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RESEARCH ARTICLE

Effects of ultrasound homogenisation on the activities of superoxide dismutase, glutathione peroxidase, catalase and levels of lipid peroxide in liver homogenates

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Öz

Özdemir DS, Başpınar N, Akalın PP. Ultrasound homojenizasyonun karaciğer homojenatlarında süperoksit dismutaz, glutasyon peroksidaz, katalaz aktiviteleri ve lipid peroksit düzeylerine etkileri.

Abstract

Ozdemir DS, Baspinar N, Akalin PP. Effects of ultrasound homogenisation on the activities of superoxide dismutase, glutathione peroxidase, catalase and levels of lipid peroxide in liver homogenates.

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Amaç: Çalışmada mekanik ve ultrasound (sonikasyon) homojenizasyon tekniklerinin karaciğer süperoksit dismutaz, glutasyon peroksidaz, katalaz aktiviteleri ile lipid peroksitleri ve total protein düzeylerine etkileri araştırılmıştır.

Gereç ve Yöntem: Bu amaçla taze dana karaciğeri küçük parçalara ayrılarak mekanik homojenizasyon (2 dk) ve sonikasyon (2, 4, 6, 8 ve 10 sn) grupları oluşturuldu. Süperoksit dismutaz, glutasyon peroksidaz, katalaz enzim aktiviteleri ile lipid peroksitleri ve total protein düzeyleri homojenatların süpernatantında spektrofotometrik yöntemlerle belirlendi.

Bulgular: Süperoksit dismutaz, glutasyon peroksidaz, katalaz aktiviteleri ile total protein düzeylerinin, mekanik homojenizasyon gruplarında, sonikasyon grubuna göre önemli düzeyde farklı (P<0.05) olduğu tespit edildi. Sonikasyon grubu süperoksit dismutaz ve glutasyon peroksidaz aktiviteleri, mekanik homojenizasyon grubuna göre yüksek, katalaz aktiviteleri ise düşük bulundu (P<0.05). Glutasyon peroksidaz aktivitesi, 8 sn sonikasyon grubunda, 2, 4, 6 ve 10 sn'lik gruplara göre düşük (P<0.05) belirlenirken, katalaz 8 sn sonikasyon grubu diğer sonikasyon gruplarına göre en yüksek (P<0.05) aktivite düzeylerini gösterdi. Total protein düzeyleri 8 sn sonikasyon grubunda diğer gruplara göre düşük olarak gözlemlendi, istatistiksel fark ise 2, 6 ve 10 sn (P<0.05) gruplar arasında belirlendi. Lipid peroksidasyonu 8 sn grupta diğer gruplara göre yüksek seyrettiği, ancak istatistiksel farkın 2 sn (P<0.05) grup ile oluştuğu belirlendi.

Öneriler: Karaciğer homojenatlarında, antioksidan enzim aktiviteleri ile lipid peroksidasyon ve total protein düzeyleri üzerine mekanik homojenizasyon ve sonikasyon tekniklerinin etkilerinin farklı olduğu, ayrıca, 8 sn sonikasyon uygulamasının, tüm parametreler için kritik bir nokta olabileceği düşünülmüştür.

Anahtar kelimeler: Ultrasound homojenizasyon, sonikasyon, mekanik homojenizasyon, antioksidan enzimler, lipid peroksidasyonu

Aim: In this study, effects of ultrasound homogenisation (Sonication) technique on the activities of superoxide dismutase, glutathione peroxidase, catalase, levels of lipid peroxidation and total protein in liver homogenates were investigated.

Materials and Methods: Postmortem healthy fresh calf liver was used as the material. Liver was sliced and grouped as mechanical homogenisation (2 min) and sonication group (2, 4, 6, 8 and 10 second sonication). Activities of superoxide dismutase, glutathione peroxidase, catalase, levels of lipid peroxidation and total protein were measured in supernatant of homogenisated samples by spectrophotometric methods.

Results: Superoxide dismutase, glutathione peroxidase, catalase activities and total protein levels in mechanical group were significantly different from sonication groups (P<0.05). In sonication groups, superoxide dismutase and glutathione peroxidase activities were higher and catalase activity was lower from mechanical group (P<0.05). As regards glutathione peroxidase activity, 8 sec sonication group was the lowest compared to 2, 4, 6 (P>0.05) and 10 sec (P<0.05) groups whereas 8 sec catalase activity was the highest compared to other sonication groups (P<0.05). Total protein level was the lowest in 8 sec group compared to the other sonication groups which significant difference was determined in 2, 6 and 10 sec (P<0.05) groups. Lipid peroxidation level was the highest in 8 sec sonication group compared to other sonication groups with a significance in 2 sec group (P<0.05).

Conclusions: In liver homogenates, antioxidant enzyme activities, lipid peroxidation and total protein levels were significantly different between mechanical and ultrasound homogenisation groups. Sonication for 8 seconds suggested to be critical point.

Keywords: Ultrasound homogenisation, sonication, mechanical homogenisation, antioxidant enzymes, lipid peroxidation

Introduction

Sonochemistry includes (sound) sonic and (ultrasound) ultrasonic wave applications. The effects of ultrasound on biological systems were first reported by Wood and Loomis in 1927. Sonication induced damage is poorly characterized owing to its potentially complex mechanisms. Ultrasonic sound waves are longitudinal waves above the limit of human hearing range of 20 kHz and produce gas bubbles. This process is named as cavitation which leads to high local temperatures and formation of free radicals by the sonolysis of water. Many applications of ultrasound are suggested to alter protein structures with a destabilizing effect of air–liquid interface of sonication-induced bubbles (Hawkins and Davies 2001, Mason and Peters 2002, Satheeshkumar and Jayakumar 2002). Formation of free radicals is suggested to be responsible in aggregate formation for some proteins (Stathopulos et al 2004).

Hydroxyl radical leads to formation of other reactive oxygen species, for example hydrogen peoxide (H_2O_2) and superoxide ($O_2\cdot$) (Akkuş 1995). In biochemical assays mechanical homogenisation of tissues has been widely used but the homogenate obtained after mechanical homogenisation does not reflect the exact content of tissue because it contains intact cells and organelles (Burden 2012). On the other hand ultrasonic homogenisation purposes the fragmentation of cell organelles more efficiently. The adverse effects induced by high temperature and free radical formation on molecules must be taken into account in the determination of oxidant or antioxidant molecules during sonication (Kavutçu 2006).

The enzymes glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) are the antioxidants that scavenge reactive oxygen species. Superoxide dismutase catalyzes the dismutation of the $O_2\cdot$ into H_2O_2 and oxygen (O_2), and then H_2O_2 is reduced to H_2O and O_2 by CAT or GPx. Lipid peroxidation (LPO) reflects the peroxidation of lipids (Kavutçu 2006).

The aim of this study was to evaluate the effects of ultrasound homogenisation technique on SOD, GPx and CAT activities, LPO and total protein levels compared to mechanical homogenisation technique in liver homogenates.

Materials and Methods

In order to minimize the enzyme activity changes, one postmortem fresh calf liver was taken from slaughterhouse during the slaughtery and used in the study. Liver were dissected out, cleaned in ice-cold normal saline (0.9%, w/v), pat dried in filter paper and were weighed (0.5 g) and stored in -86^{0}C in aluminum foil until the analysis. 0.5 g liver was taken into 5 mL tubes and ice-cold phosphate buffer (0.1 M, pH 7.4) was added. For mechanical homogenisation (n=12), samples were homogenised (Sartorius 37070, Göttingen, Germany) for 2 min in 1500 r/min. For ultrasound homogenisation, samples were sonicated (SONIC vibra cells., SONICS & MATERIALS, INC, USA, model: VCX 130 Serial no: 45822, with net power output 130 W, Frequency 20 kHz, Amplitude 100%, Prob: S&M 630-0422, Prob Model: CV18, Prob Serial No: 6837) with a procedure of: puls on: 2, 4, 6, 8, 10 sec, puls off: 30 sec. (n=12 for each) for 5 times after 30 seconds cooling period of each (Anonymous 2010). Homogenates were then centrifuged at 3000 g, for 10 min at +4°C. Supernatants were collected for the analysis of enzymes, LPO and total protein. For LPO analysis, immediately after homogenisation, butylated hydroxy toluen (0.5 mM, 10 µL) was added into each tube to prevent further peroxidation.

Determination of antioxidants, LPO and total protein

Glutathione peroxidase activity was determined spectrophotometrically (UV 2100 UV–VIS Recording Spectrophotometer Shimadzu, Japan) by using GPx-340™ Oxis Research kit (Bioxytech, CA, USA). The results are expressed as mU/g protein. Catalase activity was determined using CAT-520™ Oxis Research kit. The results are expressed as U/mg protein. Superoxide dismutase activity was determined using Ransod kit (Randox Laboratories). The results are expressed U/g protein. Lipid peroxidation levels were determined using LPO-586™ Oxis Research kit. The results are expressed as nmol/mg protein. Total protein levels were determined with commercial kit by Human Diagnostics (HUMAN Gesellschaft für Biochemica und Diagnostica, Wiesbaden, Germany). Results were expressed as mg/g liver.

Statistical analysis of differences among treatments was done using ANOVA followed by the Tukey test. Results obtained are expressed as mean ± SD. Statistical significance was set at $P<0.05$.

Results

Effects of mechanical homogenisation and sonication on SOD, GPx, CAT, activities, LPO and total protein levels are given in the Table 1.

Superoxide dismutase activity was lower in M group compared to S groups where insignificant elevations were determined in S8s and S10s groups. M group GPx activity was lower from all S groups, but only the S4s and S10s groups were statistically significant ($P<0.05$). As regards CAT activity, opposite to GPx, M group CAT activity was higher compared to all S groups ($P<0.05$) only the S8s group did not differ significantly. Lipid peroxidation levels were not different between M and S groups. As regards total protein levels, S6s group was higher ($P<0.05$) and S8s group was lower ($P<0.05$) from M group.

Table 1. Effects of mechanical homogenisation and sonication on superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) activities, lipid peroxidation (LPO) and total protein (TP) levels (Mean ± SD, n=12)

	SOD	GPx	CAT	LPO	TP
Groups	U/g protein	mU/g protein	U/mg protein	nmol/mg protein	mg/g liver
M	123±15.0^{c}	250±25.2^{c}	434±25.8^{a}	0.21±0.04^{ab}	196±7.48^{bcd}
S2s	258±18.7^{ab}	425±45.5^{abc}	130±8.20^{c}	0.10±0.01^{a}	223±14.6^{ab}
S4s	256±24.3^{ab}	476±36.5^{ab}	181±20.6^{c}	0.20±0.02^{ab}	159±3.59^{de}
S6s	291±43.9^{a}	410±31.9^{abc}	141±11.8^{c}	0.23±0.02^{ab}	245±6.16^{a}
S8s	221±32.3^{abc}	301±19.9^{bc}	381±15.0^{ab}	0.26±0.05^{b}	136±2.73^{e}
S10s	212±26.6^{abc}	499±46.4^{a}	195±12.7^{c}	0.18±0.02^{ab}	192±6.23^{bcd}

$^{a, b, c, d, e}$: Letters in the same column are statistically significant (P<0.05), M: Mechanical homogenisation, S: Sonication, s: second.

Discussion

In this study, the effects of ultrasound homogenisation at different time periods on liver GPx, CAT, SOD activities and LPO and total protein levels were evaluated and compared to mechanical homogenization (Table 1). To our knowledge, no study was evaluated the direct effects of mechanical and ultrasound homogenisation on antioxidant enzymes in liver homogenates. Mechanical activation that occurs during sonication process breaks down molecules in the liquid phase. Expansion occurs after compression of the liquid by ultrasound application, and then sudden pressure drop leads to formation of oscillating bubbles. With the each cycle of ultrasound energy, these bubbles expand and then they can collide and/or collapse (Hawkins and Davies 2001, Mason and Peters 2002, Satheeshkumar and Jayakumar 2002).

Ultrasonic cavitation and sonochemical reactions lead to highly reactive free radical formation in organic media. Most of these free radicals are stable only at the level of nano or microseconds whereas some sonochemicals like H^2O^2 remain stable for a long period (Edmonds and Sancier 1983). Reactive oxygen species produce protein radicals by reacting with many different chemical moieties on proteins, which then are likely to decrease protein stability (Hawkins and Davies 2001).

In biological systems, the most important free radicals are O_2., H_2O_2 and hydroxyl (OH.). Superoxide dismutase, CAT and GPx are important intracellular enzyme systems against free radicals (Akkuş 1995). In the study, SOD, GPx, CAT activities, LPO and total protein levels in M group were significantly different from S groups (P<0.05). In biochemical assays, mechanical or ultrasound homogenisation of tissues has been widely used. It was suggested that the adverse effects induced by high temperature and free radical formation during ultrasound homogenisation must be taken into account in the determination of oxidant or antioxidant molecules (Kavutçu 2006). In the study, ultrasound homogenisation procedure had different effects on the parameters evaluated compared to mechanical homogenisation procedure (Table 1). The increase of GPx and SOD activities may be associated to the increased free radical formation during ultrasonic cavitation and sonochemical reactions caused by ultrasound homogenisation process (Edmonds and Sancier 1983).

An effect of sonication varies depending on the energy levels of sound waves, intensity, time of administration, intermittent or continuous application and the material. Release of enzymes from organelles, activation or inactivation of enzymes may occur during sonication process. The speed of transformation of cholesterol to choleston was differently affected from the duration time of sonication; 5 sec 20 KHz sonication increased transformation speed rate by 99% whereas 10 min sonication decreased the speed rate by 40% (Bar 1988). Takatsuki et al (2003) investigated the effects of 28 KHz sonication on the H+-ATPase activity in Aloe arboreent kallus cells. Wet weights were increased at 2-5 and 10 sec sonication after 2 days compared to 30 and 60 sec administration. A significant decrease was determined in 30 and 60 sec groups. Erte (2007) reported that abiotic effects of 20 KHz sonication on the vitis vinifera L. for resveratrol yield showed different rates in periodic and continuous administration. In carp erythrocytes, Milowska et al (2005) applied 1 MHz continuous-wave ultrasound sonication at the intensities of 0.61 to 2.44 W/cm2 for 5 min into carp erythrocytes. At the intensities of 1.90 and 2.44 W/cm_2 lipid peroxidation levels were increased. In this study LPO levels were affected differently by duration time. It can be suggested that; as the sonication duration time increase until 8 sec, LPO levels may be increased because of the lipid peroxidation of organelle-cell membranes. At 10 sec duration, organelle-cell membrane integrity may completely be disrupted by sonication process and no more lipid peroxidation occur because of the formation of molecules with lower molecular weight which do not represent LPO activity, thus affecting the antioxidant enzymes.

As regards GPx, S8s activity was the lowest compared to S2s, S4s, S6s (P>0.05) and S10s (P<0.05) groups whereas S8s CAT

activity was the highest compared to other S groups (P<0.05). Total protein level was the lowest in S8s group compared to the other S groups with significant difference was determined in S2s, S6s and S10 s (P<0.05). Lipid peroxidation level was the highest in S8s group compared to other S groups with significance in S2s group. No significant difference was determined regarding SOD activity although a decrease was seen in S8s and S10s compared to the other S groups.

Conclusions

In liver homogenates, antioxidant enzyme activities, LPO and total protein levels were significantly different between mechanical and ultrasound homogenisation groups and the sonication duration time differently affected the parameters. Sonication for 8 seconds suggested to be critical point and further studies are needed to understand the exact mechanism of sonication on biological molecules.

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