

Melatonin Supplementation Could Trigger Delayed Cardiac Preconditioning Against I/R Injury in Partial Nephrectomized Rats with Emphasis to Possible Role of Cardiac NO.

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Abstract: The cardioprotective effects of melatonin are consistent with its ability to scavenge free radical. However free radicals are considered as preconditioning factors, helping the heart to withstand consequent attacks of ischemic reperfusion injury. So, this study aimed to clarify whether melatonin supplementation, concomitant with the deterioration of kidney function in experimental model of renal failure, is able to protect the isolated heart against the liability for global ischemic reperfusion (I/R) injury or its antioxidant effect interferes with proposed preconditioning effect of free radical. Moreover, the study evaluated the changes of myocardial nitric oxide (NO) system with melatonin treatment as one of the suggested triggers of preconditioning. Thirty male Albino rats were divided into three equal groups, sham- operated control rats, 5/6 subtotal nephrectomized (STNx) group and 5/6 subtotal nephrectomized melatonin- supplemented (STNx + M) group. Melatonin was given at a dose of 5 mg / kg/ day for 8 weeks. Rats in all groups were subjected to estimation of plasma urea, creatinine, malondialdehyde (MDA) and nitrate levels, followed by perfusion of isolated hearts. A period of ischemia (30 min) followed by reperfusion for another 30 min was done. The cardiac hemodynamic changes during reperfusion at 5, 15, 25 and 30 min intervals were recorded. At the end of reperfusion, the different chambers of the heart were subjected for determination of the absolute weights as well as their weights to body weight ratios. Sections from the cardiac muscle, mainly ventricle, were used for tissue reduced glutathione (GSH) and nitrate estimation. Partial nephrectomized group (STNx) exhibited significant deterioration of the baseline cardiac hemodynamic as well as more liability for ischemic reperfusion injury in early (5 min) and late reperfusion (30 min) records. Also nephrectomy caused significant cardiac remodeling (hypertrophy), manifested in the increased left ventricle and whole cardiac weights to body weight ratio. The significantly increased plasma MDA, urea and creatinine with nephrectomy showed a negative correlation with the reduced plasma and cardiac tissue nitrate. Melatonin treatment concomitant with the deterioration of renal function (in STNx +M group) showed significant higher basal coronary flow compared to STNx group but it did not improve the ameliorate basal intrinsic cardiac activity due to renal failure. Following I/R, melatonin pre treated group showed some sort of protection against deterioration of cardiac activity in particular at 30 min reperfusion. A 44.5 % decrease in HR in STNx rats versus 30.5% decrease in HR in melatonin treated has been observed. Also the percentage of decrease in peak tension and the tension /left ventricular weight due to reperfusion were significantly lower with melatonin treatment at both 5 and 30 min records of reperfusion. Also melatonin shortened the time to peak tension (TPT) in particular at 30 min reperfusion where ,the increase in TPT due to reperfusion injury was +20.3% with melatonin treatment versus +51.9% in non treated rats. Although, melatonin shortened the half relaxation time(1/2RT) and improve the myocardial flow rate(MFR) compared to non treated group in some records of reperfusion but compared to basal record ; the percentage of change was non significant. Melatonin significantly decreased urea, creatinine and MDA levels which still higher compared to sham control group. Also melatonin ameliorated the hypertrophic changes but not completely with an increase in cardiac tissue GSH and nitrate levels in hearts of melatonin treated rats as well as plasma nitrate. The increased MDA which is an indicator for free radical generation in partial nephrectomized rats did not provide the supposed preconditioning effect against ischemic reperfusion injury in isolated hearts or its effect wasn't conclusive. On the other hand, melatonin was able to improve the basal coronary flow rate and appears to offer some sort of preconditioning and/or protection against I/R injury a condition of excess free radical generation. Cardiac tissue GSH (anti-oxidant) and NO triggering by melatonin may be added to its free radical scavenging effect in the suggested protection and / or preconditioning.

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1. Introduction:

Nowadays, cardiovascular diseases represent the most important health risks as they are responsible for more than 50% of total mortality. Among them, ischemic heart disease is the leading cause of morbidity and mortality (Ostadal 2009). In coronary patients aging, diabetes and other factors interfere with most of cardioprotectants as well as with preconditioning-based interventions (Downey and Cohen 2009). Similarly renal diseases are commonly associated with accelerated cardiovascular disease such as ischemia, pericarditis and peripheral vascular disease due to uremic toxins, and electrolyte disturbances (Leineweber *et al.*, 2002), dyslipidemia, endothelial dysfunction, oxidative stress, and inflammation (Schiffirin *et al.*, 2007)

Ischemic-reperfusion injury to cardiac myocytes is mediated by an overproduction of oxygen free radicals in part during ischemia and more abundantly during early reperfusion (Petrosillo *et al.*, 2006) and chronic renal failure is commonly associated with increased free radical generation (Schiffirin *et al.*, 2007), thereafter, appropriate antioxidant strategies could be considerably useful in protection against cardiac ischemic reperfusion injury (I/R) in renal failure.

Melatonin is considerably better than the classic antioxidants in resisting free-radical-based molecular destruction as it is mentioned in several studies to be a suicidal or terminal antioxidant which sacrifices itself and does not participate in redox cycling after scavenging free radicals (Korkmaz *et al.*, 2009). Moreover, melatonin has a well known cardioprotective effect in view of its free radical-scavenging activity or through its direct interaction with its cardiovascular receptors (Paulis & Simko 2007). Some studies used melatonin before ischemia or during reperfusion (*in vitro*) (Szarszoi *et al.*, 2001) and others used it, *in vivo*, just before ischemia (Sahna *et al.*, 2005).

Free radicals has been claimed to play an important role in the triggering action of ischemic preconditioning, where, exposure of the heart to one or more short episodes of ischemia protects against subsequent long period of ischemia (Genade *et al.*, 2006 and Bolli 2007). Preconditioning protects the myocytes as well as the endothelium of large epicardial and intra myocardial coronary arteries against reperfusion injury. The short ischemic attack cause a transient increase in reactive oxygen species (ROS) which suggested to activate protein kinase C causing reduction in endothelial-neutrophil interaction and /or activation of gene expression of protective proteins such as NO synthases and antioxidant enzymes

(Laude *et al.*, 2002). Moreover, ROS suggested reducing pore opening in inner mitochondrial membrane resulting in reduction in ROS production by mitochondria during reperfusion (Matsuzaki *et al.*, 2009). However, Preconditioning is used now as generic term to encompass any protocol applied before prolonged ischemia that protect the heart during ischemic reperfusion (Halestrap *et al.*, 2007)

Nitric oxide (NO), an important cellular signaling molecular or chemical mediator, beside its well known formation by endothelium, it is formed by the coronary endothelium, endocardial endothelium, cardiac nerves and cardiomyocytes of the normal heart as all express NO synthase and have basal production of nitric oxide (Zweier & Talukder 2006). NO has been shown to play an important role in the myocardial preservation via its vasodilator, antioxidant, anti platelet and anti-neutrophil actions (Schulz *et al.*, 2004). Also it has been confirmed that NO mediates the ischemic preconditioning and many drug can alleviate myocardial ischemia-reperfusion injury by promoting NO production (Cuong *et al.*, 2006),

Thereafter, antioxidants may block the preconditioning effects of free radical and so the liability for ischemic reperfusion injury may be increased. The aim of this study was, therefore, to establish whether melatonin, in view of its free radical-scavenging has a cardioprotective effect or it interferes with the supposed preconditioning effects of free radical. Also the contribution of NO to the possible effect of melatonin is considered during this investigation.

2-Materials and Methods:

2.1-Experimental Animals:

This study was carried out on male thirty albino rats, weighing 180-200gm. They were purchased from military animal farm (Cairo) and maintaining in our hold facilities in the Physiology Department under standard conditions of boarding and given ordinary rat diet. Water was available ad libitum.

2.2-Experimental protocol:

Experimental animals were allocated into three equal groups as follows:

Group I (10 rats): Sham- operated control rats.

Group II (10 rats): Two stages subtotaly nephrectomized rats (STNx) without melatonin treatment.

Group III(10 rats):Two stages STNx rats treated with melatonin (Amon Co.), at a dose of 5 mg / kg/day by oral gavages for 8 weeks, immediately started after the second stage of the operation

2.3-Experimental procedure:

Subtotal nephrectomy was conducted according to Hatori *et al.* (2000), where anaesthetized rats using Ether were laparotomized and 2/3 of the left kidney was removed. 4-5 days later the labarotomy was repeated and the right kidney was totally removed resulting in 5/6 subtotal nephrectomy. The rats had restricted to water and standard rat chew, food consumption was checked twice a week.

Melatonin supplementation:

Rats were supplemented by Melatonin in a dose of 5 mg /kg/day according to (Lee *et al.*, 2002; Stacchiotti *et al.*, 2006). Melatonin was obtained as a powder supplied by Amon Co. Egypt. Melatonin powder was dissolved in distilled water as 5mg/10 ml and each rat received the calculated dose according to its weight by gavages. Sham control rats and STNx rats received distilled water by gavages instead of melatonin.

Blood sampling:

Eight weeks after the second stage of the operation and after overnight fasting, rats were weighed, injected with 1000 IU heparin sodium half an hour before anesthesia. Animals were anaesthetized by intraperitoneal injection of sodium thiopental in a dose of 40mg /kg B.W (Nile CO.). Labarotomy was done and the abdominal aorta was cannulated and the blood samples were collected in heparinized tube for determination of the levels of plasma creatinine, urea, MDA and nitrate.

Isolated heart ischemic –reperfusion:

Rapidly after blood sampling, the chest of the rat was opened and the heart was excised. The heart was immediately chilled in ice –cold modified Krebs-Henseleit bicarbonate buffer solution (pH 7.4) for fast cardioplegia and to prevent ischemia. The aorta was then cannulated and a retrograde perfusion with Krebs- Henseleit bicarbonate buffer solution was started under constant pressure (55 mmHg) without recirculation. The solution gassed with 95% O₂ and 5% CO₂, according to modified Langerdorff technique described elsewhere by Ayobe and Tarazi (1983) The entire system was jacketed in water to maintain a temperature of 37 C. The tension by the heart was measured by a light weight (0-50g) range D1-isometric force transducer which is connected through a strain gauge coupler FC117 to a two channel oscillograph (Washington MD2 Bioscience). One gram weight was attached to the heart apex and was left to hang freely. After 15 minutes (stabilization period), the base line activities were recorded at 50 mm/sec paper speed. Total global ischemia was

induced by stopping delivering of the perfusion fluid for 30 minutes. Afterwards, the hearts were reperfused again for additional 30 minutes. The duration of ischemic reperfusion injury selected according to Nakamura *et al.*, (1991)

Hemodynamic parameters:

In each record, Heart rate (HR, beat/min), peak developed tension (PT, gram), time to peak tension (TPT, msec.), half relaxation time ($\frac{1}{2}$ RT, msec.) and myocardial flow rate

(MFR, ml /min) were determined at different intervals of reperfusion, at 5, 15, 25 and 30 minutes. In addition, basal peak tension / LVW (g/ 100mg) and basal myocardial flow rate / LVW (ml/min/100mg) was calculated later on.

Cardiac tissue handling:

At the end of ischemic reperfusion procedure, hearts were thoroughly cleaned from fat and fibrous tissues. The atria were separated, the right ventricular wall peeled evenly and remaining left ventricle +septum were all blotted dry using absorbing paper and weighed in a 5-digit –Metler balance (AP 160). Cardiac weights of atria, right ventricle, left ventricle, and whole heart were expressed as absolute, as well as relative weights normalized to body weight (mg/g).

After weighing, the cardiac tissue of the left ventricle was homogenized according to Eissa *et al.*, (1990) in homogenization buffer (PH 7.2); for each 0.1mg cardiac tissue, 1ml buffer is added. The buffer consisted of (0.32 mmol/l Sucrose, 20 mmol /l N-2 hydroxyethyl piperzine N-2 ethansulfonic acid (HEPES),.0.5 mmol/l Ethylene diamine tetra – acetic acid (EDTA),.1 mmol /l Di Thiotheritol (DTT),1 mmol/l Phenyl methane sufonyl floride (PMSF) (Sigma).After homogenization, the homogenate was cooled for 10 min. in an iced water bath; then the samples were centrifuged for 10 min. at 4000 rpm. The supernatant was stored in aliquots at _ 80C for subsequent estimation of cardiac tissue nitrate and GSH.

Biochemical analysis:

Measuring plasma urea was formed according to the method described by Searcy *et al.*, (1967), by using kits supplied by Biolabo, France, and depending on colorimetric method, adjusted at wave length 600 nm.

Concentration of creatinine in plasma was estimated according to Jaffe reaction described by Fabiny and Ertingshausen (1971) and Labbe *et al.*, (1996) by using kits supplied by Biolabo, France, depending on colorimetric method adjusted at wave length 490 nm.

Plasma MDA level (nmol /L) was determined as an indicator of lipid peroxidation products in plasma. The

principle of the method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA, according to the technique of Esterbauer & Cheesman (1990). The concentrations were then divided by 1000 to be expressed in μmol .

Nitrate assay: Plasma samples, as well as, supernatant of cardiac tissue homogenate were subjected to nitrite assay using reagents supplied by Sigma. Nitrate used as an indicator for nitric oxide level since nitric oxide is extremely unstable lipid-soluble gas and undergoes rapid oxidative degradation to the stable breakdown products nitrate and nitrite. Nitrate determined according to a method described by Borjes and Borjes (1995). The concomitant reduction of nitrate to nitrite by NADPH was monitored by the oxidation of the coenzyme and the decrease in the absorbance at 340 nm. Plasma nitrate was expressed as $\mu\text{mol/l}$, whereas cardiac tissue nitrate levels were expressed as $\mu\text{mol/g}$.

Cardiac tissue GSH:

Supernatant of cardiac tissue was also subjected for estimation of GSH level. GSH was determined by the spectrophotometric method according to method of Jollow *et al.*, (1974). The kits provided by Bio-diagnostic and the results were expressed as nmol/g tissue.

Statistical Analysis:

Results were expressed as mean \pm SEM. The statistical significance of differences between means was determined by student's 't' test for both paired and unpaired group at a level of significance $p < 0.05$. Data was analyzed by ANOVA, Statistical analysis

and correlations were calculated by Pearson correlation (2-tailed) using SPSS program (statistical progression for social science) statistical package (SPSS Inc.) version 8.

Percentage of changes was calculated in all the studied groups, at 5 minute and 30 minute during reperfusion relative to the basal record.

3-Results:

As shown in table (1); urea and creatinine levels were significantly higher ($p < 0.05$) in partial nephrectomized group (STNx) compared to sham control group. The plasma level of MDA was significantly ($p < 0.05$) increased, while plasma nitrate concentration was significantly ($p < 0.05$) decreased. The cardiac tissue levels of both GSH and nitrate were significantly decreased. Also, the body weight was significantly decreased (% of change from initial weight was 46.43% in STNx rats compared to 71.55 % in sham control).

Melatonin treated group (STNx+M) showed significant ($p < 0.05$) lower urea and creatinin level compared to the non treated nephrectomized rats but their levels still significantly ($p < 0.05$) higher compared to sham control rats. Melatonin also significantly ($p < 0.05$) lowered the plasma MDA level and significantly ($p < 0.05$) increased plasma nitrate concentration compared to STNx rats. In addition, melatonin treatment caused significant ($p < 0.05$) increase in cardiac tissue levels of both GSH and nitrate. The body weight in the melatonin treated group (STNx+M) was significantly ($p < 0.05$) higher compared to nephrectomized non treated rats (STNx), but it still significantly ($p < 0.05$) lower compared to sham control rats.

Table (1): showing the changes in plasma Urea, Creatinine , nitrate and MDA and cardiac tissue levels of GSH and nitrate as well as the % of change in body weights(gm) in the different studied groups

	Sham control (n=10) Mean \pm S.E.M	STNx (n=10) Mean \pm S.E.M	STNx+M(n=10) Mean \pm S.E.M
Urea(mg/dl)	29.5 \pm 0.36	82.5 \pm 0.67 ^a	64.7 \pm 2.16 ^{ab}
Creatinine(mg/dl)	0.29 \pm 0.01	0.99 \pm 0.02 ^a	0.49 \pm 0.02 ^{ab}
Plasma nitrate($\mu\text{mol/L}$)	14.5 \pm 1.14	5.83 \pm 0.6 ^a	9.48 \pm 0.84 ^{ab}
Plasma MDA($\mu\text{mol/L}$)	1.76 \pm 0.09	2.77 \pm 0.15 ^a	2.15 \pm 0.05 ^{ab}
Cardiac tissue GSH. (nmol/g)	465.3 \pm 6.16	302.0 \pm 8.7 ^a	410.0 \pm 11.9 ^{ab}
Cardiac tissue nitrate. ($\mu\text{mol/g}$).	894.0 \pm 5.1	704.6 \pm 5.6 ^a	808.1 \pm 5.5 ^{ab}
Final B.W	327.5 \pm 5.1	281.0 \pm 4.6 ^a	311.5 \pm 3.2 ^{ab}
BW (% of change)	71.55%	46.43%	62.8%

Changes in cardiac weights:

As shown in table (2) and figure (1); in the STNx group, the ratios of heart weight and the left ventricle (including septum) to body weight as well as the left

ventricle to right ventricle weight ratio ,all were increased significantly ($P < 0.001$ for all) compared with those of sham control.

In melatonin treated -nephrectomized group (STNx +M), the relative weight of whole heart and left ventricle exhibited highly significant ($P < 0.001$) decrease compared to the nephrectomized group but

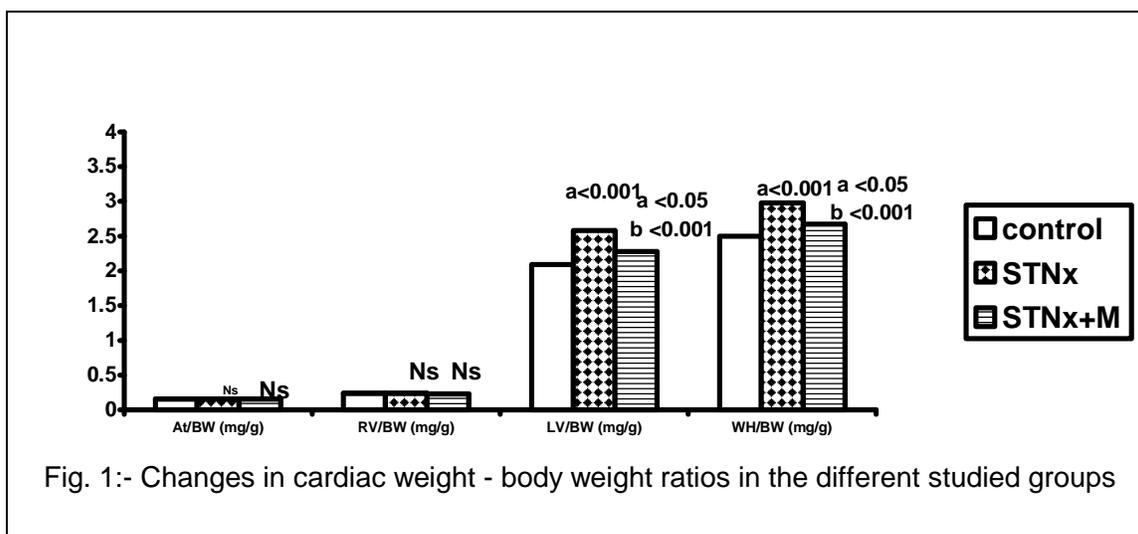
they still having significantly ($p < 0.05$) higher values compared to sham control. Melatonin also significantly ($p < 0.05$) decreased the LV/ RV ratio compared to nephrectomized rats.

Table (2) Changes in absolute cardiac weights and cardiac weight body weight ratios in control, nephrectomized(STNx) and nephrectomized melatonin- treated rats(STNx + M).

Cardiac weight	Sham controls (n=10) Mean \pm SEM	STNx(n=10) Mean \pm SEM	STNx +M(n=10) Mean \pm SEM
Atrium (At, mg)	53.8 \pm 1.19	44.97 \pm 2.11 ^a	48.02 \pm 1.56 ^a
At / BW(mg/g)	0.16 \pm 0.002	0.16 \pm 0.00	0.16 \pm 0.005
Right ventricle(RV. mg)	78.9 \pm 2.08	68.18 \pm 2.21 ^a	71.86 \pm 1.4 ^a
RV/ BW (mg/ g)	0.24 \pm 0.004	0.24 \pm 0.007	0.23 \pm 0.004
Left ventricle(LV. mg)	685.3 \pm 18.11	718.5 \pm 15.6	711.0 \pm 13.22
LV/BW (mg/g)	2.09 \pm 0.04	2.58 \pm 0.03 ^a	2.28 \pm 0.05 ^{ab}
LV/RV (mg/mg)	8.72 \pm 0.23	10.69 \pm 0.32 ^a	9.92 \pm 0.21 ^{ab}
Whole heart(WH. mg)	818.0 \pm 20.03	837.15 \pm 17.43	830.9 \pm 14.17
WH/BW(mg/g)	2.50 \pm 0.04	2.98 \pm 0.03 ^a	2.67 \pm 0.05 ^{ab}

a: Significance by LSD at $P < 0.05$ relative to control group.

b: Significance by LSD at $P < 0.05$ relative to STNx group(subtotal nephrectomy).



Base line cardiac performance:

As shown in table (3); in STNx rats, the basally recorded *in vitro* cardiac activities were clearly altered. This was manifested in the significantly decreased, heart rate, peak developed tension and myocardial flow rate compared to their sham control rats. The calculated basal, myocardial flow rate per 100 mg LVW as well as peak tension per 100mg LVW was also significantly reduced compared to sham control rats. Moreover, the basal time to peak tension and half relaxation time were significantly prolonged in

hearts of STNx rats. Melatonin treatment (in STNx+M) was able to increase significantly ($p < 0.05$) the myocardial flow rate while the myocardial flow rate per/100mg LVW was border line. Significantly ($P < 0.05$) increased.

On the other hand, in melatonin treated-nephrectomized rats (STNx+M), the values of basal heart rate, peak developed tension, tension /100mg LVW, time to peak tension and half relaxation time did not significantly differ from that observed in non treated nephrectomized rats.

Table (3): Showing basal activity of intrinsic cardiac properties in the different studied groups.

	Sham control Mean \pm SEM	STNx Mean \pm SEM	STNx +M Mean \pm SEM
HR (beat/ min) (n=10)	187.4 \pm 10.1	146.5 \pm 7.2 ^a	163.0 \pm 6.5 ^a
Peak developed tension (PT, gram) (n=10)	6.2 \pm 0.51	4.6 \pm 0.29 ^a	4.6 \pm 0.26 ^a
Peak tension/ LVW (g/100mg) (n=10)	0.91 \pm 0.08	0.64 \pm 0.04 ^a	0.65 \pm 0.04 ^a
Time to peak tension (TPT, msec) (n=10)	88.0 \pm 7.4	140.0 \pm 7.2 ^a	137.0 \pm 8.9 ^a
Half relaxation time (HRT, msec) (n=10)	82.0 \pm 7.4	176.0 \pm 7.6 ^a	156.0 \pm 8.2 ^a
Myocardial flow rate (MFR, ml/min) (n=10)	7.5 \pm 0.37	4.41 \pm 0.47 ^a	5.6 \pm 0.39 ^{ab}
MFR / LVW (ml/min/100mg) (n=10)	1.06 \pm 0.05	0.61 \pm 0.07 ^a	0.79 \pm 0.06 ^{ab}

a: Significance by LSD at P <0.05 relative to sham control group.

b: Significance by LSD at P <0.05 relative to STNx group

Ischemic reperfusion results:

As shown in tables (4, 5 & 6); compared to pre-ischemic basal level of cardiac activity, the three studied groups exhibited nearly the same pattern of response to I/R with different intensities. Significant (p<0.05) decrease in beating rate (HR), depression in peak tension (PT) and tension/100mg LVW, diminished myocardial flow rate (MFR) and flow rate / 100mg LVW, as well as significantly (p<0.05) prolonged time to peak tension (TPT) and half relaxation time (HRT) have been observed.

Chronotropic activity:

As shown in table (4-A); in STNx group during reperfusion of the isolated hearts, significant (P<0.05) decrease in heart rate was observed compared to the corresponding records in sham control (at 15, 25 & 30 minute) and compared to its basal records (P<

0.0005). Melatonin treatment in STNx+M group increased the HR compared to non treated group, the increase was significant (P<0.05) at 25 and 30 minute reperfusion records.

Peak developed tension and tension per LVW:

As shown in table (4-B & C); in STNx group the peak developed tension as well as the tension per 100mg left ventricular weight were significantly (P<0.05) reduced in all records of reperfusion when compared to the corresponding records in sham control rats and when compared to their basal records (P<0.0005 or P<0.001). In melatonin treated rats, compared to STNx rats, the tension only increased significantly (P<0.05) at 5 min. reperfusion record. Also the tension / 100mg LVW was significantly (P<0.05) increased only at 5 and 30 min.

Table (4): Shows the pre-ischemic (basal) records and the changes in heart rate (HR), peak developed tension (PT) and peak tension/ LVW (g/100mg) at different intervals of reperfusion in the different studied groups.**A- Heart rate**

Parameters	Group	Basal	5min.	15min.	25min.	30min.
HR (Beat/min)	Sham control Mean \pm SEM N=10	187.4 \pm 10.1	125.0 \pm 9.9 ^c	136.0 \pm 11.9 ^c	128.0 \pm 10.3 ^c	119.0 \pm 11.0 ^c
	STNx Mean \pm SEM N=10	146.5 \pm 7.2 ^a	102.6 \pm 7.6 ^c	98.5 \pm 6.9 ^{ac}	81.0 \pm 6.2 ^{ac}	80.5 \pm 5.9 ^{ac}
	STNx+M Mean \pm SEM N=10	163.0 \pm 6.5	129.5 \pm 11.7 ^c	121.0 \pm 7.8 ^c	112.5 \pm 7.8 ^{bc}	113.5 \pm 8.4 ^{bc}

B- Peak tension

Parameters	Group	Basal	5min.	15min.	25min.	30min.
PT (gram)	Sham control Mean \pm SEM N=10	6.2 \pm 0.51	4.12 \pm 0.33 ^C	3.85 \pm 0.29 ^C	3.89 \pm 0.26 ^C	3.65 \pm 0.2 ^C
	STNx Mean \pm SEM N=10	4.6 \pm 0.29 ^a	2.98 \pm 0.22 ^{ac}	3.15 \pm 0.20 ^{ac}	2.85 \pm 0.23 ^{ac}	2.7 \pm 0.3 ^{ac}
	STNx+M Mean \pm SEM N=10	4.6 \pm 0.26	3.8 \pm 0.22 ^b	3.3 \pm 0.20 ^c	3.1 \pm 0.17 ^c	3.04 \pm 0.2 ^c

C-Peak tension /LVW

Parameters	Group	Basal	5min.	15min.	25min.	30min.
PT/ LVW gm/100mg	Sham control Mean ± SEM N=10	0.91± 0.08	0.6 ± 0.05 ^c <0.0005	0.56 ± 0.04 ^c <0.0005	0.57± 0.04 ^c <0.0005	0.54± 0.04 ^c <0.0005
	STNx Mean ± SEM N=10	0.64± 0.04 ^a	0.41 ±0.03 ^{ac}	0.43 ± 0.03 ^{ac}	0.40 ± 0.03 ^{ac}	0.38 ± 0.04 ^{ac}
	STNx+M Mean ± SEM N=10	0.65 ± 0.04	0.53± 0.03 ^{bc}	0.46± 0.03 ^c	0.43± 0.03 ^c	0.48± 0.02 ^b

a: Significance by LSD at P <0.05 relative to sham control group. **b:** Significance by LSD at P <0.05 relative to STNx group.

c: Significance at each record, 5, 15, 25&30 minutes of reperfusion relative to baseline Values.

Time to peak tension:

As shown in table (5); changes in time to peak tension (TPT) during reperfusion showed that nephrectomized rats exhibited significant (P<0.05) prolonged TPT throughout all records of reperfusion compared to sham control. Also they demonstrated significant longer TPT compared to their TPT basal records (P<0.02, P<0.005 &P<0.0005 at 5,15 &both 25 and 30 min. respectively). Melatonin treatment did not alter the TPT significantly except for at 30 min. reperfusion record where it was significantly (P<0.05) shorter compared to nephrectomized group.

Half relaxation time:

As shown in table (5); STNx rats showed significantly prolonged half relaxation time (HRT) in all records of reperfusion compared to sham control (P<0.05), as well as compared to their basal record (P<0.01,P<0.002,P<0.005&P<0.004 at 5,15,25 and 30 min. records respectively). In the Melatonin treated rats (STNx+M), the HRT was significantly (p<0.05) shorter compared to STNx rats at 5 and 30 minutes reperfusion and shorter though none significantly at 15 and 25 min. reperfusion.

Table (5) Shows the pre-ischemic (basal) records and the changes in time to peak tension (TPT) and half relaxation time (1/2 RT) at different intervals of reperfusion in the different studied groups.

Parameters	Group	Basal	5min.	15min.	25min.	30min.
TPT (msec.)	Sham control Mean ± SEM N=10	88.0 ± 7.4	110.0± 12.9	113.0 ± 9.32 ^c	119.0 ± 7.1 ^c	120.0 ± 7.6 ^c
	STNx Mean ± SEM N=10	140.0± 7.2 ^a	162.0 ±9.97 ^{ac}	173.0 ±8.6 ^{ac}	179.0 ± 5.0 ^{ac}	210.0 ± 11.5 ^{ac}
	STNx+M Mean ± SEM N=10	137.0± 8.9	169.0 ± 16.1 ^c	164.0 ± 8.6 ^c	165.0 ± 10.0 ^c	163.0 ± 10.2 ^{cb}
HRT (msec.)	Sham control Mean ± SEM N=10	82.0 ± 7.4	97.0 ± 6.8 ^c	107.0± 8.0 ^c	116.0± 7.8 ^c	110.0 ± 7.6 ^c
	STNx Mean ± SEM N=10	176.0 ± 7.6 ^a	202.0 ±11.4 ^{ac}	209.0 ± 10.9 ^{ac}	215.0 ±14.5 ^{ac}	217.0 ±13.5 ^{ac}
	STNx+M Mean ± SEM N=10	156 ± 8.2	172.0 ± 7.9 ^{bc}	186.0 ± 9.4 ^c	185.0 ± 11.2 ^c	185.0 ±11.24 ^{bc}

Myocardial flow rate:

As shown in table (6); the myocardial flow rate and myocardial flow rate /100mg LVW were significantly(P<0.05) reduced during all reperfusion

records following ischemia in nephrectomized rats compared to sham control and compared to their basal record. Melatonin treatment increased the MFR and the corrected MFR significantly (P<0.05 for both) at

both 5 and 30min reperfusion record compared to nephrectomized non treated rats.

As shown in table (7); in STNx group, the % of change did not differ significantly compared to the corresponding % of change in sham control rats in almost all of the studied parameters except for the reduced myocardial flow rate and myocardial flow

rate per left ventricular weight in STNx rats. The MFR significantly ($P < 0.05$) decreased by -14.1 ± 3.11 % in sham control, versus -26.9 ± 3.1 in STNx. Also, the MFR/LVW significantly ($p < 0.05$) decreased by -11.7 ± 2.5 % in sham control versus -26.8 ± 3.4 in STNx group.

Table (6): Shows the pre-ischemic (basal) records and the changes in myocardial flow rate (MFR) and myocardial flow rate / LVW, at different intervals of reperfusion in the different studied groups.

parameters	Group	Basal	5min.	15min.	25min.	30min.
MFR (ml /min.)	Sham control Mean \pm SEM N=10	7.5 \pm 0.37	6.45 \pm 0.46 ^c	5.9 \pm 0.44 ^c	5.2 \pm 0.4 ^c	5.15 \pm 0.43 ^c
	STNx Mean \pm SEM N=10	4.41 \pm 0.47 ^a	3.2 \pm 0.31 ^{ac}	3.10 \pm 0.24 ^{ac}	2.8 \pm 0.22 ^{ac}	2.7 \pm 0.22 ^{ac}
	STNx+M Mean \pm SEM N=10	5.6 \pm 0.39 ^b	4.2 \pm 0.28 ^{bc}	3.97 \pm 0.29 ^c	3.5 \pm 0.35 ^c	3.96 \pm 0.29 ^{bc}
MFR/ LVW (ml /min/ 100gm)	Sham control Mean \pm SEM N=10	1.06 \pm 0.05	0.94 \pm 0.07 ^c	0.86 \pm 0.06 ^c	0.76 \pm 0.06 ^c	0.75 \pm 0.06 ^c
	STNx Mean \pm SEM N=10	0.61 \pm 0.07 ^a	0.44 \pm 0.05 ^{ac}	0.43 \pm 0.04 ^{ac}	0.38 \pm 0.03 ^{ac}	0.37 \pm 0.03 ^{ac}
	STNx+M Mean \pm SEM N=10	0.79 \pm 0.06 ^b	0.60 \pm 0.04 ^{bc}	0.56 \pm 0.04 ^{bc}	0.50 \pm 0.05 ^c	0.55 \pm 0.04 ^{bc}

a: Significance by LSD at $P < 0.05$ relative to sham control group.

b: Significance by LSD at $P < 0.05$ relative to STNx group.

c: Significance at each record, 5, 15, 25&30 minutes of reperfusion relative to baseline values

Table (7): Percentage of change for the *in vitro* studied parameters at 5minute reperfusion record (early stage) and at 30 minute reperfusion record (late stage) compared to pre-ischemic basal record.(-ve sign=decreased % while +ve sign =increased %)

	5 min. perfusion			30 min perfusion		
	Sham Control	STNx	STNx +M	Sham Control	STNx	STNx +M
HR	-31.1 \pm 4.3	-29.5 \pm 4.6	-19.4 \pm 4.9	-35.4 \pm 5.86	-44.5 \pm 4.17	-30.55 \pm 4.22 ^b
PT	-28.9 \pm 6.3	-35.5 \pm 3.7	-16.3 \pm 3.3 ^b	-38.5 \pm 4.95	-39.75 \pm 5.98	-31.58 \pm 5.17 ^b
PT/LVW	-31.8 \pm 5.3	-35.6 \pm 3.8	-17.0 \pm 3.8 ^b	-38.5 \pm 5.0	-39.7 \pm 6.1	-25.3 \pm 4.4 ^b
TPT	+26.2 \pm 10.9	+16.4 \pm 5.9	+20.4 \pm 5.7	+36.5 \pm 7.85	+51.93 \pm 8.16	+20.28 \pm 2.59 ^b
HRT	+22.04 \pm 6.1	+14.9 \pm 4.3	+12.0 \pm 2.7	+51.55 \pm 14.0	+23.35 \pm 5.63	+18.25 \pm 3.77
MFR	-14.1 \pm 3.11	-26.97 \pm 3.1 ^a	-23.5 \pm 3.8	-31.42 \pm 4.06	-35.62 \pm 4.5	-36.39 \pm 4.97
MFR/ LVW	-11.7 \pm 2.5	-26.8 \pm 3.4 ^a	-23.5 \pm 3.7	-29.5 \pm 4.2	-36.0 \pm 4.2	-29.6 \pm 3.3

a: Significance by LSD at $P < 0.05$ relative to sham control group.

b: Significance by LSD at $P < 0.05$ relative to STNx group

On the other hand, melatonin treated group exhibited less deterioration of almost all of the hemodynamic parameters with reperfusion, in particular at 30min reperfusion.

Regarding the chronotropic activity, at 30 minute reperfusion, a 44.5± 4.17 % significant (P<0.05) decrease in HR in STNx versus 30.5 ± 4.2 % decrease in HR in melatonin treated has been observed.

Also, at 5 minute reperfusion, a 35.5 % significant (P<0.05) decrease in peak tension in STNx rats met with a 16 % decrease in tension in melatonin treated group. Moreover, in the late stage of reperfusion (at 30 min reperfusion) a 39.7 % significant (P<0.05) decrease in peak tension in STNx rats met by a 24.9% decrease in tension in melatonin treated group.

Melatonin treatment also exhibited significant (P<0.05) less decrease in peak tension / 100mg of LVW with reperfusion compared to non treated group. At 5 min reperfusion the decrease (P<0.05) in corrected peak tension was 35.6% in STNx rats versus 17.0% decrease in STNx + M group. At 30 min. reperfusion the decrease (P<0.05) in corrected peak tension was 39.7 % in STNx rats versus 25.3% decrease in STNx+ M rats.

The time to peak tension at 30min record showed a +51.9% prolongation in STNx rats compared to its basal records versus +20.3 % increases(P<0.05) in melatonin treated group compared to their basal record.

However in evaluating the % of change from the basal record in the STNx and STNx+M groups, in

early and late stage of reperfusion the% of increase in HRT was nearly similar in both group (at 5 min :+14.9% in STNx group and + 12.0 % in STNx +M group, at 30 min.: +23.3% in STNx group versus + 18.25 % in STNx+M group) with non significant % of change.

Results of correlation:

As shown in table (8-a);correlation analysis in STNx group showed significant(P <0.03,P<0.01 &P<0.02) negative correlation between plasma MDA level in one side and plasma nitrate , cardiac tissue nitrate and GSH levels in other side respectively. Also, a positive significant (P<0.01) correlation between plasma MDA level and LV/RV ratio is observed together with positive correlation (though non significant) with LV/BW ratio. In addition, plasma nitrate showed a positive significant (P<0.02) correlation to cardiac tissue nitrate.

In addition, positive significant(r=0.645, P<0.04,n=10) correlation between the TPT and the whole heart /body weight ratio was observed in STNx group but not shown in the table.

As shown in table (8-b); correlation analysis in STNx+M group showed significant (P<0.009, P<0.001& P< 0.0001) negative correlation between plasma MDA and plasma & cardiac tissue nitrate as well as with cardiac tissue GSH respectively. Also, tissue nitrate level was positively (P<0.001) correlated with tissue GSH.

Table (8-a):Correlation analysis in nephrectomized group:

		urea	Creatinine	Plasma nitrate	Tissue nitrate	Tissue GSH	LV/RV	LV/BW
MDA	r	+0.057	+0.211	-- 0.674 ^a	-- 0.757 ^a	-- 0.707 ^a	+0.764 ^a	+0.270
	P	<0.87	<0.558	<0.033	<0.011	<0.022	<0.01	<0.45
	N	10	10	10	10	10	10	10
		urea	Creatinine	Tissue Nitrate	Basal MFR	Flow/100gm LVW		
Plasma Nitrate	R	-- 0.233	-- 0.041	+0.685 ^a	+0.103	+0.062		
	P	<0.517	<0.911	<0.029	<0.776	<0.865		
	N	10	10	10	10	10		

Table (8-b): Correlation analysis in nephrectomized melatonin treated group.

		Urea	Creatinine	Plasma nitrate	Tissue nitrate	Tissue GSH	LV/RV
MDA	R	+0.133	+0.188	--0.769 ^b	--0.869 ^b	--0.973 ^b	+0.689
	P	<0.714	<0.602	<0.009	<0.001	<0.0001	<0.27
	N	10	10	10	10	10	10
		urea	Creatinine	Plasma nitrate	Tissue GSH	Basal MFR	
Tissue Nitrate	R	- 0.009	-0.279	+0.614	+0.892 ^b	+0.357	
	P	< 0.98	< 0.435	0.59	0.001	0.312	
	N	10	10	10	10	10	

a: Significance by LSD at P <0.05 relative to sham control group.

b: Significance by LSD at $P < 0.05$ relative to STNx group

4-Discussion:

The ability of the heart to resist ischemic reperfusion injury either due to previous exposure to brief attacks of ischemia or due to any protocol applied before prolonged ischemia that protect the heart during ischemic reperfusion (Preconditioning), is very significant and important precaution other than cardioprotective drugs which are given during the cardiac attack, especially against acute episodes of myocardial infarction. As the onset of the attack is not predictable and cardioprotective intervention can usually implemented only at the time of reperfusion, after the appearance of the symptoms, where significant part of the damage has already occurred so preconditioning is of great therapeutic significance (Bolli 2007). Aforementioned findings, it is of interest to clarify the actual role of melatonin whether it has a cardioprotective effect or it abrogate the supposed preconditioning effects of free radicals generated during renal failure.

In this study, the increased urea and creatinin level in 5/6 partial nephrectomized rats confirming that partial nephrectomy had caused renal failure. On the other hand melatonin treatment significantly ameliorated the detrimental changes in both urea and creatinine. However, their values still higher compared to sham control, which indicate that the ability of melatonin to ameliorate the effect of nephrectomy is not complete. In coincide with this study the deterioration of renal function (plasma creatinine and proteinuria) and structure (glomerulosclerosis and tubulointerstitial damage) resulting from renal ablation was ameliorated significantly with melatonin treatment (Quiroz *et al.*, 2008).

The significantly, increased plasma level of MDA as an indicator for lipid peroxidation and free radical generation, and decreased plasma nitrate with partial nephrectomy could be correlated to each other. Reduction in nitric oxide levels during kidney failure has been related to the reaction of nitric oxide with superoxide anions to yield peroxynitrite rather than nitrate. Peroxynitrite possesses the biological activity responsible for renal damage (Kim *et al.*, 2000 and Wang *et al.*, 2003). This explanation is favored by the significant negative correlation between MDA values and levels of plasma nitrate demonstrated in 5/6 nephrectomized rats ($r=0.674$ $p < 0.033$ $n=10$). In contradiction with our result, increased NO production in the systemic circulation of uremic rats (Aiello *et al.*, 1997) and patients (Lau *et al.*, 2000) was noticed.

Melatonin treatment significantly reduced MDA level which suggests an attenuated lipid peroxidation. Melatonin reported to be effectively protects against

lipid peroxidation as well as decrease the synthesis of MDA which is an end product of lipid per oxidation (Zwirski-korczała *et al.*, 2005).

Melatonin also significantly increased plasma nitrate concentration which implies slight improvement in NO level as nitrate level was still significantly low compared to sham control. The increased plasma nitrate as well as cardiac tissue nitrate with melatonin treatment could be attributed to the ability of melatonin to stimulate the endothelial NO synthesis through increasing intracellular calcium and/or increasing NO availability by preventing the NO conversion to peroxynitrite through its free radical scavenging activity (Pogan *et al.*, 2002). The reduced MDA together with an increase in NO levels after melatonin treatment as well as the significant negative correlation between them may be supportive for the previous explanation.

Regarding the basal cardiac properties in the form of significantly altered basal cardiac hemodynamic in nephrectomized non treated rats which is manifested in the significantly diminished basal peak tension and tension per LVW as well as in the significantly prolonged time to peak tension, all quantify myocardial systolic dysfunction with nephrectomy. Previous study reported an alteration in Ca^{2+} handling and an increase in the rate of inactivation of the L-type Ca^{2+} current in isolated cardiac myocytes from STNx rats (Donohoe *et al.*, 2000).

In addition, the significantly prolonged half relaxation time in nephrectomized non treated group may quantify diastolic impairment. Both systolic and diastolic dysfunctions have been reported before with renal failure which was attributed to the associated left ventricular hypertrophy (Zoccali *et al.*, 2004). In this study, the significantly increased left ventricular and whole heart weights to body weight ratios in nephrectomized non treated rats as well as the significantly reduced myocardial flow rate implies non physiological hypertrophy and may explain the systolic and diastolic impairment. Abnormalities of the microcirculation or of the main coronary circulation with uremia may lead to ischemia and fibrosis and to stiffness of the myocardium with subsequent occurrence of diastolic dysfunction (Malik *et al.*, 2009).

The significantly prolonged time to peak tension and half relaxation time in nephrectomized rats is reminiscent with the simultaneous negative chronotropic effect, since these times represent parts of the prolonged duration of the whole cycle. Moreover, the positive significant correlation between the TPT and the whole heart /body weight ratio in STNx group may explain this prolonged time to reach peak tension ($r=0.645$, $p < 0.04$, $N=10$)

The significantly reduced basal myocardial flow rate in nephrectomized rats could be attributed to the increased liability of ischemic heart diseases due to reduction in coronary reserve and capillary density with chronic kidney diseases (Amann *et al.*, 1998). Also the highly significant ($p < 0.0001$ $r = 0.614$) positive correlation between the coronary flow and the plasma nitrate level in all groups and positive though non significant in STNx group all could lead to a suggest that reduced plasma nitrate level and hence its reduced vasodilator effect in nephrectomized rate may be compromised in this reduced basal coronary flow.

In contradiction with our result, in acute experimental uremia several indices of myocardial contractility have been found to be increased but the experimental model was acute rather than chronic (Nivatpumin *et al.*, 1975). Also, some studies showed structural changes but no cardiac functional changes with uremia (Hatori *et al.*, 2000; Kennedy *et al.*, 2006). However, in line with our result, cardiac function in an isolated perfused working heart, was significantly impaired 4 weeks after 5/6 nephrectomy (raine *et al.*, 1993).

The baseline activity of hearts isolated from nephrectomized melatonin treated rats (STNx+M) was not significantly different from that of nephrectomized non treated rats as regards heart rate, peak tension development, time to peak tension as well as half relaxation time.

Melatonin treatment improved the basal coronary flow rate significantly compared to the STNx rats but it caused border line significant increase in MFR/100 mg LVW which could be attributed to the inability of melatonin to ameliorate completely the cardiac hypertrophy associated with renal failure. Thus melatonin treatment concomitant with renal impairment did not alter the basal intrinsic properties of the heart, but its effect on the baseline coronary flow may be due to its vascular effect.

Melatonin showed to inhibit the development of nitrate tolerance (diminished blood vessel responsiveness to the vasodilator effect of nitroglycerin) in coronary arteries via MT1- or MT2-melatonin receptors and this requires the presence of endothelial cells (O'Rourke *et al.*, 2003) or via increasing NO availability which stimulates guanylate cyclase in smooth muscle cells leading to vasodilatation (Pogan *et al.*, 2002).

In all studied groups the pattern of response to reperfusion after a period of ischemia was in the form of decreasing in beating rate (HR), depression in peak tension (PT), diminished myocardial flow rate (MFR) as well as significantly prolonged time to peak tension (TPT) and half relaxation time (HRT). This response varied in intensity in the three studied groups. Also, it varied during early (5 min.) and late stage (30 min.) of

reperfusion. during ischemia ATP declines resulting in decrease in PH due to anaerobic glycolysis. The increased proton stimulates $\text{Na}^+ - \text{H}^+$ exchanger resulting in increased intracellular Na^+ which reverse the mode of $\text{Na}^+ - \text{Ca}^{++}$ exchanger leading to increased intracellular and intra mitochondrial Ca^{++} level. This in turn activates channel protein (MPT) in inner mitochondrial membrane, leading to loss of ATP which falls to very low levels that causes inhibition of some process and cellular death. In addition, generation of reactive oxygen species (ROS) during ischemia may play a role in this impairment. However the generation of ROS during ischemia is very low as oxygen is required for this process (Murphy and Steenbergen 2008).

On the other hand during reperfusion, a large burst of reactive oxygen species (ROS) has been consistently shown to occur with extensive tissue damage. Cardiac myocytes, endothelial cells, and infiltrating neutrophils contribute to this enhanced ROS production. These radicals may exceed the capacity of the cellular intrinsic free radical scavenging systems (Petrosillo *et al.*, 2006 and Murphy & steenbergen 2008). The altered cardiac hemodynamic with ischemic reperfusion agree with other findings where depressed contractile function, coronary flow as well as altered vascular reactivity occurs with I/R (Zweier *et al.*, 2006).

In this study, 5/6 nephrectomized rats showed more liability for reperfusion injury which may be related to the observed reduced cardiac GSH (anti-oxidant) which may be exhausted in defending the free radicals supposed to associate renal damage and could be dictated by the increased plasma MDA level. Also the reduced plasma and cardiac tissue nitrate levels may quantify NO involvement in peroxynitrite formation. The negative significant correlation between MDA level and cardiac tissue GSH & both plasma and cardiac tissue nitrate level is supportive to the previous explanation.

Despite of the previously mentioned deteriorated cardiac activity in STNx rats during reperfusion of their hearts, the % of change in all of its cardiac parameters from the basal record at 5 min. and 30 min not differ significantly from that percentage of change in sham control hearts (table 7). This may refers to possibility of preconditioning but the increased free radicals dictated in increased MDA level in STNx group could not provide the suspected preconditioning effect suggested by other studies (Yellon and Downey 2003; Genade *et al.*, 2006) which appeared markedly in the significantly reduced myocardial flow rate and significant decrease in myocardial flow rate/ left ventricular weight. Thus the preconditioning effect of free radical could be suggested to be inconclusive.

Melatonin treatment improved the deteriorated chronotropic activity in STNx+ M group both in early stage of reperfusion (though non significant) and late stage of reperfusion (significant). Also it improved the inotropic activity manifested in the significantly better % of change in PT& PT/100mg LVW both at early and late stage of reperfusion. Also this was manifested in shorter time to peak tension with melatonin treatment. Thus melatonin could be suggested to provide preconditioning and /or protection against ischemic reperfusion injury.

Preconditioning was reported to occur in two phases, very early phase within a few minutes after the preconditioning stimulus and lasts only 1–2 hours. The second window of protection develops more slowly, 12–24 hours after the preconditioning stimulus, but it lasts much longer, for 3–4 days and requires the synthesis of new proteins (Liem *et al.*, 2007). The effect of melatonin could be suggested in this study to stimulate the late phase preconditioning, and increasing cardiac tissue GSH and nitrate may be their possible tools.

On the other hand, melatonin did not show significant better % of change in half relaxation time (HRT) or coronary flow rate during reperfusion which suggests its inability to abrogate the diastolic dysfunction and the impaired myocardial flow rate which observed in nephrectomized non treated rats during reperfusion. In other study, it was reported that reversibly injured regions in heart can demonstrate persistent diastolic dysfunction despite complete systolic functional recovery 24 h after reperfusion, demonstrating a direct relationship between micro vascular obstruction and greater post-infarct LV diastolic dysfunction (Azevedo *et al.*, 2004).

In addition to the effect of melatonin to decrease the liability for reperfusion injury in this study , melatonin also in other studies reduced myocardial I/R injury (Reiter and Tan 2003 and Ceyran *et al.*, 2008) and decreased the myocardial infarct size in isolated rat heart (Sahna *et al.*, 2005)

Cardiac tissue reduced glutathione (GSH), as anti oxidative defense system, was reduced in I/R hearts in nephrectomized rats, but it was significantly increased in the nephrectomized melatonin-treated group. In line with this result, Melatonin was reported not only to supports several intracellular enzymatic antioxidant enzymes, including SOD and glutathione peroxidase (GSH-Px) but also induces the activity of -glutamylcysteine synthetase, thereby stimulating the production of another intracellular antioxidant glutathione (GSH). (Winiarska *et al.*, 2006)

The significantly decreased cardiac tissue nitrate in nephrectomized rats reflects the effect of I/R. ischemia-reperfusion can induce endothelial cell injury and activate endogenous NOS inhibitors,

leading to eNOS activity inhibition and NO production dysfunction. Reperfusion may produce also significant reactive oxygen species, such as the superoxide anion and hydroxyl radicals, which damage lipids and proteins. NO is a strong antioxidant and can substantially be degraded by reacting directly with free radicals. (Sun, *et al.*, 2009)

On the other hand, the significant increase in cardiac tissue nitrate, an indicator for nitric oxide level, with melatonin treatment may be considered as a triggering factor in preconditioning. NO has been shown to have an important role in PC and cardioprotection (Bolli 2001; Jones and Bolli 2006 and Murphy & Steenbergen 2008) plausible mechanisms whereby NO enhances resistance to cell death following ischemia-reperfusion include inhibition of calcium influx, antagonism of adrenergic stimulation, reduction in myocardial oxygen demands, opening of sarcolemmal and/or mitochondrial ATP-sensitive K(KATP) channels, and possibly direct antioxidant actions, such as inhibition of the effects of superoxide anion (O₂[•]) and peroxynitrite (ONOO) (Bolli , 2007). Pretreatment with melatonin increased NO bioavailability and decreased endothelin expression and consequently suggested to play a protective role in preserving both liver function and structure during ischemia and reperfusion injury (Zhang *et al.*, 2006) Moreover, it was postulated that the fundamental role of NO is in late PC: that is, NO plays a dual role in the late phase of this phenomenon, acting initially as the trigger and subsequently as the mediator of late PC (Bolli 2007).

The whole heart and left ventricular hypertrophic changes in the nephrectomized group is in line with the results in multiple studies. Cardiovascular remodeling has been described in STNx rats (Törnig *et al.*, 1996) and in uremic patients (Amann *et al.*, 1998). Also, cardiac hypertrophy and histological evidence of myocardial fibrosis has been seen in absence of hypertension 8 weeks after subtotal nephrectomy in rats (Donohoe *et al.*, 2000). Cardiac hypertrophy may be due to the associated volume overload leading to eccentric hypertrophy with a resultant myocyte to arteriolar capillary mismatch or due to the possible associated hypertension or higher angiotension II concentration leading to concentric hypertrophy (Berl and Henrich 2006) .

The ability of melatonin treatment to ameliorate these hypertrophic changes may be attributed to its anti- oxidant capacity. A previous study showed that oxidative stress plays an important role in cardiac hypertrophy (Araujo *et al.*, 2008).

Also the increased cardiac tissue nitrate, an indicator for nitric oxide level in melatonin treated group may be contributed in the reduced cardiac hypertrophy. NO has been shown to inhibit human

endothelial cell apoptosis and Myocardial apoptosis reported to be one of many important factors leading to cardiac remodeling as apoptosis is followed by compensatory hypertrophy (Sata *et al.*, 2000 and Agata *et al.*, 2002) however the effect was not sufficient compared to normal control rats.

Melatonin was found to prevent cardiac hypertrophy in hyperthyroid rats which may occurs as a result of the effect of melatonin on hemodynamic overload, NO availability, free radicals and lipid profile which were suggested all to modify myocardial remodeling (Ghosh *et a.*, 2007)

5-Conclusion:

The increased MDA which is an indicator for free radical generation in partial nephrectomized rats did not provide the supposed preconditioning effect against ischemic reperfusion injury in isolated hearts later on or its effect was inconclusive. Also, melatonin as a cardio-protective therapy improved the impairment of coronary flow rate due to renal failure under basal condition, but it was unable to ameliorate the basal systolic and diastolic dysfunction. However with I/R a situation of generation of more free radical, melatonin treatment exhibited less deterioration in the chronotropic and inotropic activity compared to nephrectomized non treated group. Melatonin did not support the diastolic dysfunction compared to its effect on systolic function. In addition, long term therapy of melatonin concomitant with deterioration of kidney function, appears to offer some sort of preconditioning and protection against I/R injury in particular during late reperfusion. Cardiac tissue GSH (anti-oxidant) and NO triggering by melatonin may be added to its free radical scavenging effect in the suggested protection and / or preconditioning.

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6-References:

- 1-Agata J, Chao L and Chao J (2002): Kallikrein gene delivery improves cardiac reserve and attenuates remodeling after myocardial infarction. *Hypertension*; 40(5):653-9.
- 2-Aiello S, Noris M, Todeschini M, Zappella S, Fogliani C, Benigni A, Corna D, Zoja C, Cavallotti D and Remuzzi G (1997): Renal and systemic nitric oxide synthesis in rats with renal mass reduction. *Kidney Int.* 50: 171–181.
- 3-Amann K, Breitbach M, Ritz E and Mall G (1998): Myocyte/capillary mismatch in the heart of uremic patients. *J. Am. Soc. Nephrol.*; 9(6):1018-22.
- 4-Araujo AS, Schenkel P, Enzweiler AT, Fernandes TR, Partata WA, Llesuy S, Ribeiro MF, Khaper N, Singal PK and Belló-Klein A (2008): The role of redox signaling in cardiac hypertrophy induced by experimental hyperthyroidism. *J. Mol. Endocrinol.*; 41(6):423-30.
- 5-Ayobe MH and Tarazi R C (1983): Beta receptors and contractility reserve in left ventricular hypertrophy. *Hypertension*; 5: 192- 197.
- 6-Azevedo CF, Amado LC, Kraitchman DL, Gerber BL, Osman NF, Rochitte CE, Edvardsen T and Lima JA (2004): Persistent diastolic dysfunction despite complete systolic functional recovery after reperfused acute myocardial infarction demonstrated by tagged magnetic resonance imaging. *Eur. Heart J.*; 25(16):1419-27.
- 7-Berl T and Henrich W (2006): Kidney-heart interactions: epidemiology, pathogenesis, and treatment. *Clin. J. Am. Soc. Nephrol.*; 1(1):8-18.
- 8-Bolli R (2001): Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: an overview of a decade of research. *J. Mol. Cell Cardio.*; 133: 1897–1918.
- 9-Bolli R (2007): Preconditioning: a paradigm shift in the biology of myocardial ischemia. *Am J Physiol Heart Circ Physiol.*; 292 (1):H19-27.
- 10-Bories PN and Bories C (1995): Nitrate determination in biological fluids by an enzymatic one step assay with nitrate reductase. *Clin. Chem.*; 41: 904-907.
- 11-Ceyran H, Narin F, Narin N, Akgun H, Ceyran AB, Ozturk F and Akcali Y.(2008) :The effect of high dose melatonin on cardiac ischemia-reperfusion Injury. *Yonsei. Med. J.*; 49(5):735-41.
- 12-Cuong DV, Kim N, Youm JB, Joo H, Warda M, Lee JW, Park WS, Kim T, Kang S, Kim H and Han J (2006): Nitric oxide-cGMP-protein kinase G signaling pathway induces anoxic preconditioning through activation of ATP-sensitive K channels in rat hearts. *Am. J. Physiol. Heart Circ. Physiol.*; 290: H1808-H1817.
- 13-Donohoe P, McMahon AC, Walgama OV, Bertaso F, Dockrell ME, Cramp HA, Mullen AM, Shattock MJ, Hendry BM and James AF (2000): L-type calcium current of isolated rat cardiac myocytes in experimental uraemia. *Nephrol. Dial. Transplant*; 15(6):791-8.
- 14-Downey JM and Cohen MV (2009): Why do we still not have cardioprotective drugs? *Circ. J.*; 73: 1171 – 1177.
- 15-Eissa S, Khalifa A and EL-Ahmady O (1990): Tumor markers in benign and malignant breast tissue CA15.3, CEA and ferritin in cytosol and membrane enriched fractions. *J.Tumor Marker Oncol.*; 5:220.

- 16-Esterbauer H and Cheeseman KH (1990): Determination of lipid peroxides. *Methods enzymol.*; 186: 407-421.
- 17-Fabiny DL and Ertingshausen G (1971): Automated Reaction-Rate Method for Determination of Serum Creatinine with the Centrifuge. *Clinical Chemistry*; (17):696-700.
- 18-Genade S, Ytrehus K and Lochner A (2006): Melatonin prevents cardioprotection induced by a multi-cycle ischemic preconditioning protocol in the isolated perfused rat heart. *Cardiovasc. J. S. Afr.*; 17(5):239-44.
- 19-Ghosh G, De K, Maity S, Bandyopadhyay D, Bhattacharya S, Reiter RJ and Bandyopadhyay A (2007): Melatonin protects against oxidative damage and restores expression of GLUT4 gene in the hyperthyroid rat heart. *J. Pineal Res.*; 42: 71-82.
- 20-Halestrap A.P, Clarke S.J, and Khaliulin I (2007): The role of mitochondria in protection of the heart by preconditioning. *Biochim. Biophys. Acta.*; 1767(8): 1007-1031.
- 21-Hatori N, Havu N, Hofman-Bang C, Clyne N and Pehrsson SK (2000): Myocardial morphology and cardiac function in rats with renal failure. *Jpn. Circ. J.*; 64: 606- 610.
- 22-Jollow DJ, Mitchell JR, Zampaglione N and Gillette JR (1974): Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology*; 11: 151-69.
- 23-Jones SP and Bolli R (2006): The ubiquitous role of nitric oxide in cardioprotection. *J. Mol. Cell Cardiol.*; 40: 16-23.
- 24-Kennedy D J, Vetteth S, Periyasamy S M, Kanj M, Fedorova L, Khouri S, Kahaleh M B, Xie Z, Malhotra D, Kolodkin NI, Lakatta E G, Fedorova O V, Bagrov AY and Shapiro J I(2006): Central Role for the Cardiotonic Steroid Marinobufagenin in the Pathogenesis of Experimental Uremic Cardiomyopathy Hypertension; 47:488-495.
- 25-Kim SW, Lee J, Paek YW, Kang DG and Choi KC (2000): Decreased nitric oxide synthesis in rats with chronic renal failure. *Korean Med Sci.*; 15(4):425-30.
- 26-Korkmaz A, Reiter RJ, Topal T, Manchester LC, Oter S and Tan D (2009): Melatonin: An Established Antioxidant Worthy of Use in Clinical Trials. *M O L M E D* 15(1): 2 4 3 - 5 0.
- 27-Labbé D, Vassault A, Cherruau B, Baltassat P, Bonète R, Carroger G, Costantini A, Guérin S, Houot O, Lacour B, Nicolas A, Thioulouse E and Trépo D (1996): Method selected for the determination of creatinine in plasma or serum. Choice of optimal conditions of measurement. *Ann. Biol. Clin.*; 54(8-9):285-298.
- 28-Lau T, Owen W, Yu YM, Noviski N, Lyons J, Zurkowski D, Tsay R, Ajami A, Young VR and Castillo L (2000): Arginine, citrulline, and nitric oxide metabolism in end-stage renal disease patients. *J. Clin. Invest.* 105: 1217-1225.
- 29-Lau T, Beauchamp P, Thuillez C and Richard V (2002): Endothelial protective effects of preconditioning. *Cardiovascular Research*; 55:466-473.
- 30-Lee YM, Chen HR, Hsiao G, Sheu JR, Wang JJ and Yen MH (2002): Protective effects of melatonin on myocardial ischemia reperfusion injury in vivo. *J. pineal Res.*; 33(2): 72-80.
- 31-Leineweber K, Heinroth-Hoffmann I, Pönicke K, Abraham G, Osten B and Brodde OE (2002): Cardiac beta-adrenoceptor desensitization due to increased beta-adrenoceptor kinase activity in chronic uremia. *J. Am. Soc. Nephrol.*; 13(1):117-24.
- 32-Liem DA, Honda HM, Zhang J, Woo D and Ping P (2007): Past and present course of cardioprotection against ischemia-reperfusion injury. *J. Appl. Physiol.*; 103: 2129-2136.
- 33-Malik J, Tuka V, Mokrejsova M, Holaj R and Tesar V (2009): Mechanisms of Chronic Heart Failure Development in End-Stage Renal Disease Patients on Chronic Hemodialysis . *Physiol. Res.* 58: 613-621.
- 34-Matsuzaki S, Szweda P.A, Szweda L.I, and Humphries K.M (2009): Regulated production of free radicals by the mitochondrial electron transport chain: Cardiac ischemic preconditioning. *Adv. Drug Deliv. Rev.*30; 61(14): 1324-1331.
- 35-Murphy E and Steenbegen C (2008): Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. *Physiol. Rev.*; 88: 581-609.
- 36-Nakamura M , Tahoma M , Yaoshimura H, Kubo H , Yobiki S and Machir K(1991): Coronary collateral vessels during the early period of acute myocardial infarction. Their development. *J. Cardiol.* 21: 605: 612.
- 37-Nivatpaumin T, Yipintsoi T, Penpargkul S and Scheuer J (1975): Increased cardiac contractility in acute uremia: Interrelationship with hypertension. *Am. J. Physiol.*; 299: 501-505.
- 38-O'Rourke ST, Hammad H, Delagrangre P, Scalbert E and Vanhoutte PM (2003): Melatonin inhibits nitrate tolerance in isolated coronary arteries. *British Journal of Pharmacology*; 139:1326-1332.
- 39-Ostadal B (2009): The past, the present and the future of experimental research on myocardial ischemia and protection. *Pharmacol. Rep.*; 61(1):3-12.
- 40-Paulis L and Šimko F (2007): Blood Pressure Modulation and Cardiovascular Protection by

- Melatonin: Potential Mechanisms Behind. *Physiol. Res.*; 56: 671-684.
- 41-Petrosillo G, Di Venosa N, Pistolese M, Casanova G, Tiravanti E, Colantuono G, Federici A, Paradies G and Ruggiero FM (2006): Protective effect of melatonin against mitochondrial dysfunction associated with cardiac ischemia reperfusion: role of cardiolipin. *FASEB J.*; 20: 269–276.
- 42-Pogan L, Bissonnette P, Parent L and Sauve R (2002): The effects of melatonin on Ca²⁺ homeostasis in endothelial cells. *J. Pineal Res.*; 33: 37-47.
- 43-Quiroz Y, Ferrebuz A, Romero F, Vaziri ND and Rodriguez-Iturbe B (2008): Melatonin ameliorates oxidative stress, inflammation, proteinuria, and progression of renal damage in rats with renal mass reduction. *Am. J. Physiol. Renal Physiol.*; 294(2):F336-44.
- 44-Raine AEG, Seymour AML, Roberts AFC, Radda GK and Ledingham JGG (1993): Impairment of cardiac function and energetics in experimental renal failure. *J. Clin. Invest*; 92:2934- 2940.
- 45-Reiter RJ and Tan DX (2003): Melatonin: a novel protective agent against oxidative injury of the ischemic/reperfused heart. *Cardiovasc Res.* 1; 58(1):10-9.
- 46-Sahna E, Parlakpinar H, Turkoz Y and Acet A (2005): Protective effects of melatonin on myocardial ischemia/reperfusion induced infarct size and oxidative changes. *Physiol. Res.*; 54(5):491-5.
- 47-Sata M, Kakoki M, Nagata D, Nishimatsu H, Suzuki E, Aoyagi T, Sugiura S, Kojima H, Nagano T, Kangawa K, Matsuo H, Omata M, Nagai R and Hirata Y(2000). Adrenomedullin and nitric oxide inhibit human endothelial cell apoptosis via a cyclic GMP-independent mechanism. *Hypertension*; 36(1):83-8.
- 48-Schiffirin EL, Lipman ML and Mann JF (2007): Cardiovascular involvement in general medical conditions. Chronic kidney disease: effects on the cardiovascular system. *Circulation.* 3; 116(1):85-97.
- 49-Schulz R, Kelm M and Heusch G (2004): Nitric oxide in myocardial ischemia/reperfusion injury. *Cardiovasc. Res.*; 61:402-413.
- 50-Searcy RL, Reardon JE and Foreman JA (1967): A new photometric method for serum urea nitrogen determination. *Am. J. Med. Technol.*; 33(1):15-20.
- 51-Stacchiotti A, Ricci F, Rezzani R, Li Volti G, Borsani E, Lavazza A, Bianchi R and Rodella LF (2006): Tubular stress proteins and nitric oxide synthase expression in rat kidney exposed to mercuric chloride and melatonin. *J. Histochem. Cytochem.*; 54(10):1149-57.
- 52-Sun HY, Xue FS, Xu YC, Li CW, Xiong J, Liao X and Zhang YM(2009): Propofol improves cardiac functional recovery after ischemia-reperfusion by upregulating nitric oxide synthase activity in the isolated rat hearts. *Chin. Med. J. (Engl.)*; 122(24):3048-54.
- 53-Szarszoi O, Asemu G, Vanecek J, Ostadal B and Kolar F (2001): Effects of melatonin on ischemia and reperfusion injury of the rat heart. *Cardiovasc. Drugs Ther.*1; 5:251–257.
- 54-Törnig J, Amann K, Ritz E, Nichols C, Zeier M and Mall G (1996): Arteriolar wall thickening, capillary rarefaction and interstitial fibrosis in the heart of rats with renal failure: the effects of ramipril, nifedipine and moxonidine. *J. Am. Soc. Nephrol.*; 7(5):667-75.
- 55-Wang W, Jittikanont S, Falk SA, Li P, Feng L, Gengaro PE, Poole BD, Bowler RP, Day BJ, Crapo JD and Schrier RW(2003): Interaction among nitric oxide, reactive oxygen species, and antioxidants during endotoxemia-related acute renal failure. *Am. J. Physiol. Renal Physiol.*; 284(3):F532-7.
- 56-Winiarska K, Fraczyk T, Malinska D, Drozak J and Bryla J (2006): Melatonin attenuates diabetes induced oxidative stress in rabbits. *J. Pineal Res.*40:168–76.
- 57-Yellon DM, Downey JM.(2003): Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev.*;83 (4):1113-51.
- 58-Zhang WH, Li JY and Zhou Y (2006): Melatonin abates liver ischemia/reperfusion injury by improving the balance between nitric oxide and endothelin. *Hepatobiliary Pancreat. Dis. Int.*; 5(4):574-9.
- 59-Zoccali C, Mallamaci F and Tripepi G (2004): Novel cardiovascular risk factors in end-stage renal disease. *J. Am. Soc. Nephrol.*; 15 (1):S77-80.
- 60-Zweier JL and Talukder MAH (2006): The role of oxidants and free radicals in reperfusion injury. *Cardiovascular Research*; 70: 181 – 190.
- 61-Zwirska-Korczala K, Jochem J, Adamczyk-Sowa M, Sowa P, Polaniak R, Birkner E, Latocha M, Pilc K and Suchanek R (2005): Influence of melatonin on cell proliferation, antioxidative enzyme activities and lipid peroxidation in 3T3-L1 preadipocytes--an in vitro study. *J. Physiol. Pharmacol.*; 56 (6):91-9.

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