (NBT) in both testes identified the presence of oxygen free radicals¹⁹. Exogenous glutathione given in this study was Tationil® according to the human dose which was converted into a

dose of Wistar rats which was 25 mg. This drug provides antioxidant activity effects, the chemical reactions will eliminate lipid peroxide, thus preventing reperfusion injury.

Prillman and Turner also reported that oxygen radical scavengers combined with superoxide dismutase, catalase, allopurinol, and verapamil can improve testicular function after an acute torsion trial²⁰. According to Feza et al., from their biochemical research, it was found that therapy with Allopurinol before detection could prevent reperfusion injury. Greenstein et al., in his histopathology study also found a significant difference between the group which was given polyethylene glycol-superoxide dismutase and the control group²¹. Viguera et al., administered naloxone before testicular deterioration was significantly reduced the damage to the seminiferous tubules histopathologically, this is probably the effect of free radical protection, blockade of endorphins, and stabilization of membrane cells undergoing ischemia²². Deliktaş et al., also reported the testicular torsion-detorsion study, administration of L-Carnitine known as antiradical and antioxidant activity could prevent contralateral testicular damage associated with ischemia / reperfusion²³. Similarly, Ranade et al., showed an important role of vitamin E in reducing histological damage and reducing peeling of germ cells and also increasing spermatogenesis after induction of ischemia for three hours followed by one hour of reperfusion²⁴.

CONCLUSION

Based on the results of this study it can be concluded that the administration of Tationil® as exogenous glutathione in torsion-detorsion testes of Wistar rats, in the seminiferous tubules of the contralateral testes will give a better degree of histopathological features according to the Cosentino classification than those which was not given Tationil®.

Conflict of Interest : None

Ethical Clearance : From Institute Committee

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