The protective effect of melatonin on nicotine-induced myocardial injury in newborn rats whose mothers received nicotine

Annesi nikotin almış yeni doğan ratlarda melatoninin nikotine bağlı miyokardiyal hasarı önlemedeki rolü

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ABSTRACT

Objective: Nicotine, one of the most dangerous substances in tobacco, can pass the placenta and affect the fetal hemodynamics. The aim of this study was to evaluate the protective effects of melatonin on hearts of nicotine exposed newborn rats whose mothers received nicotine.

Methods: This is an experimental, randomized, controlled study. Study groups were composed of five groups of rats; high-dose nicotine (HDN), HDN+melatonin (HDNM), low-dose nicotine (LDN), LDN+melatonin (LDNM), control. Myocardial and plasma malondialdehyde (MDA), nitric oxide(NO), glutathione peroxidase (GSHPx) and superoxide dismutase (SOD) were analyzed and myocardial tissue was examined histopathologically. Comparisons of groups were done with Kruskal-Wallis one way analysis test. All pairwise multiple comparisons and the comparisons between control and other groups were done with Dunn's nonparametric multiple comparison test.

Results: Plasma and tissue MDA levels among groups were different ($p=0.001$ for plasma MDA and $p=0.001$ for tissue MDA). Plasma MDA levels of HDN, HDNM, LDN, and tissue MDA levels of HDN and LDN were significantly higher than in control group ($p<0.05$ for plasma MDA and for tissue MDA). Plasma and tissue NO levels among groups were also different ($p=0.011$ for plasma NO and $p=0.001$ for tissue NO). Plasma NO of LDN group was higher than of LDNM group, and plasma NO of LDNM group was lower than in control group ($p<0.05$). Tissue NO levels of HDN and LDN groups were higher than of control group ($p<0.05$). There was no difference between plasma GSHPx levels among groups ($p=0.221$) but statistically significant different was detected between tissue GSHPx levels among groups ($p=0.001$). Tissue GSHPx level was found lower in HDN group than in control group ($p<0.05$). Tissue GSHPx level of LDNM group was higher than of LDN group, and tissue GSHPx level of HDNM group was higher than of HDN group ($p<0.05$). A difference was found between plasma and tissue SOD among groups ($p=0.005$ for plasma SOD and $p=0.001$ for tissue SOD). Plasma SOD of LDN group was significantly lower than of HDNM and LDNM groups ($p<0.05$). Tissue SOD analyzes revealed lower levels in HDN and LDN groups than in control group ($p<0.05$). Severe cardiomyopathy was determined in HDN and LDN groups ($p<0.05$).

Conclusion: Nicotine exposure depletes myocardial antioxidant enzymes and increases free radicals and lipid peroxidation products. Melatonin particularly prevents the nicotine-induced cardiac injury as an antioxidant. *(Anadolu Kardiyol Derg 2008; 8: 243-8)*

Key words: Nicotine, secondary myocardial disease, oxidants, antioxidants, melatonin

ÖZET

Amaç: Tütündeki önemli zararlı maddelerden biri olan nikotin plasentayı geçebilir ve fetal hemodinamik dengeyi etkiler. Bu çalışmanın amacı: Hamilelik ve emzirme döneminde annesi nikotin almış yeni doğan ratlarda melatoninin nikotine bağlı miyokardiyal hasarı önlemedeki rolünü araştırmaktır.

Yöntemler: Bu çalışma deneysel, randomize ve kontrollü bir çalışmadır. Çalışma grupları sırasıyla yüksek doz nikotin (HDN), HDN+melatonin (HDNM), düşük doz nikotin (LDN), LDN+melatonin (LDNM), kontrol olmak üzere 5 grup rattan oluşmuştur. Miyokardiyal ve plazma malondialdehit (MDA), nitrik oksit (NO), glutatyon peroksidaz (GSHPx) ve süperoksit dismutaz (SOD) çalışıldı ve kalp dokularından histopatolojik analizler yapıldı. Grupların çoklu karşılaştırması Kruskal-Wallis varyans analizi ile yapıldı. Kontrol grubu ile diğer grupların karşılaştırılmasında ve kontrol grubu dışındaki grupların ikili karşılaştırılmasında Dunn's nonparametrik testi kullanıldı.

Bulgular: Gruplardaki plazma ve doku MDA düzeyleri arasında fark bulundu (plazma MDA düzeyleri için $p=0.001$ ve doku MDA düzeyleri için $p=0.001$). Plazma MDA seviyeleri HDN, HDNM ve LDN gruplarında, doku MDA düzeyleri HDN ve LDN grubunda kontrol grubuna göre yüksek bulunmuştur (plazma ve doku MDA düzeyleri için $p<0.05$). Gruplar arasındaki plazma ve doku NO düzeyleri de farklıydı (plazma NO düzeyleri için $p=0.011$ ve doku NO düzeyleri için $p\leq0.001$). Düşük doz nikotin grubu plazma NO seviyesi LDNM grubuna göre anlamlı yüksek, LDNM grubu plazma NO seviyesi kontrol grubundan düşük bulunmuştur ($p<0.05$). Yüksek doz nikotin ve LDN gruplarında doku NO seviyesi kontrol grubundan yüksek bulunmuştur ($p<0.05$).

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Gruplarda plazma GSHPx düzeyleri arasında fark saptanmazken (p=0.221) doku GSHPx düzeyleri arasında istatistiksel olarak anlamlı fark saptandı (p=0.001). Doku GSHPx seviyesi HDN grubunda kontrol grubuna göre belirgin azalmıştır (p<0.05). Yüksek doz nikotin grubuna göre HDNM grubunda LDN grubuna göre LDNM grubunda doku GSHPx seviyeleri yüksek bulunmuştur (p<0.05). Gruplar arasında plazma ve doku SOD değerleri için farklılık saptanmıştır (plazma SOD düzeyleri için p=0.005 ve doku SOD değerleri için p=0.001). Düşük doz nikotin grubu plazma SOD değeri HDNM ve LDNM gruplarına göre anlamlı düşük bulunmuştur (p<0.05). Doku SOD seviyeleri incelendiğinde HDN ve LDN grubunda kontrol grubuna göre belirgin azalma tespit edilmiştir (p<0.05). Histopatolojik olarak HDN ve LDN gruplarında ağır kardiyomiyopati tespit edilmiştir (p<0.05).

Sonuç: Nikotine maruz kalmak miyokardiyal antioksidan enzimlerde azalmaya, serbest radikal ve lipid peroksidasyon ürünlerinde artışa neden olmaktadır. Melatonin antioksidan olarak kardiyak hasarı kısmen önlemektedir. *(Anadolu Kardiyol Derg 2008; 8: 243-8)*

Anahtar kelimeler: Nikotin, sekonder miyokardiyal hastalık, oksidan, antioksidan, melatonin

Introduction

The increase in smoking rate in women, results in increase in pregnant smokers. Hazardous effects of cigarette smoking on pregnant women and fetus are important and must be prevented. Cigarette contains lot of toxins and the most known toxic component is nicotine. Nicotine enters the fetal circulation by passing the placenta (1, 2). Maternal injection of nicotine increases vascular resistance, decreases fetal heart rate and umbilical blood flow. In animal studies, it was demonstrated that nicotine deteriorates the fetal blood flow, oxygenation, and acid-base balance (3). In rat and human studies, it was shown that nicotine causes vasopressin release, which results in umbilical vasoconstriction (4, 5).

The penetration of nicotine to breast milk has been known since 1933 (6). Nicotine concentration in breast milk is 3 times more than plasma concentration and cotinine concentrations in the urine of breastfed babies were found higher (6). In animal studies, the free oxygen radicals and lipid peroxidation products were found to be increased by choric nicotine exposure (7).

The pineal hormone melatonin neutralizes free oxygen radicals, reactive oxygen products, and also stimulates antioxidant enzymes: superoxide dismutase, glutathione peroxidase and glutathione reductase (8).

There are some studies about the relation between smoke/nicotine exposure and sudden infant death, nicotine related hypoxia and cardiac changes (9), but there are few studies about the effects of pregestational and prenatal smoking on fetal cardiovascular system, and the effects of free oxygen radicals, lipid peroxidation products, and the protective effects of melatonin on nicotine-induced cardiac toxicity.

The aim of this study was to evaluate the relation between nicotine exposure with free oxygen radicals and lipid peroxidation products, and the protective effects of melatonin on nicotine exposed rat infants.

Methods

This is an experimental, randomized, and controlled study. Three months old, female, 170-220gr weighed Sprague- Dawley rats (n=49) were divided into 5 groups. The rats were divided into five groups randomly (random numbers method). High dose nicotine (HDN) (n=10), high dose nicotine and melatonin (HDNM) (n=9), low dose nicotine (LDN) (n=10), low dose nicotine and melatonin (LDNM) (n=10), and control group (distilled water) (n=10). All procedures were performed in the Experimental Animals Breeding and Research Center of Medical Faculty of the Erciyes University. Animal care was carried out with the prior approval of the Animal experimental Ethics Committee of the Erciyes University. and was in full compliance with Turkish Law 6343/2, Veterinary Medicine Deontology Regulation 6.7.26, and with Helsinki Declaration of Animal Rights. In HDNM group 1 rat died in the second day of the study, but the cause could not be detected.

Nicotine in dose of 6 mg/kg/day was injected by subcutaneous way to high dose groups, and 1 mg/kg/day - to low dose groups, from the beginning of randomization to the end of lactation period, (average 11 week). Melatonin (Sigma Chemical Company, Sigma, St. Louis. MO) was given in dose of 10 mg/kg/day by intraperitoneal route after mating, during pregnancy and lactation period (10), and control group received distilled water. At the end of lactation period, on 21st day of infant rats, one infant rat was selected from each cage randomly and rats were sacrificed, blood and heart tissue samples were taken for analysis of glutathione peroxidase (GSHPx), superoxide dismutase (SOD), malondialdehyde (MDA), nitric oxide(NO) and, histopathological study.

Plasma and myocardial GSHPx activity was calculated by Paglia and Valentine's combined enzymatic method (11) by measuring the peroxidation rate of H_2O_2 to glutathione reaction. Plasma and myocardial SOD activity levels were measured by Sun et al.'s method (12): xanthine oxidase was used for superoxide producer, and inhibition of nitro blue tetrazolium (NBT) reduction. Myocardial SOD activity was calculated by division of specific activity to total protein, which was calculated by Lowry's method (13). Tissue MDA levels were measured by Ohkawa et al's method (14). Measured activities were divided by total tissue protein. Plasma MDA activity, was measured by Jain's method (15). Tissue NO levels were analyzed by Moshage et al.'s method (16).

Hematoxylene/eosin stained myocardial tissue samples were evaluated histopathologically with light microscope. Severity of the histopathological changes and cardiomyopathy degree was used to determine the total score (0 to 3 points) (17). Scoring was done according to the respective changes: a. Myocardial swelling and interstitial edema (+1), b. Myocardial fiber disorganization (+1), c. Myocardial fiber necrosis (+1), d. Myocardial fiber vacuolization (+1). Total scores were calculated using these parameters and were represented as 0 point- no evident cardiomyopathy, 1 point- mild cardiomyopathy, 2 points- moderate cardiomyopathy, 3 points- severe cardiomyopathy.

Statistical Analysis

Analyses were performed with the statistical package for scientist SIGMA STAT for Windows version 3.10 (Systat Software Inc. San Jose, CA, USA). Data were expressed as median,

minimum and maximum values. Comparison of plasma and tissue levels of MDA, NO, GSHPx, SOD between groups were made by using Kruskal-Wallis One Way Analysis. All pairwise multiple comparisons and the comparisons between control and other groups were done with Dunn's nonparametric multiple comparison test. Chi-square test was used to evaluate the difference between discrete parameters. Statistical significance was set at $p < 0.05$.

Results

The plasma MDA levels of HDN, HDNM, LDN groups and tissue MDA levels of HDN and LDN groups were significant higher than İN control group ($p<0.05$, Table 1). Pairwise multiple comparisons of groups revealed no significant difference within groups.

When NO levels of groups were compared, the plasma NO level of LDNM group was significantly lower than İN control group ($p<0.05$). Plasma NO level of LDNM group was significantly lower than of LDN group. Tissue NO levels of HDN and LDN groups were significantly higher than of control group ($p<0.05$). Tissue NO levels in LDNM group was lower than in HDN group ($p<0.05$, Table 2).

Table 1. Plasma and tissue MDA levels

Group	n	Plasma, µmol/L Median (Min-Max)	Tissue, nmol/µgr protein Median (Min-Max)
HDN	10	0.81 (0.34-1.58)[a]	0.29 (0.20-0.41)[a]
HDNM	9	0.60 (0.26-0.77)[a]	0.14 (0.10-0.25)
LDN	10	0.59 (0.32-1.52)[a]	0.20 (0.16-0.49)[a]
LDNM	10	0.46 (0.30-0.65)	0.15 (0.10-0.21)
Control	10	0.36 (0.24-0.48)	0.12 (0.08-0.18)
*Chi-square		17.587	30.390
*p		<0.05	<0.05

* - Kruskal Wallis test used to compare plasma and tissue MDA levels among groups
[a] p<0.05- differences are significant, Dunn's nonparametric multiple comparison test between control and other groups
HDN- high-dose nicotine, HDNM- high-dose nicotine+melatonin, LDN- low-dose nicotine, LDNM- low-dose nicotine+ melatonin, MDA- malondialdehyde

Table 2. Plasma and tissue NO levels

Group	n	Plasma, µmol/L Median (Min-Max)	Tissue, nmol/µgr protein Median (Min-Max)
HDN	10	113.50 (77.50-257.50)	2.90 (2.10-4.10)[a, c]
HDNM	9	85.50 (52.50-230.50)	1.96 (1.24-3.00)
LDN	10	186.00 (100.50-291.50)[b]	2.53 (2.11-2.91)[a]
LDNM	10	93.50 (33.50-125.50)[a]	1.68 (1.41-2.34)
Control	10	133.50 (48.50-235.50)	1.47 (1.00-2.07)
*Chi-square		13.033	29.170
*p		<0.05	<0.05

* -Kruskal Wallis test used to compare plasma and tissue NO levels among groups
[a] p<0.05, Dunn's non parametric multiple comparison test between control and other groups
[b] p<0.05, Dunn's non parametric multiple comparison test within groups, LDN versus LDNM
[c] p<0.05, Dunn's non parametric multiple comparison test within groups, HDN versus LDNM
HDN - high-dose nicotine, HDNM- high-dose nicotine+melatonin, LDN- low-dose nicotine, LDNM- low-dose nicotine+ melatonin, NO- nitric oxide

The comparison of plasma GSHPx levels of groups with control group and pairwise multiple comparisons of groups revealed no significant difference ($p>0.05$). The tissue GSHPx levels were found decreased in HDN group as compared with control group ($p<0.05$). Tissue GSHPx levels of LDNM group was higher than of LDN and HDN groups, and tissue GSHPx levels of HDNM group was higher than of LDN and HDN groups ($p<0.05$, Table 3).

The comparison of plasma SOD levels of groups with control group revealed no significant difference ($p>0.05$). When the groups were compared with each other according to the plasma SOD levels, in LDN group plasma SOD level was higher than in HDNM and LDNM groups ($p<0.05$). Tissue SOD level of LDNM group was higher than of LDN and HDN groups, and tissue SOD levels of HDNM group was higher than of LDN and HDN groups ($p<0.05$, Table 4).

When histopathological findings were compared (Fig. 1, 2) the cardiomyopathy scores of HDN and LDN groups were significantly

Table 3. Plasma and tissue GSHPx levels

Group	n	Plasma, µmol/L Median (Min-Max)	Tissue, nmol/µgr protein Median (Min-Max)
HDN	10	0.15 (0.10-0.96)	0.09 (0.01-0.20)[a]
HDNM	9	0.26 (0.13-0.37)	0.37 (0.20-0.59)[d, e]
LDN	10	0.16 (0.11-0.61)	0.14 (0.02-0.27)
LDNM	10	0.23 (0.09-0.79)	0.36 (0.25-0.59)[b, c]
Control	10	0.26 (0.11-0.35)	0.24 (0.13-0.44)
*Chi-square		5.719	31.373
*p		>0.05	<0.05

* -Kruskal Wallis test used to compare plasma and tissue GSHPx levels among groups
[a] p<0.05, Dunn's non parametric multiple comparison test between control and other groups
[b] p<0.05, Dunn's nonparametric multiplecomparison test within groups, LDNM versus LDN
[c] p<0.05, Dunn's nonparametric multiple comparison test within groups, LDNM versus HDN
[d] p<0.05, Dunn's nonparametric multiple comparison test within groups, HDNM versus HDN
[e] p<0.05, Dunn's nonparametric multiple comparison test within groups, HDNM versus LDN
GSHPx- glutathione peroxidase, HDN- high-dose nicotine, HDNM- high-dose nicotine+melatonin, LDN- low-dose nicotine, LDNM- low-dose nicotine+ melatonin

Table 4. Plasma and tissue SOD levels

Group	n	Plasma, µmol/L Median (Min-Max)	Tissue, nmol/µgr protein Median (Min-Max)
HDN	10	1.05(0.68-1.24)	12.63(6.63-18.60)[a]
HDNM	9	1.24(0.83-2.70)[b]	18.89(15.00-27.77)[f, g]
LDN	10	0.92(0.66-1.97)	14.39(8.33-18.72)[a]
LDNM	10	1.21(1.05-2.96)[c]	20.57(16.45-27.04)[d, e]
Control	10	1.11(0.88-1.47)	18.69(11.70-21.62)
*Chi-square		14.729	21.796
*p		<0.05	<0.05

* -Kruskal Wallis test used to compare plasma and tissue SOD among groups
[a] p<0.05, Dunn's non parametric multiple comparison test between control and other groups
[b] p<0.05, Dunn's nonparametric multiple comparison test within groups, HDNM versus LDN
[c] p<0.05, Dunn's nonparametric multiple comparison test within groups, LDNM versus LDN
[d] p<0.05, Dunn's nonparametric multiple comparison test within groups, LDNM versus HDN
[e] p<0.05, Dunn's nonparametric multiple comparison test within groups, LDNM versus LDN
[fp]<0.05, Dunn's nonparametric multiple comparison test within groups, HDNM versus HDN
[gp]<0.05, Dunn's nonparametric multiple comparison test within groups, HDNM versus LDN
HDN- high-dose nicotine, HDNM- high-dose nicotine+melatonin, LDN- low-dose nicotine, LDNM- low-dose nicotine+ melatonin, SOD- superoxide dismutase

higher than of other groups and the HDN group cardiomyopathy score was higher than of LDN group ($p<0.05$). The cardiomyopathy scores of HDN and HDNM were similar, while the cardiomyopathy score of LDNM was lower than of LDN group ($p<0.05$, Table 5).

Discussion

Smoking is becoming more prevalent in woman population. Smoker women also smoke in pregnancy and pregestational period. Although the detrimental effects of smoking are known, few is known on smoking effects on fetus in pregnancy. The hazardous effects of smoking on fetus and infant may be preventable by quitting of smoking. In recent years, several experimental studies on preventing strategies and agents for smoke related toxicity on fetus and infant have been published. Nicotine, the main toxic component of tobacco, was used in experimental studies, because nicotine can penetrate to breast milk, and affect the infant. Smoking in pregnancy, results in chronic hypoxia, which causes organ damage in developing organism (2). In this study nicotine administration to the mother rats during perinatal period, resulted in nicotine exposure to the infant rats. By penetration of nicotine through breast milk to the infants the MDA and NO levels in the organism increased and caused oxidative damage. By melatonin administration, GSHPx and SOD levels increased partially. The effect of melatonin was significant in LDNM group, but melatonin was less effective in HDNM group. So, the increased dose of nicotine causes much more damage.

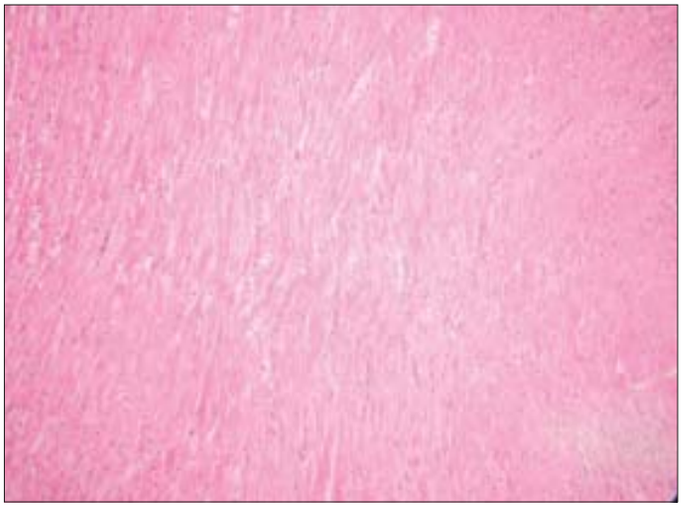

Figure 1. Normal myocardial tissue (Control Group, Hematoxylene/eosin x 100)

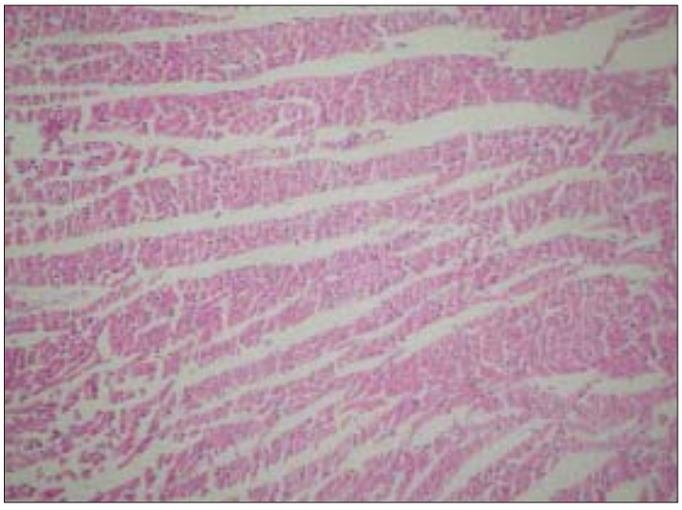

Figure 2. Myocardial swelling, interstitial edema, disorganization, and necrosis (High Dose Nicotine Group, Hematoxylene/eosinx100)

In nicotine toxicity, the main mechanism of action is altering the cellular oxidant-antioxidant system (7). There are many studies that declare the pathophysiology of nicotine-induced organ damage, which is related with the increase in lipid peroxidation (19, 20). Ashakumary and Vijayammal demonstrated that, nicotine promotes atherogenesis by increasing lipid peroxidation in a rat model (7). In present study, tissue MDA levels of nicotine-received groups were higher than control group, which is in parallel to literature reports. The tissue MDA levels were lower in melatonin-received groups; which allows us to consider the melatonin has a protective effect on heart by altering the lipid peroxidation and decreasing the nicotine-induced cardiomyopathy. This is a dose dependent effect because high dose nicotine groups had higher MDA levels. Plasma MDA levels were similar in LDNM group and control group, but were significantly higher in HDNM group than in control group.

Increased NO production results in oxidative injury, apoptosis, and/or necrosis of heart tissue (21). High levels of NO with superoxide radicals produce a very toxic compound peroxynitrite, which causes severe cytotoxicity (22, 23). In our study the tissue NO levels of HDN and LDN groups were found higher than of control group. Higher levels of NO have an important role in the pathogenesis of nicotine-induced myocardial injury.

There are several studies about the detoxifying effect of melatonin on NO (24, 25). Gilad et al. (24) reported the inhibitor activity of melatonin on peroxynitrite related oxidant process of NO and superoxide reaction. Blanchard et al. (25) demonstrated that NO can be inhibited by melatonin with one molecule oxygen. It was supposed that melatonin is the substrate of peroxynitrite and decreases the NO toxicity by competitive inhibition. In our study melatonin application decreased the NO and MDA levels.

The SOD and GSHPx have effective role in free oxygen radical detoxification (26). Florek et al. (27) studied SOD and

Table 5. Percentages of cardiomyopathy scores in groups

Group	n	No evident cardiomyopathy	Mild	Moderate	Severe
HDN	10	-	0	30	70[a]
HDNM	9	12	33	11	44[a]
LDN	10	-	30	0	70[a]
LDNM	10	-	10	70	20[a, b]
Control	10	80	20	0	0

Chi-square test, [a]$p<0.05$ when compared with control group, [b]$p<0.05$ when compared with LDN group
HDN- high-dose nicotine, HDNM- high-dose nicotine+melatonin, LDN- low-dose nicotine, LDNM- low-dose nicotine+ melatonin

GSHPx in heart, liver, kidneys and placenta of 21–day-old smoke exposed rats, and found SOD and GSHPx hypoactivation in these tissues. Also chronic hypoxia caused by smoking results in myocardial antioxidant system failure. In our study, tissue GSHPx levels of HDN group were found decreased than of control group. In hypoxic conditions, GSHPx and SOD activities were decreased by nicotine administration. We think that oxidants and antioxidants have an important role in myocardial injury pathogenesis.

Melatonin can penetrate cellular compartments by its lipid solubility. By this way, melatonin can protect DNA and membrane lipids from free oxygen radicals, also acts by activating antioxidants enzymes like GSHPx, SOD, and catalase (28). Previous studies revealed that melatonin acts as an antioxidant by: a) direct free oxygen radical scavenger, b) stimulation of antioxidant enzymes, c) decreasing the electron transport (by this way decreasing superoxide radical production) and increasing the affectivity of mitochondrial oxidative phosphorylation, and d) increasing the other antioxidant activities. Melatonin also can easily penetrate the placenta and increase the antioxidant activity in fetus. Kotler et al. and Antolin et al. studied the activity of antioxidant enzyme gene expression property of melatonin and found a positive effect on gene expression of antioxidant enzymes (29, 30). In our study, the pharmacological doses of melatonin (10 mg/kg) increased the GSHPx and SOD activities in HDNM and LDNM groups and decreased NO and MDA levels

Lough et al (31), exposed to smoke pigs for 12-15 weeks (8 cigarettes daily for 5 day per week) and at the end of the study the heart weight of study group was found increased as compared with control group. Swelling, mitochondrial lipid deposition, and increased autophagolysosomal activity were found in histopathological examination of heart tissues (31). Rajs et al. (32) studied the pericardial cotinine concentrations in subjects died from SIDS whose mother and father were smokers, and using myocardial histological analyses revealed focal necrosis, swelling, and inflammatory changes in high cotinine detected group. In our study, the histopathological examination revealed severe cardiomyopathy characterized by swelling, interstitial edema, disorganization, and necrosis in all rats of HDN group and in 7 rats of the LDN group. The higher cardiomyopathy scores in HDN group than in LDN group provide evidence the nicotine-induced cardiomyopathy is dose dependent. In LDNM group there was no necrosis on histopathological examination, and the cardiomyopathy scores of LDNM group were significantly lower than of LDN group.

Limitations of the study

This study has some limitations: smoking conditions were not identical to the real smoking habit, it would be better to expose the nicotine by inhaling cigarette smoke instead of direct nicotine administration. However, we wanted to be sure that we gave enough nicotine. Secondly, the sample size might affect our results, but there were economical limitations.

Conclusion

This study demonstrated some toxic effects of nicotine and antioxidant effects of melatonin in nicotine-induced myocardial injury. It was detected that melatonin acts by increasing GSHPx and SOD activities, and decreasing the myocardial NO and MDA levels. Studies on cigarette smoking and its toxicity may reveal the injury mechanism, and may aid to establish protective strategies, and declaration of the studied results may reduce the smoking rate in the world.

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