

Effects of Melatonin on Noncardiogenic Pulmonary Edema Secondary to Adnexial Ischemia-Reperfusion in Guinea Pig

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Abstract

OBJECTIVES: Pulmonary edema has been shown to occur following ischemia-reperfusion injury in a variety of organs and effects of several pharmacological agents on ischemia-reperfusion-induced damage has been investigated previously. However, there are only a few studies in the literature about pulmonary injury following adnexial ischemia-reperfusion. In this study we aimed at investigating pulmonary changes following adnexial ischemia-reperfusion and the effects of melatonin on noncardiogenic pulmonary edema secondary to adnexial ischemia-reperfusion.

METHODS: A total of 32 Dunkin-Harley guinea pigs were randomly divided into four groups. In group I, oophorectomy was performed following adnexial torsion of three hours (ischemia). Adnexial torsion of three hours followed by a 3 hours detorsion period (reperfusion) and then oophorectomy was performed in remaining three groups. No therapy was given in group one and two while isotonic saline and melatonin was applied intraperitoneally in groups three and four, respectively. Serum malondialdehyde (MDA) levels of operated ovaries and lungs and polimorphonuclear leucocyte infiltration of lungs were determined.

RESULTS: MDA levels in serum, ovary and lungs were higher in detorsion groups (groups II, III) than torsion group (group I) ($p < 0.01$). Melatonin administration significantly decreased the polimorphonuclear leukocytes infiltration of lung parenchime and MDA levels in serum, ovaries and lungs (groups II and IV; groups III and IV; $p < 0.01$, $p < 0.01$). MDA levels and lung tissue PNL infiltration levels of melatonin administered detorsioned group was similar to those levels of only salphingo-oophorectomy performed group (Group I and IV, $p > 0.05$).

CONCLUSION: Pathophysiology of ischemia-reperfusion must be considered in the cases of adnexial torsion where detorsion is thought. As an antioxidant, melatonin administration might be helpfull in decreasing post-operative morbidity by decreasing reperfusion injury of lungs.

Abbreviations & units

g	gram,
mg	milli gram,
kg	kilo gram,
°C	centigrade degrees,
EDTA	Ethylenediaminetetraacetic acide,
MDA	Malondialdehyde ,
Nm	nano meter,
Nmol	nano mol,
PNL	polimorphonuclear leucocyte,
h	hour,
I	ischemia,
R	reperfusion,
M	Melatonin,
PS	physiological saline

Introduction

Reperfusion injury develops as a result of reperfusion of tissues following a certain period of ischemia [1]. Free radicals are released into systemic circulation which gives harm to the other parts of body as well as the reperfused tissue. Lungs may also be affected. Non-cardiogenic pulmonary edema was shown to occur following ischemia-reperfusion of gut and extremities [2-6]. Free radical scavengers had been proposed to lessen pulmonary injury secondary to ischemia-reperfusion of tissues [3].

Adnexial torsion is a serious gynecologic problem where conservative management include detorsion of the involved segments. Detorsion of torsioned ovaries was shown to cause reperfusion injury histopathologically in our previous study performed in rat [7].

In this study, effect of an antioxidant, melatonin (N-Acetyl-5-methoxytryptamine) [8, 9] on pulmonary injury secondary to adnexial ischemia-reperfusion was investigated.

Material methods

The protocol of this study was approved by the Local Ethics Committee. Total of 32 virgin Dunkin Hartley guinea pigs (obtained from Elazig Veterinary Research Institute) weighing 350–400 g were used in this study. They had regular oestrus cycles monitored by vaginal smearing. Animals were kept under 12 hours light- 12 hours dark, 23±2°C and 55±15% humid conditions and unrestricted access to food.

Animals were anesthetized by intramuscular administration of ketamine-hydrochloride (Ketalar, Eczacibasi, Istanbul, Turkey) 50 mg/kg plus xylazine-hydrochloride (Rompun, Bayer, Istanbul, Turkey) 5 mg/kg.

In prone position, after shaving and appropriate cleaning, skin and muscular layers were incised one cm lateral to midline to explore right adnexes of the animals. Right adnexes were rotated by 360–720° including tuba and ovarian ves-

sels and fixed to abdominal muscles by 5/0 silk (Ethicon, UK). Skin was sutured with 5/0 silk. Three hours later, animals in all groups were re-anaesthetized and abdomens were opened from previous incision sites. Adnexes were excised in group 1 at the end of three hours. Detorsion was performed and reperfusion was allowed for additional three hours in remaining three groups. Animals in groups 1 and 2 received no treatment. Isotonic saline and melatonin 30 mg/kg was given intraperitoneally to the group 3 and 4, respectively.

All groups were reanesthetised at the end of ischemia period in group I and reperfusion was performed in groups 2, 3, and 4 for 3 hours. Abdominal and thoracic cavities were opened by median incisions and animals were killed by intracardiac blood aspiration. Lungs and operated ovaries were rapidly excised.

Blood samples were collected into glass tubes containing Ethylenediaminetetraacetic acid (EDTA) and plasma were obtained and stored at -70° C until assayed. Malondialdehyde (MDA) concentrations were measured by spectrophotometer (Schimadzu UV-1201) at 532 nm wave length and given in nmol/ml for blood and nmol/g for tissue.

After MDA measurement, remaining lung tissues were fixed in 10% formalin and embedded in paraffin following appropriate processing. Sections in three micrometer thickness were stained by hematoxyline-eosine. Histopathologic findings of non-cardiogenic pulmonary oedema were determined by the method described by Rahman *et al.* [10]. Polymorphonuclear leukocyte infiltration of lung parenchyme were evaluated under a light microscope (Olympus BX-50). PNL number was determined by taking the average of PNL number counted in five different view fields of 10x40 magnification under the light microscope.

Results are given as mean ± standard deviation. Kruskal-Wallis analysis of variance were used for comparison between groups and the Mann Whitney *U* test was applied to those with $p < 0.05$ and $p < 0.01$ was accepted as statistically significant. Correlation between MDA levels and polimorphonuclear leucocyte (PNL) numbers in lung parenchyme was analyzed by spearman correlation analysis. "SPSS 9.0 for windows" was used for statistical analysis of data.

Table 1. Plasma, ovary and lung MDA levels and neutrophil numbers in lung parenchyme

Groups	n	Ovarian tissue MDA concentration (nmol/ml)	Lung MDA concentration (nmol/g)	Plasma MDA concentration (nmol/g)	PNL infiltration of lung parenchyma (Number)
I (3 h I)	8	1.8 ± 0.1	0.9 ± 0.1	1 ± 0.2	69 ± 3
II (3 h I, 3hR)	8	3.3 ± 0.3	1.9 ± 0.1	1.7 ± 0.2	95 ± 2
III (3h I, 3hR, PS)	8	3.1 ± 0.5	1.9 ± 0.1	1.8 ± 0.1	96 ± 4
IV (3h I, 3hR, M)	8	2.07 ± 0.2	1 ± 0.2	1.1 ± 0.2	73 ± 6

h: hour, I: ischemia, R: reperfusion, M: Melatonin, PS: physiological saline, MDA: Malondialdehyde, PNL: Polimorphonuclear leukocytes.

Results

None of the animals died or had abnormal vital signs throughout the experimental period. Operation time was 5 ± 1 minutes after induction of anesthesia. Macroscopically torsioned ovaries had cherry-red color and hemorrhagic appearance.

Histopathologic examination of lungs revealed interstitial and intraalveolar edema, congestion in alveolar walls, focal intraalveolar hemorrhage and fibrin deposition and proliferation of capillaries. MDA levels of plasma, ovaries and lungs and the degree of PNL infiltration of lungs in groups are given in table I (Figures 1, 2 and 3).

When comparison was made between the groups with respect to PNL infiltration in lung tissue and MDA levels it was determined that ovary, lung tissue and plasma MDA levels of detorsion performed groups were significantly higher than those of the salphingo-oophorectomy performed group. PNL findings were also in pallel with groups MDA levels (Groups I and II, I and III, I and IV, $p < 0.01$, Table 1).

The MDA levels of plasma, ovary and lung tissue and PNL infiltration in lung tissue was significantly lower in groups, which received melatonin in addition to detorsioning, than the groups in which only detorsioning was performed (Group II and IV, Group III and IV, respectively; $p < 0.01$, $p < 0.01$). MDA levels and lung tissue PNL infiltration levels of the melatonin administered detorsioned group was similar to those levels of only salphingo-oophorectomy performed group (Group I and IV, $p > 0.05$).

Discussion

In treatment of adnexal torsion, some gynecologists used to avoid detorsioning of adnexis because of the risk of thromboembolic complications [11]. Nowadays detorsion of the torsioned adnexes is the method of choice and no serious thromboembolic complication have been reported in the literature up to now [12–16]. However, reperfusion of torsioned tissue by detorsion would have some local and systemic consequences due to reperfusion of tissues [17]. Lungs are among the tissues that

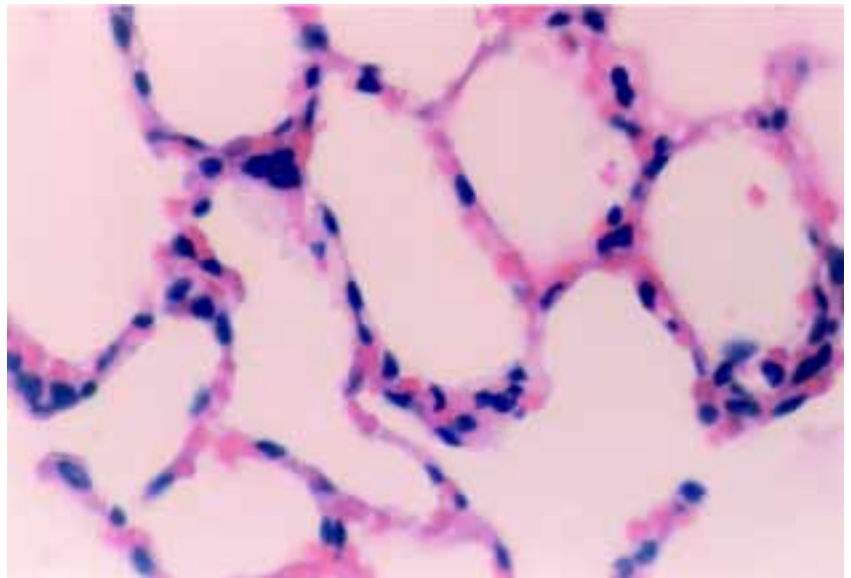


Figure 1. Polimorpho nuclear leucocyte infiltration of lung parenchyma in salphingo-oophorectomy performed group. (HEX400).

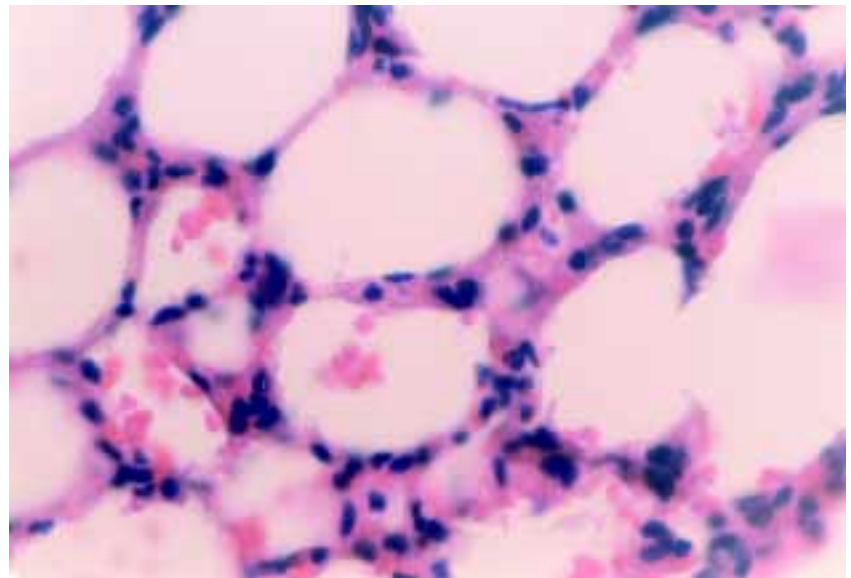


Figure 2. Polimorpho nuclear leucocyte infiltration of lung parenchyma in detorsion performed and physiological saline administered group. (HEX400).

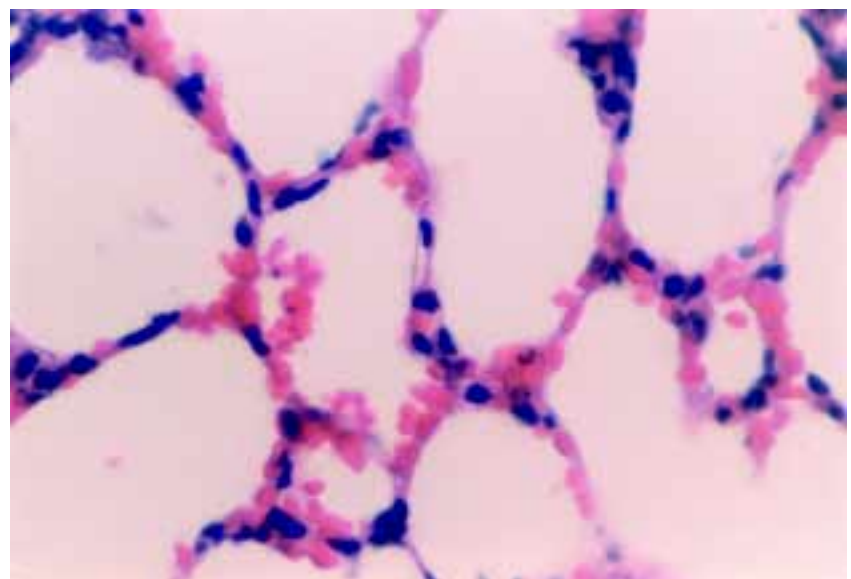


Figure 3. Polimorpho nuclear leucocyte infiltration of lung parenchyma in detorsion performed and melatonin administered group. (HEX400).

are vulnerable to the reperfusion effect. In the present study, in agreement to our previous study [18], histopathological signs of non-cardiogenic pulmonary edema were detected.

Pulmonary effects of operation-induced pain, general anesthetics, and operation procedures are well known [19]. In this study, subjects in all groups were affected in some degrees. Detorsion procedure increased pulmonary edema compared to control group, however.

In pathophysiology of non-cardiogenic pulmonary edema, the primary inciting event is injury in capillary site of the alveolar capillary membrane. As a result, plasma leaks from capillaries into the interstitial and intra-alveolar spaces [20]. Proinflammatory vasoactive amines, xanthine oxidase enzyme and neutrophil activation and infiltration of tissues resulting in increased capillary permeability may be responsible [15,6,21, 22].

Consistent with the literature [9] in this study, after experimental adnexial torsion-detorsion, MDA levels in plasma, ovary and lung tissue were significantly increased compared to those of salpingo-oophorectomy alone which is parallel to our results. In addition, we found that pulmonary parenchymal PNL infiltration was higher in detorsion groups in which melatonin administration was protective to some extent.

Although inflammatory mediators were not determined, in the light of literature our findings provide evidence for participation of free radicals as the primary event in the pathophysiology of alveolar injury

in adnexial detorsion. Here, the mechanism of action of melatonin is pivotal. The first and receptor independent effect of melatonin is to decrease superoxide ion production [9,23,24]. Second, by binding to its receptors, it induces detoxifying enzymes of free radicals. Melatonin also decreases oxidative damage by inhibiting nitric oxide synthase [23,25].

Early diagnosis and treatment of non-cardiogenic pulmonary edema is essential. In the cases especially if the underlying cause is detected and treated within the first 48 hours, clinical outcomes may be reversible with some sequels. After this period, pulmonary fibrosis develops. Mortality exceeds 80% after 10 days [25]. In consideration of differences between the species, clinical evaluation of our results put forward that, in the cases of adnexial torsion treated by detorsion, consideration of those pulmonary changes may help in the early diagnosis of those pathologic consequences and decrease post-operative morbidity.

Although there is no report about pulmonary complications of detorsion of adnexial torsion in the literature, this may be simply because the evaluation of these cases was based on only the clinical findings. Among those, no post-mortem evaluation exists. Possible sub-clinical changes might have been undiagnosed.

In conclusion, detorsion of torsioned adnexes increased pulmonary edema compared to controls but melatonin administration significantly prevented this reperfusion-induced damage to the lungs. Our findings may be of importance in the clinical management of torsioned adnexes by detorsion.

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