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THE ANALYSIS OF FATTY ACİDS LEVEL IN RAT HEARTH TISSUES PERFORMED MYOCARDIAL ISCHEMIA-REPERFUSION INJURY AND MELATONIN

ABSTRACT

Melatonin (N-acetyl-5-methoxytryptamine),the main secretory product of the pineal gland, is a free radical scavenger that has been found to protect against lipid peroxidation in many experimental models. In the present many study the effect of melatonin on lipid peroxidation of long chain polyunsaturated fatty acids located in rat. The most sensitive fatty acids in the heart were the monounsaturated eicosenoik acid (C20:1 n-6), polyunsaturated arachidonic acid (C20:4 n-6) docosapenthonoic acid (C22:5 n-3) and docosahexanoic acid (C22:6 n-3) which compared with the corresponding control values. I/R performed group showed remarkable differerences ($p<0.05$).In this study, myokardial- iscemia reperfusion is determined to cause increase or decreasa in different types of fatty acids.

Keywords: Melatonin, Myocardial Ischemia-Reperfusion, Tissues, Fatty acids, Gas Chromatography

SIÇANLARIN KALP DOKULARINDA YAĞ ASİTLERİ DÜZEYLERİNİN I/R VE MELATONİN UYGULAMALARIYLA DEĞİŞİMLERİNİN İNCELENMESİ

ÖZET

Melatonin- beyin epifizinin ana ürünü olan salgısı-birçok deneyde yağ peroksidasyonuna karşı savaşan serbest radikal bir temizleyici olduğu gözlemlendi. Mevcut birçok çalışmalarda-farede yapılan-melatoninin çoklu doymamış yağ asitlerine etkisi olduğu belirlendi. Araşidonik asit (C20:4 n-6) dokosapentaenoik asit (C22:5 n-3, tekli doymamış yağ asitleri, eicosenoik asit (C20:1 n-6)ve dokosaheksaenoik asit (C22:6 n-3) karşılık gelen kontrol değeri ile karşılaştırıldı. I/R çalışılan grup büyük farklılıklar gösterdi. Bu çalışmada miyokardiyal iskemi reperfüzyonunun farklı yağ asitleri türlerinde artmalara veya azalmalara sebep olduğu belirlendi.

Anahtar Kelimeler: Melatonin, Miyokardiyal İskemi Reperfüzyon, Doku, Yağ Asitleri, GC.

1. INTRODUCTION (GİRİŞ)

Melatonin (N-acetyl-5-metoxytryptamine) is an indole synthesized mainly by the the pineal gland of all mammals including humans (Reiter RJ 2003) and also, it is produced in a limited number of organs in tract (Tang PL 1997).Melatonin possesses free radical scavenging activity. Eperimental studies have shown that melatonin directly schavengens the hydroxyl radical, peroxyl radical, peroxynitrite anion and singlet oxygen. Furthermore, this tryptophan derivative stimulates a number of antioxdative enzymes and stabilizes cell membranes (Allegra 2003).It is demosrated that myocardial ischemia-reperfusion injury is reated to increasad free radical generated and intracellular calcium overload especially during the period of reperfusion (Sahna E,2006). Moreover, melatonin have been shown to reduced the reduce the ischemia-reperfusion injury in various model of model of experimental I/R injuries (Deniz İ, 2006).

Several publication indicated that populations in Greenland and Japan exhibited a low incidence of sudden cardiac death (Bang, 1971).This was associadet whit a high n-3 PUFA intake. Since these early studies, numerous epidemiological. Investigations have been carried out. Most have reported the protective effect of n-3 PUFAs (Bang H. O 1981, Hodgson J., 1993, Osler M 2000) but others have not shown any correlation between the intake n-3 PUFAs and CHD mortality (Keys A 1986, Oomen C. M 2000, Marckmann P 1999) The firs reference to the cardioprotective effects of fish oil (rich in *n* − 3 PUFA) comes from Greenland Eskimo population and dates back to 1976—the year when also first statin was invented (H.O. Bang 1976 A. Endo 1976) Beside statins and life style changes, the *n*−3 PUFAs, espe- cially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), could play an important role in cardiovascular disease prevention (particularly in patients with hearth failure R.B. Singh 2002). Lipid peroxidation is a free radical chain reaction (Kappus HA, (1987)) which causes the degeneration of cell membranes. Most products of lipid peroxidation are known to have mutagenic and/or carcinogenic properties (Fang, J.L. 1997). Free radical species affect all species affect all importanat componenets o cells shuch as lipids, proteins, carbohydrates and nucleic acids (Sarkar, 1997). Lipids are oxidized by free radical attack, and henece membranes are damaged (Cheesman, 1993).

The ability of melatonin lipid peroxidation has been studied in various oxdatives stres models of rat(Tang, 1997 and Deniz, 2006). Tang et al (3)reporte that the direct effect of iron on lipid peroxidation in Cell membranes from the brain, heart, kidney and liver of the male Sprague-Dawley rat was markedly reduced by melatonin in Tang 1997.The action of melatonin on the peroxidation of lipids in biological membranes has been studied in many laboratories (Garcia JJ 1997, Rudzite V 1999)but to a few studied have shown how long chain PUFA such as C20:4 n-6, C22:5 n-3 and C22:6 n-3 are protected by this indole or its derivatives during the lipid peroxidation process (Leaden, 2002 and Gavazza, 2004). Polyunsaturated fatty acids, specifically the n-3 series have been implicated in the prevention of various human diseases, including obesity, diabetes, coronary heart disease and stroke and inffammatory and neurologic diseases and regulate the expression of genes in various tissues, including the liver, heart, adipose tissue and brain (Sampath, 2004). The presented data offer the support to the hypothesis that pharmacological amounts of melatonin effectively reduce oxidative stress and display antihyperlipidemic activity. The decreased levels of thiobarbituric acid reactive substances found in the brain and liver tissues of melatonin-treated rats suggest that melatonin may provide an effective protection against lipid peroxidation. In addition, melatonin reduces the free radical-induced alteration of microsomal membrane fluidity during induced lipid peroxidation (Garcia, 1997 and Longoni, 1998). A remarkable body of evidenc indicates that melatonin experts antioxidant protection in different experimental systems both in vitro and in vivo (Pandi-Perumal, 2006, Reiter, 2007 and Tan, 2007).

2. RESEARCH SIGNIFICANCE (ÇALIŞMANIN ÖNEMİ)

Melatonin (N-acetyl-5-methoxytryptamine), the main secretory product of the pineal gland, is a free radical scavenger that has been found to protect against lipid peroxidation in many experimental models. Cardiac-Ischemia reperfusion damage can appear in most clinic occassional; likes iscemic heart disease. We hope that this study will provide guidance to related with regulation of iscemic heart disease.

3. MATERİALS AND METHODS (MATERYAL VE METOT)

3.1. Experimental Groups (Deney Grubları)

Male Wistar rats weighing 200–250 g were placed in a temperature-(21□□2^{O}C) and humidty-(60 □5%) controlled room in which a 12–12h light-dark cycle was maintained. Thirty rats were divided into six groups equally:-group1 (contro) received normal saline intraperitoneally(i.p) for 15 days-group 2 (L-NAME group) received non-specific NOS-N inhibitor LAME (40mg/kg, i.p.) for 15 days and melatonin's vehicle (ethanol) the last 5 days;-group 3 (melatonin group) received both L-NAME (40 mg/kg) for 15 days and melatonin (10 mg/kg, i.p) for the last 5 days of this time; and groups 4–6 were similar to groups 1–3, respectively, but at the end of 15 days, these groups were subjected to the myocardial IR.L-NAME (Fluka Chemie, Switzerland) was dissolved in normal saline (0.09% NaCl wt/vol). Melatonin (Sigma Chemical Co., St Louis, Missouri, USA) was dissolved in ethanol and Downloaded By:(TÜBTAK EKUAL) At: 14:01 15 October 2008 Hypertension, Reperfusion Injury, Myeloperoxidase 675 further diluted in salina(0.09% NaCl wt/vol) to give a final concentration of 1%. All experiments in this study were performed in accordancewith the guidelines for animal research from the National Institutes of Health and were approved by the Local Committee on Animal Research.

3.2. Ischemia-Reperfusion Procedure (İskemi-Reperfuzyon Uygulama)

Rats were anesthetized with urethane (1.2–1.4 g/kg) admimistered intraperitoneally. The trache was cannulated for artifical respiration. The chest was opened by a left thoracotomy. Positive-pressure artificial respiration was started immidiately with room air, using a volume of 1.5mL/100g body weight at a rate 60 beats/min to maintain normal pCO2, pO2, and pH parameters. A6/0 silk suture attached to a 10-mm micropoint reverse-cutting needle was quickly placed under the left main coronary artery. The artery was occluded for 30 min and then reperfused for 120 min (Sahna E, 2005).

3.3. Measurement of Fatty Acid Composition (Yağ Asitleri Ölçümü)

The lipids of the tissue samples were extracted with chlorophorm-metanol (2:1v/v) according to the method of Christie ve Folch et al. Tissue sample was homogenized with 10 ml hexane-isopropanol mixture. The homogenate was centrifuged at 5000 rpm for 5 min at 4°C and parts of tissue remnants were precipitated. Fatty acids in the lipid extracts were converted into methyl esters including 2%sulfuric acid (v/v) in methanol. The mixture was vortexed and then kept at 50°C for 12h.Then, after being cooled to room temperature, 5ml of 5%sodium chloride was added and then it was vortexed. Fatty acid methyl esters were extracted with 2×5ml hexane. Fatty acid methyl esters were treated with 5 ml 2% KHCO3 solution and then the hexane phase was evaporated by the nitrogen flow and then by dissolving in 0.5ml fresh hexane (Christie, Folch, Peter 1990). They werw taken to auto sampler vials.

3.4. Gas Chromatographic Analysis of Fatty Acid Methyl Esters (Gas Kromatografisi İle Yağ Asitleri Tayini)

Methyl esters werw analyzed with the Shimadzu GC-17 Ver. 3gas chromatography (Kyoto, Japan). For this analysis, 25 m of long Permobond® FFAP-0.1 µm capillary column (Machery-Nagel, Germany) with an iner diameter of 0.25 µm and a thickness of 25 micron film was used. During the analysis, the colon temperature was kept at 120-220 °C and the increment of temperature was 3°C/min, injection temperature was kept at 240°C and the detector temperature was kept at 280°C. The nitrogen carrier gas flow was 1 ml/min. The methyl esters of fatty acids were identified by comparison with authentic external standard mixtures analyzed under the same conditions. After this process, the necessary programming was made and the Class GC 10 software version 2.01 was used to process the data.

3.5. Statistical Analyses (İstatistiksel Analiz)

Data are expressed as the mean±SD. All statistical analyses were performed using SPSS 10.0 pack program (Chicago, IL, USA). Statistical comparisons were made with Mann- Whitney-U test determining differences among groups. A value of $P<0.05$ was considered significant.

Table 1. Chemical composition (relative % peak area) of the fatty acids in the Cardiac-Ischemia reperfusion, melatonin-Ischemia and control

Fatty Acids		Control X ± Sx(n=4)	C-Ischemia X ± Sx(n=4)	Mel-Ischemia X ± Sx(n=4)	
Miristik acid	(C14:0)	0.56±0.29	0.59±0.20	0.37±0.04	b
Pentadecanoic	(C15:0)	0.20±0.02	0.22±0.01	0.19±0.03	
Palmitic acid	(C16:0)	16.30±1.29	15.80±1.74	14.77±0.15	ab
Palmitoleic acid	(C16:1)	1.50±0.27	1.36±0.19	0.94±0.27	ab
Heptadecanoic	(C17:0)	0.82±0.07	0.75±0.05	0.5 ±0.08	ab
Stearic acid	(C18:0)	17.63±1.65	16.49±0.87	18.70±0.76	b
Oleic acid	(C18:1)	12.58±1.09	12.73±1.84	9.52±0.74	ab
Linoleic acid	(C18:2)	26.33±1.79	26.96±1.15	24.32±1.06	b
Linolenic acid	(C18:3)	0.45±0.15	0.62±0.12	0.48 ±0.07	
Eicosenoic	(C20:1)	0.58±0.18	0.75±0.06	0.62±0.09	b
Eicosatetraenoic	(C20:4)	11.27±0.82	11.54±1.49	13.59±0.93	ab
Eicosapentaenoic	(C20:5)	0.44±0.08	0.42±0.08	0.43±0.10	
Docosenoic acid	(C22:1)	0.33±0.15	0.26±0.09	0.34±0.11	
Arachidonic acid	(C22:4)	0.15±0.04	0.13±0.04	0.12±0.02	
Docosapentaenoic	(C22:5)	1.65±0.16	1.63±0.15	1.94±0.17	ab
Docosahexaenoic	(C22:6)	9.20±1.14	9.63±0.81	12.90±0.84	ab
ΣSATURATE		35.38±0.31	33.86±1.22	34.63±0.81	
ΣSATURATE		64.61±0.31	66.15±1.22	65.35±0.80	
ΣMUFA (1)		15.10±1.02	15.35±2.33	11.46±1.10	ab
ΣPUFA (2, 3, 4, 5, 6)		49.51±1.92	50.98±3.20	53.92±0.94	ab

a: $p<0.05$,
b: $p<0.001$,
*: not detectable, Values are means ± SE

4. RESULTS (SONUÇ)

I/R performed group showed remarkable differerences ($p<0.05$).In this study, myokardial- iskemi reperfusion is determined to cause increase or decreasa in different types of fatty acids. Tablo.1 summarize the results obtained in the heart respectively. Melatonin at groups caused a significant differents in the levels of Ischemia, free fatty acids in the heart when compared to controls. The most sensitive fatty acids in the heart were the monounsaturated eicosenoik acid (C20:1 n-6), polyunsaturated arachidonic acid (C20:4 n-6) docosapenthonoic acid (C22:5 n-3) and docosahexanoic acid (C22:6 n-3) which compared with the corresponding control values, had increased The addition of melatonin protected these PUFA specially arachidonic and docosahexaenoic acids located in heart.

5. DISCUSSION (TARTIŞMA)

Cardiac-Ischemia reperfusion damage can appear in most clinic occassional; likes iscemic heart disease, trombolic treatment, percutaneous caranory interventions (ballon angioplasty), cronory arter by-pass surgery, heart valve operations. (Sahna, Acet et al. 2002; Sahna, Parlakpinar et al. 2005). Most aerobic cells suffer oxidative damage as a consequence of the formation of reactive species that are generated as secondary products during respiration or from specific enzymatic reactions (Kohen, 2002). Damage to lipid deleteriously alters and modifes cellular membranes and, therefore, cellular function (Porter, 1984). Numerous studies have reported melatonin's protection against lipid peroxidation and DNA damage induced by ROS, both in vivo and in vitro (Karbownik, 2001 and Tan, 1993) but fewer workers have focused on melatonin's efficacy in reducing fatty acid damage analyzing the degradation of specific fatty acids located in selected membranes (Leaden, 2002 and Gavazza, 2004). Melatonin suppresses the ascorbate-Fe^{++} induced lipid peroxidation process in rat liver, kidney and brain microsomal preparations and protects the most common PUFAs, arachidonic and docosahexaenoic acid, from damage. Melatonin seems to be equally efficient in vitro in the protection of fatty acids that belong either to the n-6 and/or to the n-3 family. The physiological potential of melatonin in preventing lipid degradation of PUFAs in different organelles isolated from different tissues deserves further investigation (Patricio, 2005). The effect of a daily administration of melatonin for 45 days at two doses (0.5 and 1.0 mg/kg body wt.) on antioxidant status, lipid peroxidation and lipid profile in the brain and liver in rats. Both doses of melatonin caused a significant decrease in lipid peroxidation and the levels of cholesterol, phospholipids, triglycerides and free fatty acids in the examined tissues (Perumal Subramanian 2007). The decrease in the levels of cholesterol, phospholipids, triglycerides and free fatty acids found in the brain and liver of melatonin-treated rats underlines a significant antihyperlipi-demic action of melatonin (Tunez, 2002) which could be exerted by augmenting the clearance of endogenous cholesterol (Hoyos, 2000). Mortality and morbidity from coronary heart disease (CHD), diabetes mellitus (DM) and essential hypertension (HTN) are higher in people of South Asian descent than in other groups. There is evidence to believe that essential fatty acids and their metabolites may have a role in the pathobiology of CHD, DM and HTN. (Das, 1995).

NOTICE(NOT)

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