Effects of Torsion, Detorsion and Melatonin on Testicular Malondialdehyde Level

F. I. O. Duru, C. C. Noronha, A. I. Akinwande, A. O. Okanlawon

ABSTRACT
BACKGROUND: Unilateral testicular torsion is a cause of bilateral testicular damage, which has ischaemic and reperfusion components. The damage may involve lipid peroxidation leading to production of lipid peroxides in the testes, including malondialdehyde (MDA).
OBJECTIVE: To investigate the MDA variations in the ipsilateral and contralateral testes following ischaemia-reperfusion and the effect of melatonin.
METHODS: Mature adult male Sprague-Dawley rats were divided into 13 groups of 10 each. One control group underwent sham operation. Three groups were subjected to right sided testicular torsion by twisting the testes 720° counterclockwise for one, three and five hours; three groups were subjected to de-torsion following torsion lasting one, three and five hours; three groups were treated with intra-peritoneal melatonin (1mg/kg) before torsion lasting one, three and five hours, and three groups were treated with intra-peritoneal melatonin before de-torsion following torsion lasting one, three and five hours. At the end of the experiment all animals were sacrificed by decapitation and testes were collected for MDA level estimation.
RESULTS: The MDA level was significantly higher in ipsilateral torted testis than the control testis in all groups (P<0.05), with the levels increasing with the duration of torsion. Detorsion significantly increased the MDA level only if the initial torsion was for less than three hours. Melatonin did not significantly affect the MDA level in the ipsilateral testis if administered before torsion, but significantly reduced the level if administered before detorsion.
CONCLUSION: Malondialdehyde levels are altered in the both testes following unilateral testicular torsion-detorsion injuries. The reperfusion component of the injury is significant and may be reduced by melatonin. WAJM 2007; 26(4): 312–315.

Keywords: Testes, Ischaemia, reperfusion, Melatonin, Malondialdehyde

RESUME
CONTEXTE: torsion testiculaire unilatérale est une cause de dommages testiculaires bilatéraux, qui a reperfusion ischémiques et composants. Les dommages peuvent impliquer peroxysydation lipitique conduisant à la production de peroxydes lipitiques dans les testicules, notamment malondialdehyde (MDA).
OBJECTIF: Etudier les variations de la MDA et controlatéralepsisicteral testicules suivants ischémie-reperfusion et de l’effet de la mélatonine.
MÉTHODES: Mature adulte mâle Sprague-Dawley ont été répartis en 13 groupes de 10 chacun. Un groupe de contrôle ont subi une simulacre opération. Trois groupes ont été soumis à la torsion du testicule droit unilatéral par torsion testiculaire 720° antihoraire pour un, trois et cinq heures, trois groupes ont été soumis à la suite de la torsion de torsion d’une durée d’un, trois et cinq heures, trois groupes ont été traités avec la mélatonine intra-péritonéale (1mg/kg) avant de torsion d’une durée d’un, trois et cinq heures, et les trois groupes ont été traités avec la mélatonine intra-péritonéale avant de torsion suivants torsion d’une durée d’un, trois et cinq heures. À la fin de l’expérience, tous les animaux ont été sacrifiés par décapsulation et les testicules ont été recueillies pour la MDA niveau estimation.
RÉSULTATS: La MDA niveau était nettement plus élevé dans les testicules ipsilateral tordu que le testicule contrôle dans tous les groupes (P<0.05), avec des niveaux de plus en plus avec la durée de la torsion. Detorsion considérablement augmenté la MDA que si le niveau initial de torsion a été pendant moins de trois heures. Mélatonine n’a pas d’influence significative sur le niveau de la MDA dans le testicule ipsilateral s’ils sont administrés avant la torsion, mais a sensiblement réduit le niveau s’ils sont administrés avant detorsion.
Mots-clés: testicule, Ischaemia, reperfusion, Mélatonine, Malondialdehyde.
INTRODUCTION

Torsion of the testis is an important acutely painful surgical emergency with significant long-term implications if not promptly identified and treated. The condition represents one of the most common ischaemia-reperfusion situations encountered in clinical practice. Unilateral testicular torsion is known to eventually cause damage to the contra-lateral testis and may lead to infertility. An interesting finding in testicular ischaemia-reperfusion is the fact that deleterious changes in the tormented testis is eventually mirrored by events in the contra-lateral non-tormented testis. This has been demonstrated hist-pathologically and with physical and biochemical markers. Tissue malondialdehyde levels correlate positively with damage and is widely used as a marker for lipid peroxidation. Despite the considerable volume of data available on ischaemia-reperfusion injury of the testes, there is a dearth of information regarding some aspects of this condition. For example, there is little data to show how the testicular levels of MDA in the tormented testis compare with the contra-lateral non-tormented testis and how MDA evolves if testis is re-perfused after a sub-lethal duration of ischaemia. Secondly it is not known whether the reperfusion injury has a role in the injury of the contra-lateral testis, and thirdly the effectiveness of an anti-oxidant and broad spectrum free-radical scavenger like melatonin in minimizing or preventing ipsi-lateral and contra-lateral damage following testicular torsion-detorsion especially if administered before detorsion.

We therefore used an experimental ischaemia-reperfusion rat testis model to study the malondialdehyde variations in the ipsilateral and contralateral testis and to evaluate the effectiveness of melatonin in reducing both ischaemic and reperfusion injuries.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats weighing 180-200g were divided into 13 groups, each made up of 10 rats. One group underwent sham operation and served as the control. Three groups were subjected to right sided testicular torsion by twisting the testes 720 degrees counterclockwise for 1, 3 and 5 hours; 3 groups were subjected to de-torsion following torsion lasting 1, 3 and 5 hours; 2 groups were treated with intra-peritoneal melatonin (1mg/kg) before torsion lasting 1, 3, and 5 hours, and 3 groups were treated with intra-peritoneal melatonin (1mg/kg) before de-torsion following torsion lasting 1, 3 and 5 hours. At the end of the experiment all animals were sacrificed by decapitation and ipsi-lateral and contra-lateral testes were collected for malondialdehyde level determination and testicular histology.

Determination of Testicular Malondialdehyde

Testicular malondialdehyde (MDA) levels were determined using the modified thiobarbituric acid method of Bueno and Aust (1978). MDA reacts with thiobarbituric acid to give a red compound absorbing at 535nm. The stock reagent contains 2mM 15%w/v trichloroacetic acid, 0.375%w/v thiobarbituric acid and 0.25 mol/L hydrochloric acid. A 0.5g testicular tissue sample was homogenized in 5ml of 0.15M KCl and the homogenate centrifuged at 1000g for 10minutes in a Uniscrope laboratory centrifuge and the supernatant collected. An aliquot of 2ml of the stock reagent was added to 1ml of testicular homogenate supernatant and mixed thoroughly and placed in an Ectiron water bath (80-90°C) for 15minutes. It was then cooled and the flocculent precipitate removed by centrifugation at 1000g for 10minutes and the absorbance of the supernatant determined with a Spectronic spectrophotometer at 535nm against blank containing all the reagents. Concentration of malondialdehyde was calculated using the molar absorptivity coefficient of malondialdehyde which is 1.56x10⁵M⁻¹cm⁻¹.

Statistical analysis

Data are expressed as mean ± SD. Differences between groups were assessed using t-test with p<0.05 considered significant.

RESULTS

MDA levels with ipsilateral torsion alone. There was no significant difference between the MDA levels in the ipsilateral and contralateral testes of the control rats. The MDA level was higher in ipsilateral tormented testis than the control testis in all groups with the levels increasing with the duration of torsion. Also, the MDA levels in the ipsilateral tormented testes were significantly higher than the levels in the contra-lateral non-tormented testis in all groups. MDA levels began to increase significantly in the contralateral testis following more than three hours of torsion (Table 1).

Table 1: Malondialdehyde levels in ipsi-lateral tormented and contra-lateral non-tormented testis

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA, Mean (SD) μmol/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right testis</td>
</tr>
<tr>
<td>A</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>B</td>
<td>0.46 ± 0.04</td>
</tr>
<tr>
<td>C</td>
<td>1.28 ± 0.32*</td>
</tr>
<tr>
<td>D</td>
<td>3.10 ± 0.64*</td>
</tr>
</tbody>
</table>

*A, B, C, and D = Control right testicular torsion for one, three, and five hours respectively. p<0.05.

Table 2: Malondialdehyde in ipsi-lateral and contra-lateral testis following torsion-de-torsion.

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA, Mean (SD) μmol/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right testis</td>
</tr>
<tr>
<td>A</td>
<td>0.38 ± 0.01</td>
</tr>
<tr>
<td>E</td>
<td>0.89 ± 0.22*</td>
</tr>
<tr>
<td>F</td>
<td>2.06 ± 0.12*</td>
</tr>
<tr>
<td>G</td>
<td>3.26 ± 1.01*</td>
</tr>
</tbody>
</table>

*E, F, and G = Control, detorsion, following testicular torsion for one, three, and five hours respectively, followed by detorsion for 90 minutes. p<0.05

MDA levels following torsion-detorsion. There was significantly higher MDA levels in the testes tormented for one and three hours (groups E and F) but not in that tormented for five hours (group G) (Table 2) compared to their corresponding groups in Table 1. There was no significant difference between the MDA level in the contra-lateral testis in all groups (Table 2) and their corresponding groups in Table 1.

MDA levels with melatonin administered before torsion. There was no significant difference between MDA
levels in the perted testis of groups H, I and J (Table 3), compared to their corresponding testis in groups B, C and 
D (Table 1). There was a significant decrease in the MDA levels in the centralateral testis in all groups (Table 3) 
compared to their corresponding groups in Table 1.

Table 3: Malondialdehyde in ipsi-lateral and contra-lateral testis with melatonin administered before 
torsion

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA, Mean (SD) µmol/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right testis</td>
</tr>
<tr>
<td>A</td>
<td>0.38±0.01</td>
</tr>
<tr>
<td>B</td>
<td>0.48±0.14</td>
</tr>
<tr>
<td>I</td>
<td>1.04±0.30</td>
</tr>
<tr>
<td>J</td>
<td>3.14±0.62</td>
</tr>
</tbody>
</table>

*A, H, I, and J = Control; C. i.p. melatonin (1mg/kg) before torsion lasting one, three and 
five hours. *p<0.05

Table 4: Malondialdehyde in ipsi-lateral and contra-lateral testis with melatonin administered 
befo re de-torsion

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA, Mean (SD) µmol/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right testis</td>
</tr>
<tr>
<td>A</td>
<td>0.38±0.01</td>
</tr>
<tr>
<td>K</td>
<td>0.50±0.14</td>
</tr>
<tr>
<td>L</td>
<td>1.40±0.04</td>
</tr>
<tr>
<td>M</td>
<td>3.20±0.62</td>
</tr>
</tbody>
</table>

*A, K, L, and M = Control; - torsion for one, three and five hours respectively, followed by 
intraperitoneal melatonin 1mg/kg before de-torsion lasting 90 minutes. *p<0.05

MDA levels with melatonin administered before de-torsion. There was a 
significant decrease in MDA levels in the 
torted testis in groups K and L (Table 4) 
but not in group M compared to their corresponding groups in Table 2. There was no 
significant difference between the MDA levels in the centralateral testis in 
groups K, I, M (Table 4), compared to their corresponding groups in Table 2.

Histology of the testis following 720° 
counter clockwise torsion lasting one, 
three and five hours.

Transverse section of testes of intact rat showed normal interstitium, 
seminiferous tubules, and well differentiated seminiferous epithelium.

After 1 hour of 720° counterclockwise tor- 
sion, oedema of the interstitial tissues and 
disruption of the seminiferous tubule was 
evident. After 1 hour of 720° counter-clock-
wise torsion, followed by de-torsion for 
90 minutes, there was further disruption of 
the testicular architecture with evidence of 
destruction of the seminiferous tubular 
epithelium. After 3 hours of 720° count-

clockwise torsion, there was severe 
oedema of the seminiferous tubules. Af-

er 3 hours of 720° counterclockwise tor-
sion, followed by de-torsion for 90 min-
utes, oedema was reduced but there was 
more disruption of the seminiferous tu-

bular structure. After 5 hours of 720° counterclockwise, there was severe necrosis 
of the seminiferous tubules.

DISCUSSION

Melatonin is a free radical scavenger that 
directly neutralizes a number of free 
radiicals and reactive oxygen and nitrogen 
species, and stimulates antioxidant 
enzymes. It has therefore been used as 
a protective agent against a wide variety of 
processes and agents that damage 
tissues via free radical mechanisms.

This study demonstrates the adverse 
effect of testicular torsion and de-torsion 
on ipsilateral and contralateral testicular lipid peroxidation, and its amelioration by 
melatonin administration. These 
damaging effects have in previous reports 
been attributed to reduced blood flow, increased generation of reactive oxygen 
species, DNA damage, germ-cell specific apoptosis and increased adhesion of neutrophils to testicular 
subtunical vessels. In this study, there 
was contradicting reports about the role of lipid peroxidation in torsion-de torsion 
injuries, with some authors supporting a role for free radicals and others denying such a role. The present study supports a role for free radicals in testicular injury following ischaemia-reperfusion. The lipid peroxidation in the torted testis increased with the duration of ischaemia with a more gradual increase in the 
contralateral testis. In our model, 
detorsion caused a greater increase in 
testicular MDA in the ipsilateral testis, but 
only when duration of torsion was not 
more than three hours.

Reperfusion is a benefical process overall, 
following relatively brief ischaemia or hypoxia, as much of the 
damaged tissue can be salvaged by 
re-perfusing with blood and re-introducing oxygen and nutrients. Our data supports 
the notion that re-perfusion contributes significantly to the testicular injury 
following ischaemia-reperfusion. This can be attributed to the effect of free radicals, and 
increased migration of neutrophils to the 
previously ischaemic tissue. However 
in the present study, the role of 
reperfusion seems to become relatively 
insignificant after three hours of complete 
ischaemia. This is probably because the 
testis becomes infarcted and therefore 
can no longer be damaged further as it is 
not possible to further damage a dead tissue. 
The duration of ischaemia from which the 
testis is able to recover depends on the 
extent of the arterial twist. The more the 
twist, the less the duration from which 
the testis is able to recover.

For arterial occlusion to occur there 
must be multiple twisting of the spermatic 
cord, whereas arteriolar stasis develops 
secondarily to venous occlusion with fewer twists. Experimentally, complete 
cessation of arterial inflow occurs at 305-
540 degrees of torsion and 3-4 complete 
turns (1080-1440 degrees) produces 
irreversible damage after 2 hours. Torsion of 90 degrees for periods as long as 7 days 
fail to cause necrosis in 50%, and 260 
degrees causes necrosis in all cases 
within 24 hours.

In this study, intra-peritoneal 
melatonin administered before torsion was unable to significantly reduce the 
lipid peroxidation in the ipsilateral testis, 
but significantly reduced the level in the 
contra-lateral testis toward the control value. Torsion of the ipsilateral testicular artery 
dramatically reduces the melatonin 
available to the torted testis and therefore 
denies it of any beneficial effects, but by 
reducing the lipid peroxidation in the 
contra-lateral testis, suggests that free 
radiicals play a role in the contra-lateral 
testicular injury resulting from ipsilateral 
testicular torsion.

However, melatonin significantly 
reduced the lipid peroxidation resulting 
from the reperfusion of the ipsilateral 
testis. Reperfusion injury has been 
attributed to a number of factors,
particularly polymorphonuclear leucocyte accumulation in the reperfused tissue as evidenced by increased myeloperoxidase activity. The leucocytes are attracted by various chemoattractants released into the ischaemic tissue and accumulate as a result of ischaemia induced up-regulation of endothelial adhesion molecules. They soon degranulate releasing various chemical agents including free radicals into the tissue causing further damage. The administration of melatonin in this study was able to reduce the damage due to torsion followed by de-torsion apparently by mopping up free radical released from the invading leucocytes, but interestingly in a previous report, melatonin was also able to reduce leucocyte recruitment into the reperfused tissue. Melatonin is particularly suitable in this regard because of its ability to penetrate all morpho-physiological barriers including blood-testis barrier, and enters all parts of every cell where it prevents oxidative damage and preserves mitochondrial function. This is of critical importance to reproductive function because it has been shown that even the early stages of spermatogenesis are sensitive to a moderate, acute reduction in testicular blood flow.

We conclude that lipid peroxidation plays an important role in ischaemia-reperfusion injuries of the testis and that the reperfusion injury is significant and sensitive to melatonin which if administered before reperfusion, can significantly decrease the reperfusion injury.

REFERENCES