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ARTÍCULO DE INVESTIGACIÓN / RESEARCH ARTICLE

THE DOSE-DEPENDENT EFFECTS OF CAFFEINE AND BETAINE ON TELOMERASE ENZYME ACTIVITY IN MICE

Los efectos dependientes de la dosis de cafeína y betaína sobre la actividad de la enzima telomerasa en ratones

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ABSTRACT

The telomerase enzyme, which extends the Deoxyribonucleic Acid (DNA) regions called telomeres at the ends of the chromosomes, has an important place in aging, cancer and stem cell studies. In this study, the effects of betaine and caffeine on telomerase enzyme activity in mice were investigated. Telomerase activity was measured by a Polymerase Chain Reaction-Enzyme Linked Immunosorbent Assay (PCR-ELISA) based method. The activity of Superoxide Dismutase (SOD) and Catalase enzymes in the liver and kidney and the amount of Malondialdehyde (MDA) were also investigated. The results show that betaine has a slightly inhibitory effect (not significant) on telomerase activity, especially at high doses. Caffeine may act as an inhibitor in high doses but may have an activator effect at low doses. Also, it was observed that SOD and Catalase enzyme activities were parallel to the increase/decrease in telomerase enzyme activity in the liver.

Keywords: Betaine, Caffeine, Catalase, SOD, Telomerase

RESUMEN

La enzima telomerasa, que extiende las regiones del ácido desoxirribonucleico (ADN) llamadas telómeros en los extremos de los cromosomas, ocupa un lugar importante en los estudios sobre el envejecimiento, el cáncer y las células madre. En este estudio, se investigaron los efectos de la betaína y la cafeína sobre la actividad de la enzima telomerasa en ratones. La actividad de la telomerasa se midió mediante un método basado en PCR-ELISA. También se investigó la actividad de las enzimas SOD y catalasa en hígado y riñón y la cantidad de MDA. Los resultados muestran que la betaína tiene un efecto levemente inhibidor (no significativo) sobre la actividad de la telomerasa, especialmente a dosis altas. La cafeína puede actuar como inhibidor en dosis altas, pero puede tener un efecto activador en dosis bajas. Se observó que las actividades de la enzima SOD y catalasa eran paralelas al aumento / disminución de la actividad de la enzima telomerasa en el hígado.

Palabras clave: Betaína, Cafeína, Catalasa, SOD, Telomerasa

INTRODUCTION

Telomerase is the only DNA polymerase that functions as reverse transcriptase, completely covering the ends of linear chromosomes and rebuilding telomeric sequences (Sanchis-Gomar and Lucia 2015). It is a very important enzyme for the aging process and carcinogenesis (Artandi and Depinho 2010). Telomerase is activated in embryonic stem cells and maintains telomere length. However, the level of telomerase activity in most adult stem cells is lower than in embryonic stem cells. Therefore, telomere shortening occurs even in adult stem cells (Hiyama and Hiyama 2007). The telomerase enzyme has been a molecular target since its discovery. Most chemotherapy drugs used today are disadvantageous because they do not have a distinctive feature for the cell and are effective on normal healthy cells as well as cancer cells. Therefore, since the telomerase enzyme is not detected in most normal tissues, the idea of telomerase inhibition will provide a more specific target (Shay and Wright 2002, Buseman *et al*. 2012). In addition, it is thought that telomerase inhibitors may be one of the most effective methods in combination with other traditional or experimental cancer treatments (Shay and Wright 2002, Mutlu 2017).

On the other hand, telomere shortening is associated with cellular aging (Sandin and Rhodes 2014) and it is thought to be a protective mechanism against cancer. However, it has been proven that telomere dysfunction in later stages may cause tumor formation at the end of life (Tárkányi and Aradi 2008). In addition, many epidemiological studies show that short telomeres in humans are associated with many age-related diseases (Blasco 2005, Boccardi and Paolisso 2014). Therefore, it was emphasized that telomerase activators are also important for antiaging and telomerase-dependent disease treatments (De Jesus *et al*. 2012, De Jesus and Blasco 2013, Mutlu 2017).

Betaine is a methylated derivative of the glycine amino acid and is an amino acid with three hydrophobic methyl groups (-CH3) and a hydrophilic carboxyl group (-COOH) (Mahmoudnia and Madani 2012). The methyl groups provided by betaine, which acts as the donor of methyl groups such as choline and methionine, are used in transmethylation reactions for various metabolic processes such as protein synthesis, energy metabolism, carnitine and creatine synthesis (Ratriyanto *et al*. 2009, Mahmoudnia and Madani 2012). Betaine can be taken into the human body with different food sources. Especially shellfish, spinach, sugar beet and especially whole grain foods contain high amounts of betaine (Zeisel 2003, Preedy 2015). Betaine taken orally is rapidly absorbed in the body, distributed and metabolized and excreted in the urine (Schwahn *et al*. 2003).

Caffeine is a natural alkaloid compound found mainly in coffee beans, tea leaves, cocoa beans and some plants (Iarc 1991). It has been reported that caffeine is a central nervous system stimulant that affects various brain functions such as attention, memory and sensation and is consumed quite a lot worldwide (Tsunoda *et al*. 2019). The primary site of caffeine metabolism is the liver (Arnaud 1999). After the caffeine is taken into the body, it quickly enters the bloodstream from the gastrointestinal tract and is completely absorbed. The amount of caffeine in the blood reaches the maximum level within one or two hours (Arnaud 1999). It is known that caffeine has effects on adenosine receptors, central nervous system, cardiovascular system, respiratory system and endocrine system.

Reactive oxygen derivatives can be produced in some natural processes inside the cell, as well as an increase in production due to the influence of some external factors. These oxygen radicals are neutralized by the body's antioxidant defense system. However, if there is more radical production than the antioxidant system can handle, the balance is disturbed and oxidative stress occurs. The relationship between oxidative stress with aging and some diseases has been known for a long time (LiguorI *et al*. 2018). It has been suggested that exposure to oxidative stress causes the shortening of telomeres, and a decrease in stress can provide protection for telomeres (Kim *et al*. 2016). In their reviews, Reichert and Stier (2017) stated that in the literature, most studies on this topic have shown that there may be a link between telomere shortening and oxidative stress.

In this study, the effects of caffeine and betaine on telomerase enzyme activity were investigated. Although some different biological effects of caffeine and betaine have been extensively studied, there is not much information in the literature on how they affect telomerase enzyme activity. However, few articles are available regarding the effects of caffeine on TERT expression or telomere length. Tao *et al*. (2021) reported that caffeine increased the expression of TERT, the active site of telomerase, at the mRNA and protein levels, and also improved telomere length in mice. Results of a human study showed that caffeine intake decreased telomere length in US adults, while coffee intake increased telomere length (Tucker 2017). Liu *et al*. (2016) found that higher coffee intake was associated with longer telomeres in female nurses. No article has been found in the literature on the direct effects of betaine and caffeine on telomerase enzyme activity. It is important to know what direct effects of caffeine and betaine, which many people consume more or less in their daily lives, on the telomerase enzyme, which is known to have effects on cancer and aging.

MATERIALS AND METHODS

Experimental design, mouse care and applications

Mus musculus Swiss albino mice were used in this study (Burdur Mehmet Akif Ersoy University animal experiments ethics committee, decision dated 15.08.2018, numbered 390).

Betaine and Caffeine were started to be applied to 10-18 weeks old adult male mice after seven days acclimation period. The animals were housed in plastic cages on sawdust

bedding, and fed with standard diet and tap water *ad libitum*. The animal room temperature was a 23 ± 2°C, with relative humidity of 45 %, and a 12h day-night-light cycle. The mice were housed and treated by national and institutional guidelines.

The betaine dose to be given to the mice was determined as 180 mg/kg and 90 mg/kg. Betaine (SIGMA B2629) solution was prepared in distilled water so that this dose was given to the mice by micropipette in 10 µL of liquid as orally. In the literature, dose ranges for betaine administration to mice are quite wide. There are studies in which very different doses are applied, from 10 mg/kg to 1000 mg/kg (Nemmar *et al*. 2017, Khodayar *et al*. 2018, Shadmehr *et al*. 2018, Hagar *et al*. 2019). In this study, we preferred more moderate doses such as 90 and 180 mg/kg in order to be applicable to humans.

The caffeine dose was determined as 30 mg / kg and 15 mg / kg and caffeine (SIGMA C0750) solution was prepared by dissolving it in a DMSO-Oil (50 %-50 %) mixture. The mixture, which was prepared in such a way that the dose to be administered was 10 µL, was given orally to the mice with a micropipette. We wanted to limit the caffeine doses to the amount of coffee a person can drink, which corresponds to about four and a half and nine cups of filter coffee per day.

Thus, six experimental groups were formed including high and low doses of betaine, high and low doses of caffeine and two vehicle controls (water for betaine and DMSO+oil for caffeine).

Agents were administered to the mice by automatic pipette for 30 days. Experiments were carried out with 6 mice in each experimental group. At the end of the 30-day application period, the mice were exposed to ether anesthesia and dissected, and blood, liver, and kidney tissues were taken. The reason for using liver tissues is that the highest telomerase activity in mice is found in liver and testicular tissues (Prowse and Greider 1995).

MEASUREMENT OF ENZYME ACTIVITIES AND MDA AMOUNT

Roche Telomerase PCR-ELISA kit was used to measure telomerase activity and SIGMA 19160 SOD assay kit was used to measure SOD activity. Applications were carried out according to the instructions included in the kit procedure. The measurement of catalase activity was made according to Luck (1963). This method is based on measuring the breakdown of H2O2 by catalase in tissue extract and the decrease in absorbance at 240 nm. MDA amount analysis was performed in serum according to Wasowicz *et al*. (1993). This fluorimetric method measures the amount of thiobarbituric acid reactive substances (TBARS) in the serum based on the reaction between malondialdehyde and thiobarbituric acid. The product of this reaction is extracted into the butanol phase and measured at 525 nm excitation and 545 nm emission wavelengths.

Protein determination was performed on tissue extracts using the Lowry *et al.* (1951) method. Non-parametric tests were used for statistical analysis using the Minitab program.

RESULTS AND DISCUSSION

While evaluating the relative telomerase activity results, the compound applied for each group was compared with both its own solvent and the control group, where nothing was given. In addition, two different doses of the same compound were compared.

The results show that betaine has a slightly inhibitory effect, especially at high doses (not statistically significant). Caffeine may act as an inhibitor in high doses but may have an activator effect at low doses. Relative telomerase activity results are given in Table 1.

Table 1. Relative telomerase activity results obtained from application and control groups

Groups	Relative Telomerase Activity ± SE
Betaine H (180 mg/kg)	21.57 ± 1.1.5
Betaine L (90 mg/kg)	23.88 ± 0.88
Caffeine H (30 mg/kg)	22.24 ± 1.31*
Caffeine L (15 mg/kg)	30.52 ± 2.77
Vehicle 1 (Water) / Control	24.08 ± 1.36
Vehicle 2 (DMSO+Oil)	25.76 ± 1.01

* Statistically different from low dose (p <0.05)

According to antioxidant enzyme analysis in mice liver and kidneys, high dose caffeine and both high and low dose betaine mostly reduced antioxidant enzyme activities in the liver (Table 2). In addition, it was determined that high dose caffeine increased membrane damage compared to low dose, and betaine significantly decreased membrane damage at high doses.

Telomerase enzyme activity is very important in aging process, cancer and stem cells (Jiang *et al.* 2018). In various organisms such as mice, zebrafish and yeast, telomere shortening has been found to be associated with genomic instability and longevity (Hemann 2001, Blasco 2007). Telomere shortening occurs in both proliferative and anti-proliferative cells due to biological phenomena such as aging, genetic diseases such as early aging syndrome, or especially negative environmental factors such as stress. In the aging process, due to the loss of functional cells and the accumulation of surviving but abnormally damaged cells, dysfunction occurs in regenerative capacity and tissues. Telomerase enzyme may also play an important role in the treatment of cancer, a disease characterized by uncontrolled proliferation of cells in certain tissues in the human

Table 2. SOD and Catalase Enzyme Activities and MDA Results (Betaine H 180 mg / kg; Betain L 90 mg / kg, Caffeine H 30 mg / kg, Caffeine L 15 mg / kg)

GROUPS	CAT Kidney	CAT Liver	SOD Kidney	SOD Liver	MDA
Betaine H	4257.5 ± 316	1220 ± 108Δ	257.9 ± 35.1	84.2 ± 15.8Δ	8.46 ± 1.26*
Betaine L	4023.2 ± 276	1929 ± 461Δ	224.7 ± 16.2	95.7 ± 13.5Δ	11.23 ± 1.22
Caffeine H	3946.7 ± 205.4	2606 ± 281*	235.2 ± 12	98 ± 5.8*	14.16 ± 0.47*
Caffeine L	3310.3 ± 247.6Δ	3711 ± 361	230 ± 11.9	130 ± 8.4	10.39 ± 0.21
Vehicle 1 (Water)	4795.5 ± 342.6	3722 ± 169	249.8 ± 16	140.9 ± 12	10.37 ± 0.94
Vehicle 2 (DMSO+Oil)	4479 ± 191.8	2974 ± 135	232.6 ± 9.7	113 ± 9.2	11.09 ± 1.37

* Statistically different from low dose (p <0.05)

Δ Statistically different from its own control/vehicle (p <0.05)

body. Unlimited proliferation of cancerous cells occurs mostly by telomerase expression. Telomerase activity has been shown to be positive in almost all malignant tumors. Most benign tumors are found to be characterized by limited reproductive capacity and telomerase inactivation (Olovnikov 1996, Shay and Wright 2001, Granger *et al*. 2002, De Jesus and Blasco 2013).

In the literature, it has been stated that in case of betaine deficiency required for methylation, negative effects such as genetic instability, aging and cancer may occur (Newberne and Rogers 1986, Cooney 1993). In addition, betaine has been found to inhibit mitochondria-dependent cell death and apoptosis (Graf *et al.* 2002, Ji and Kaplowitz 2003, Garrett *et al*. 2013). Also, betaine is thought to have hepatoprotective potential with antioxidant and anti-inflammatory properties and also plays a role in epigenetic modification (Kim *et al*. 2009, Ueland 2011, Jung *et al*. 2013, Chen *et al*. 2015, Day and Kempson 2016). There is no information in the literature about the effects of betaine on telomerase enzyme activity.

According to the results obtained from this study, betaine slightly inhibits the telomerase enzyme, especially at its high dose, but this inhibition was not statistically significant. While determining the caffeine and betaine doses applied in this research, it was paid attention to the doses that can be exposed in daily life. Perhaps higher doses of betaine would have shown that telomerase inhibition would have become more pronounced.

According to our study, a high dose of caffeine has an inhibitory effect on telomerase enzyme activity, while a low dose has an activator effect. There are similar situations in many natural compounds that have been found to have activator and inhibitory effects on telomerase (Mutlu, 2017). Various studies have shown that various compounds have different effects on telomerase activity at high and low doses, and also cause different effects in terms of telomerase activity in cancerous or healthy cells. (Sprouse *et al*. 2012). According to the literature, resveratrol has been found to inhibit telomerase activity in some cancer cells, but to activate telomerase in epithelial and endothelial progenitor cells (Xia *et al*. 2008). Also, genistein acts as a telomerase activator at low doses and acts as a telomerase inhibitor at high doses (Sprouse *et al*. 2012).

In this study, caffeine has also been observed to show a dose-related effect in relation to the activation and inhibition of telomerase. Also, it was observed that SOD and Catalase enzyme activities were parallel to the increase/decrease in telomerase enzyme activity in the liver.

There are a few articles in the literature on the effects of caffeine on telomere length and TERT expression. Tao *et al.* (2021), showed that caffeine increased the expression of the TERT subunit of telomerase at the mRNA and protein levels. The activity of telomerase enzyme was not investigated in the study. Also in this study, a dose of 5 mg/kg was given to rats for eight months, so the effect of higher doses was not determined. The results of the study support that low dose of caffeine act as a telomerase activator, similar to our results.

As seen from the results of our current study and literature information, the applied dose is as effective as the nature of the applied compound in the modification of telomerase activity. In addition, given that caffeine alone or drinking it as coffee reveals different effects on telomere lengths (Tucker 2017), they are likely to have different effects on telomerase enzyme activity.

CONCLUSIONS

Telomerase activity results show that betaine has a slightly inhibitory effect, especially at high doses (not statistically significant). Caffeine may act as an inhibitor in high doses, but have an activator effect at low doses. The low dose of caffeine increased the telomerase activity while also increasing the SOD and catalase activities in the liver. It was observed that SOD and Catalase enzyme activities were parallel

to the increase/decrease in telomerase enzyme activity in the liver. Moderate coffee drinking can increase telomerase enzyme activity and improve antioxidant enzyme activities in the liver.

AUTHOR'S PARTICIPATION

All authors contributed to every stage of the study.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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