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The effect of caffeine on oxidative stress in liver and heart tissues of rats

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Aim: To investigate the effect of caffeine on the levels of malondialdehyde (MDA), nitric oxide (NO), and advanced oxidation protein products (AOPP) in the liver and heart tissues of rats.

Materials and methods: The current study included 30 rats, which were divided into 3 groups: a control group and 2 caffeine-treated groups. Group 1 was given caffeine at 30 mg/kg and Group 2 was given caffeine at 100 mg/kg (a high nontoxic dose) for 14 days.

Results: MDA and AOPP levels in the liver tissue of the caffeine-treated groups decreased significantly as a result of the dose. MDA and AOPP levels in the heart tissue also decreased, but this effect was not significantly affected by the dose. NO levels in the liver tissue of the caffeine-treated groups were higher than those in the control group; in the heart tissues, however, NO levels were not significantly affected by caffeine.

Conclusion: These results show that the short-term consumption of 2 different doses of caffeine may potentially protect against oxidative stress in the liver. This effect is related to the dose of caffeine in the liver tissue. Further studies will be needed to discover the mechanisms responsible for these findings.

Key words: Caffeine, malondialdehyde, AOPP, nitric oxide

Kafeinin rat karaciğer ve kalp dokusunda oksidan stres üzerine etkisi

Amaç: Biz bu çalışmada kafeinin rat karaciğer ve kalp dokusunda malondialdehit (MDA), nitrik oksit (NO) ve gelişmiş okside protein ürünleri (AOPP) düzeylerine etkisini araştırdık.

Yöntem ve gereç: Çalışmaya 30 rat dahil edilmiştir. Ratlar eşit 3 gruba ayrılmıştır, kontrol grubu ve 2 kafeinli grup. Grup 1'e 30 mg/kg kafein, grup 2'ye 100 mg/kg kafein 14 gün boyunca verilmiştir.

Bulgular: MDA ve AOPP düzeyleri karaciğerde kafeinli gruplarda dozla ilişkili olarak anlamlı azalmalar göstermiştir. Kalp dokusunda MDA ve AOPP düzeyleri kafeinle azalmıştır ancak bu etki dozdan bağımsızdır. NO düzeyleri karaciğerde kafeinli gruplarda kontrol grubuna göre artmıştır ancak kalp dokusunda kafeinin NO düzeylerine etkisi önemsizdir.

Sonuç: Bu sonuçlar göstermiştir ki kısa süreli kafein alımı karaciğer dokusunda oksidatif stresten koruyucu etki göstermektedir. Bu etkiler karaciğerde doz ile ilişkilidir. Kafeinin etki mekanizmalarının bilinmesi için daha ileri çalışmalara ihtiyaç vardır.

Anahtar sözcükler: Kafein, malondialdehit, AOPP, nitrik oksit

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Introduction

Caffeine (1,3,7-trimethylxanthine) is present in several food and beverage products, such as coffee and tea. It is widely consumed, primarily for its stimulating effect on the central nervous system. Caffeine and other methylxanthines are used in clinical medicine as diuretics, analgesics, and muscle relaxants, and they can aid in the treatment of brain disorders such as headaches and Parkinson's disease (1-3). Moreover, caffeine is used as an ergogenic aid, as multiple well-controlled experiments have found that moderate doses of caffeine can improve performance in athletes (4). The cortical stimulation produced by small amounts of caffeine results in mental alertness and reduces drowsiness and fatigue. In contrast, high doses of caffeine induce nervousness, insomnia, tremors, and other effects (5).

Some of the effects of caffeine may favor the production of free radicals and lead to a subsequent increase of lipid peroxidation by increasing oxidative stress (6-8). Some articles, however, claim that caffeine protects against cellular damage by producing beneficial antioxidant effects (9,10). Unlike lipids, protein reactions with various oxidants have not been extensively studied. Proteins are vulnerable to attack by a variety of free radicals and related oxidants. The oxidative modification of proteins in vivo may affect a variety of cellular processes and involve many kinds of proteins, such as receptors, transport systems, or signal mechanisms. The products of the oxidative modification of proteins are known as advanced oxidation protein products (AOPP) (11).

Nitric oxide (NO) is a messenger molecule functioning in vascular regulation, immunity, and neurotransmission, among other processes (12). Studies have revealed that NO may play an important role in the pathophysiologic mechanisms of diseases (13,14). High concentrations of NO, produced as a result of inducible nitric oxide synthase (iNOS) induction and peroxynitrite formation, are capable of causing lipopolysaccharide (LP) and protein oxidation (14).

While some studies in the literature have shown that caffeine improves performance, these results are not clear. Additionally, no previous study has investigated the changes in the AOPP levels of liver tissue upon exposure to caffeine.

In light of this, the aim of this study was to evaluate the effects of 2 different doses of caffeine in short-term consumption on the levels of MDA, NO (nitrite + nitrate), and AOPP in the liver and heart tissue of rats.

Materials and methods

All chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and were of analytical grade or the highest grade available.

The experimental protocols were conducted with the approval of the Animal Research Committee of Gazi University, Ankara, Turkey (Code No. G.U.E.T-07.031).

The study included 30 male Wistar rats, each weighing about 250 g. The animals were divided into 3 groups, each consisting of 10 members. Animals in the control group were given drinking water without caffeine. The first experimental group of animals (Group 1) was given a caffeine dose of 30 mg/kg, which was dissolved in distilled water. The second (Group 2) was given a dose of 100 mg/kg of caffeine solution. The total daily dosage was divided into 2 smaller doses, given in the morning and afternoon. After 14 days, all of the rats were sacrificed and liver and heart tissues were quickly removed and frozen at –80 °C.

The tissues were separately weighed and homogenized in 10 volumes of cold 0.01 M Tris-HCL buffer (pH 7.4), using an automatic homogenizer. The homogenates were then centrifuged at 15,000 rpm for 15 min at 4 °C. Clear supernatants were used for the MDA, NO, and AOPP assays. Tissue protein levels were also measured at this step according to the method used by Lowry et al. (15).

Tissue MDA assays were performed according to the guidelines of Ohkawa et al. (16). MDA is a product of lipid peroxidation that reacts with TBA under acidic conditions at 95 °C, forming a pink complex that absorbs at 532 nm. 1,1,3,3-Tetraethoxypropane was used as the standard. The results are expressed as nmol/g tissue.

NO (nitrite + nitrate) was detected in tissues by measuring the formation of nitrite by a nitrate/nitrite colorimetric assay kit (Cayman Chemical

Company, Ann Arbor, MI, USA). Concentrations were calculated by comparison to nitrite standards. The results of the NO assays are expressed as μmol/g tissue.

Tissue AOPP assays were performed as described by Witko-Sarsat et al. (17). Each well of a 96-well microtiter plate received 200 μL of supernatant diluted at a ratio of 1:5 in PBS, or chloramine-T standard solutions (0-100 μmol/L). Afterwards, 10 μL of 1.16 M potassium iodide (KI) was added, followed by 20 μL of acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm in a microplate reader against a blank containing 200 μL of PBS, 10 μL of KI, and 20 μL of acetic acid. AOPP concentrations are expressed in μmol/L of chloramine-T equivalents. The results of the AOPP assays are expressed as μmol/mg protein.

Statistical evaluation

All data are expressed as mean ± SE. Comparisons between the groups were done using the Mann-Whitney U test, followed by Bonferroni's multiple comparison test. Probability values of less than 0.05 were accepted as significant.

The correlation between MDA, AOPP, and NO values in the tissues was determined by Spearman's correlation test. All analyses were done with the SPSS 11.5 statistical software package.

Results

The results of the study are presented in the Table.

Liver MDA levels were significantly lower in Group 2 compared to the control group and Group 1 ($P < 0.05$). AOPP levels in the liver were lower in both of the caffeine-treated groups than in the control group and, furthermore, the levels were lower in Group 2 than in Group 1. Liver tissue analysis also revealed that NO levels were higher in Group 1 and Group 2 than in the control group ($P < 0.05$) (Table).

The AOPP and MDA levels of heart tissue were significantly lower in the caffeine-treated groups than in the control group ($P < 0.05$). However, NO levels in the heart tissues were not significantly different between the groups (Table).

Statistically significant correlations ($P < 0.05$) were found between:

1) AOPP and MDA levels in liver tissues ($P = 0.000$, $r = 0.629$), and
2) AOPP and MDA levels in heart tissues ($P = 0.001$, $r = 0.574$).

Table. MDA, AOPP, and NO levels in the rat tissues.

Tissue	Group	MDA (nmol/g tissue)	AOPP (μmol/mg protein)	NO (nitrite + nitrate) (μmol/g tissue)
LIVER	Control	115.78 ± 5.01	75.65 ± 3.02	0.137 ± 0.004
	Group 1	103.57 ± 7.79	60.67 ± 4.02[a]	0.229 ± 0.029[a]
	Group 2	67.48 ± 2.56[a,b]	31.96 ± 7.62[a,b]	0.267 ± 0.011[a]
HEART	Control	75.60 ± 5.32	36.78 ± 1.58	0.179 ± 0.011
	Group 1	54.60 ± 4.02[a]	30.96 ± 1.69[a]	0.203 ± 0.016
	Group 2	48.44 ± 3.68[a]	30.84 ± 1.74[a]	0.205 ± 0.024

Results are expressed as mean ± SE.
[a]: $P < 0.05$ as compared to the control.
[b]: $P < 0.05$ as compared to Group 1.

Discussion

Caffeine is a stimulant of the central nervous system. After oral ingestion, caffeine concentration in the plasma peaks within 30-120 min. Furthermore, the hydrophobic properties of caffeine allow it to pass through all biological membranes (5).

It has been reported that caffeine acts on various tissues, in particular the central nervous system (10,18) and skeletal (19) and cardiac muscle (20). The mechanism by which caffeine improves performance is not clear, but several possibilities have been proposed, such as antagonizing adenosine receptors (18) and increasing catecholamine release (19).

There are reports that suggest that caffeine is capable of inducing certain forms of oxidative damage by increasing LP (21,22). Al Moutaery et al. (22) showed that, in rats that had been given experimental head trauma, a high intraperitoneal dose (100-150 mg/kg) of caffeine increased LP in the cortex. Karas et al. (23) stated that the administration of caffeine (150 mg/kg orally) increased MDA levels in the livers of rats that had been treated with allyl alcohol to reduce the hepatotoxicity of the high dose of caffeine. On the other hand, caffeine has also been reported to be a protective substance against cellular damage (9).

We treated rats with 2 different dosages of caffeine (30 and 100 mg/kg) for 14 days. In previous studies, Al Moutaery et al. (22) showed that, in rats that had experimental head trauma, the administration of a high intraperitoneal dose of caffeine (100-150 mg/kg) increased lipid peroxidation in the cortex and caused oxidative stress. In their in vivo research, Karas et al. (23) found that the oral administration of caffeine (150 mg/kg) increased MDA levels in the livers of rats that had been treated with allyl alcohol to reduce the hepatotoxicity of the high dose of caffeine. Mukhopadhyay et al. (24) stated that caffeine administration to rats (20 mg/kg for 30 days) caused augmentation of the activities of hepatic catalase and superoxide dismutase (SOD) and a reduction of MDA levels. Additionally, Lee et al. (25) stated that adding caffeine (40 mg/kg for 1 day) to the drinking water given to rats reduced Na+/K+-ATPase activities and increased NO (nitrite + nitrate) levels by raising the endothelial NOS expression in the kidneys. After reviewing these studies, we chose 2 different dosages of caffeine (30 and 100 mg/kg) to be administered for 14 days in order to investigate possible alternative results. In some previous studies, it has been noted that combining low doses of ethanol and caffeine (10 mg/kg caffeinol) protects the brain's cortical domains against any damage that can occur as a result of distal focal ischemia in rats (26). The treatment of rats with a sustained lower dose of caffeine (10 mg/kg) reduced ischemic brain damage and global ischemia (27). Alasehirli et al. (28) found that NO production increased when increasing doses of intraperitoneally injected caffeine were given to pregnant rats (25, 50, and 100 mg/kg for 21 days).

It is known that a chronic low dose of caffeine (10 mg/kg) in rats reduces ischemic brain damage and global ischemia. It is thought that the neuroprotective effect of chronic caffeine is mediated by both its own inhibitory effect and the ability of the adenosine receptor to adapt. An acute low dose of theophylline in rats decreases brain damage after cerebral hypoxia-ischemia. Caffeine and its metabolites could have different effects on the treatment of ischemia depending on the dose, the application, and the model of ischemia (29).

In our research, MDA levels in the heart tissue of the caffeine-treated groups were lower than those in the control group. MDA levels in liver tissues were significantly lower in Group 2 when compared to those of the control group. These levels were also lower in Group 1 than in the control group, but this difference was not statistically significant. There were no statistically significant differences between the caffeine-treated groups in terms of heart MDA levels. The MDA levels in both the liver and heart were lower in Group 2 than in Group 1.

Our results suggest that short-term caffeine consumption in each of the 2 different doses used in this study decreases MDA levels in these tissues. The level of MDA in the liver is especially sensitive to dosage.

Kamat et al. (30) showed that caffeine in millimolar concentrations inhibits LP by reducing lipid hydroperoxides in the rat liver microsomes. Yukawa et al. (31) suggested that consuming 150 mL (8 g) of coffee 3 times a day for 7 days leads to reduced levels of total cholesterol, LDL-cholesterol, and MDA in the serum.

These results, in addition to our own results, suggest that the effect of caffeine may be dependent on dose, method of administration (oral or injection), and duration of treatment, among other factors.

We found that the levels of NO in the liver increased significantly after caffeine administration, but this increase was not sensitive to the caffeine dosage. The heart tissues of the caffeine-treated group had NO levels that were slightly higher than the control group, but this difference was not statistically significant.

NO is synthesized from L-arginine by NO synthase (NOS). It is a messenger molecule functioning in immunity, neurotransmission, and other processes. Diseases such as vascular dysfunctions are associated with the impaired production of NO, whereas septic shock, cerebral infarction, diabetes mellitus, and neurodegenerative disorders are associated with NO overproduction. A reduced release of NO is one of the earliest signs of endothelial dysfunction (12). NO synthesis is also decreased in rats with chronic renal failure (32).

Physiological studies have shown a significant decrease in exhaled NO after caffeine ingestion (33). Corsetti et al. (34) reported that acute caffeine administration decreases NOS and Bcl2 expression in rat skeletal muscles. Kayir et al. (35) observed that significant dose-dependent changes in locomotor activity occur in mice that are treated with caffeine; they suggested that this caffeine-induced locomotor activity might be modulated by NO in mice. This theory seems to be supported by the finding that NO levels in the brains of mice (36) and rats (10) treated with caffeine were found to be significantly higher than those of animals in the control group.

In a previous paper (37), we published results that agreed with the findings of Nikolic et al. (10) and showed that short-term treatment of animals with small doses of caffeine decreases brain arginase activity. This result suggests that caffeine allows more arginine to be consumed in other metabolic pathways, such as during NO production.

There currently exists little data on the relationship between caffeine and oxidative stress in the heart. It remains unclear how NO metabolism and arginase modulate NOS activity in the heart tissue, as well as how the NOS enzyme controls cardiac function (38). Recent work has demonstrated that arginase modulates myocardial contractility through a NOS-dependent mechanism (39,40). NOS1 deficiency leads to an increase in the xanthine oxidase-dependent reactive-oxygen species activity, which dramatically depresses the myocardial contractile function (40). Because NOS1 positively modulates myocardial contractility, Steppan et al. (39) attempted to determine whether arginase inhibition would increase basal myocardial contractility. Corsetti et al. (41) suggested that improved caffeine-induced physical performance could also be related to caffeine's ability to interfere with endogenous myocardial NO synthesis. Furthermore, the myocardial cell plays an effective antiapoptotic role against acute caffeine administration.

In our study, we did not observe significant changes in heart NO levels upon treatment with caffeine, perhaps because of a difference between the metabolism and/or regulation of NO in the heart tissue versus liver tissue.

A new marker of protein oxidation, AOPP, has recently attracted the attention of various investigators (11,42). The oxidative damage done to proteins is marked by an increase in the levels of protein carbonyls and a decrease in the levels of protein thiols (11). Witko-Sarsat et al. (17) investigated the relationship between AOPP and chronic inflammation in patients with chronic renal failure and found that AOPP more accurately marks oxidative stress than markers of lipid peroxidation. Due to their relatively early stability and longer lifespan, AOPPs have been used as a marker of oxidative stress; the effect of caffeine on AOPP levels in rat tissues, however, had not previously been measured.

We found that the AOPP levels in a caffeine-treated sample were significantly decreased in the liver and heart tissues relative to a control group. We also found that Group 2 had significantly lower levels of AOPP in liver tissues than Group 1. These results show that decreased AOPP levels in tissues may reflect the cell's ability to protect against the oxidative injury imposed by caffeine; this ability appears to be related to the dose of caffeine in the liver tissue, although the dosage does not seem to be important with regard to the heart tissue. In this study, AOPP

and MDA concentrations were positively correlated in the liver tissue. Further investigation will be needed to understand the cause of this correlation.

In conclusion, these data suggest that short-term consumption of 2 different doses of caffeine may protect against oxidative stress in the liver tissue of rats. This effect is also related to the caffeine dose in the liver tissue. Further studies will be needed to understand the mechanism of caffeine's effects on different tissues.

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