Islets

ISSN: 1938-2014 (Print) 1938-2022 (Online) Journal homepage: https://www.tandfonline.com/loi/kisl20

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To cite this article: Asli San Dagli Gul, Ersin Fadillioglu, Ismail Karabulut, Ahmet Yesilyurt & Tuncay Delibasi (2013) The effects of oral carvacrol treatment against H_2O_2 induced injury on isolated pancreas islet cells of rats, Islets, 5:4, 149-155, DOI: 10.4161/isl.25519

To link to this article: https://doi.org/10.4161/isl.25519

RESEARCH PAPER

Islets 5:4, 149–155; July/August 2013; © 2013 Landes Bioscience

The effects of oral carvacrol treatment against H_2O_2 induced injury on isolated pancreas islet cells of rats

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Keywords: pancreas, islets of langerhans, oxidative stress, carvacrol

Pancreatic islet transplantation is an alternative treatment of insulin replacement therapy in diabetes mellitus, but the islets are exposed to many chemical, mechanical damages, and oxidative stress before transplantation. Carvacrol is a well-known essential oil for its antioxidant, antimicrobial, antifungal and antiinflammatory properties. The aim of this study was to investigate the possible protective effects of carvacrol against H_2O_2 induced cellular injury on isolated pancreas islets. After carvacrol (20, 40 and 80 mg/kg/day) treatment, the pancreas islets were isolated by enzyme digestion. The isolated islets were incubated within 0, 150 and 300 μM H_2O_2 containing medium at +4°C for 15 min. Then, the islets were examined with fluorescein diacetate and propidium iodide mixture stains for viability. A number of islets were stored for lipid peroxidation, protein oxidation and DNA fragmentation analysis. The cell viability ratio of Carvacrol 20 mg/kg/day group was increased in comparison to control and vehicle (DMSO) groups. Additionally, carvacrol application protected the cells from lipid peroxidation and protein oxidation induced by H_2O_2. H_2O_2 caused tissue injury and DNA fragmentation. There was only one DNA fragmentation band from islet cells of 20 mg/kg/day carvacrol treated group, however there were more than one bands from control and DMSO groups. In conclusion, carvacrol treatment ameliorates islet cell injury induced by H_2O_2. However, the dose of carvacrol is important and our results suggest that 20 mg/kg/day dose is more effective than doses of 40 or 80 mg/kg/day.

Introduction

The transplantation of isolated pancreatic islets is a viable option for the treatment of type 1 diabetes.[1] The major limitations of this procedure are loss of viability and increased apoptosis during surgical removal (of pancreas), isolation and purification process.[2] A significant portion of the islet mass is lost due to stress from multiple sources. One of them is oxidative stress. Pancreas Langerhans islets are particularly vulnerable to oxidative stress because of low antioxidant enzyme levels.[3] Apoptosis is a process of programmed cell death in which the cell activates an intrinsic suicide cascade system. Morphologically, cells starting a programmed cell death exhibit typical chromatin and cytoplasmic condensation followed by nuclear fragmentation and generation of one or more apoptotic bodies.[4] The islets are highly vulnerable to early apoptosis with the help of imbalance between oxidant/antioxidant systems due to the inevitable-prolonged time required for islets isolation and low antioxidant protection systems in the islets. The ischemia, due to isolation procedure, induces high oxygen radical formation.[5] The loss of intact islets is mainly due to early cell death partially mediated by oxidative stress during transplantation process. The pancreatic islet transplantation mainly depends on the presence of intact islets and their survival after transplantation. In this regard, treatment of the donor with some supportive agents may help to increase stability of islets before transplantation.

Carvacrol (2-methyl-5-[1-methylethyl]phenol) is monoterpene phenolic constituent of essential oil produced by numerous aromatic plants.[6] Carvacrol is a very small and lipophilic, and easily pass cell membrane.[7] It has variety of pharmacological properties including anti-inflammatory, antioxidant and antimicrobial activities.[8-10] Carvacrol administration significantly improves the glutathione (GSH) level whereby the maintenance of GSH by carvacrol takes place mainly due to inactivation of reactive oxygen species via its radical scavenging effects.[11] Aydın et al. found that calvacrol treatment caused increases of total antioxidant capacity levels in cultured primary rat neurons.[12] Landa et al.[13] reported that the inhibition of prostaglandin production-mediated via arachidonic acid pathway- could be responsible for the biological (anti-inflammatory) effects of this natural compound. Slamenova et al.[14] examined the DNA-protective effects of carvacrol on the basis of the increased resistance of isolated hepatocytes and testicular cells against the oxidative agent hydrogen peroxide (H_2O_2). Oral treatment with

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Submitted: 03/14/13; Revised: 06/20/13; Accepted: 06/24/13
http://dx.doi.org/10.4161/isl.25519

Table 1. The cell viability percentage of groups after FDA/PI examination with fluorescence microscopy is listed

Groups	Without H_2O_2	H_2O_2	
		150 μM	300 μM
Control	78.64 ± 7.50	69.13 ± 12.42[a]	70.25 ± 8.35[a]
DMSO	73.77 ± 8.74	61.07 ± 12.31[a]	56.08 ± 11.88[a]
Carvacrol 20 mg/kg	87.22 ± 6.42	78.68 ± 8.59[a]	80.72 ± 8.69[a]
Carvacrol 40 mg/kg	84.45 ± 8.48	79.48 ± 9.27	73.45 ± 11.69[a,b]
Carvacrol 80 mg/kg	73.19 ± 10.81	67.41 ± 11.72	58.60 ± 11.69[a,b]

[a]$p < 0.05$ in comparison with "Without H_2O_2 group"; [b]$p < 0.05$ in comparison with "H_2O_2 (150 μM) group".

carvacrol provided significantly more resistant rat DNA against the damaging effects of H_2O_2. The oxidative stress can easily be induced by extracellular H_2O_2 application to cell culture with cell medium.[14]

Accordingly, the aim of this study was to investigate the protective effects of oral carvacrol treatment against H_2O_2 induced injury on isolated pancreas islets in rats.

Results

Florescein diacetate and propidium iodine (FDA/PI) examination with fluorescence microscopy for viability

The cell viability of groups showed that viability was decreased in both 150 and 300 μM H_2O_2 groups (in comparison with that of without H_2O_2) in control and dimethyl sulfoxide (DMSO) groups ($p < 0.05$, **Table 1**; **Fig. 1**). Without H_2O_2, the carvacrol treatment (with doses of 20 and 40 mg) significantly increased the viability when compared with control and DMSO groups ($p < 0.05$). On the other hand, the increase in concentration of carvacrol decreased the cell viability. By increasing the concentration of H_2O_2, the cell viabilities were decreased in control, vehicle, and 40 and 80 mg carvacrol groups significantly ($p < 0.05$). Dose of 20 mg carvacrol showed cell viability of 78.68 (± 8.59)% for 150 μM and 80.72 (± 8.69)% for 300 μM H_2O_2. These results were significantly lower than those without H_2O_2 ($p < 0.05$). However, the viabilities of 20 mg carvacrol with H_2O_2 were significantly higher than all other groups including 40 and 80 mg carvacrol treated groups ($p < 0.05$).

Apoptosis

According to cell viability results, 20 mg carvacrol had higher protection against H_2O_2 induced injury than other carvacrol dosages and the DNA fragmentations were performed on four control samples and four 20 mg carvacrol samples to see the protection for apoptosis. The gel pictures are given in **Figure 2**. As seen in **Figure 2A**, control samples showed fragmentation formation with oxidative stress induction of 150 and 300 μM H_2O_2. The 20 mg carvacrol treatment almost completely protected islets from DNA fragmentation induced with 150 μM H_2O_2 (**Fig. 2B**). Similarly, same dose of carvacrol was still protective against DNA fragmentation induced with 300 μM H_2O_2. There was only small amount of DNA fragmentation in carvacrol treated groups after 300 μM H_2O_2.

Lipid peroxidation and protein oxidation

The oxidative injury indices, malondialdehyde (MDA) and protein carbonyl, are presented in **Figure 3**. When without H_2O_2 group was considered, the control and DMSO groups had higher MDA and protein carbonyl levels than 20 mg carvacrol treated group ($p < 0.05$). 150 or 300 μM H_2O_2 induced oxidative stress in all groups ($p < 0.05$). In control and vehicle groups, there were 2 times significant increases with 150 μM H_2O_2 and more than 3 times significant increases with 300 μM H_2O_2 in protein carbonyl contents (as compared with those without H_2O_2 induction groups) ($p < 0.05$). On the other hand, protein carbonyl content increased less in 20 mg carvacrol treatment group when compared with other groups with H_2O_2 induction ($p < 0.05$).

Similar results were obtained concerning MDA levels from all groups. They were significantly increased with H_2O_2 induction; however the increase was less in carvacrol group with H_2O_2 induction than in control and vehicle groups ($p < 0.05$, **Fig. 3B**).

Discussion

Pancreas Langerhans islet transplantation has become a viable therapy for improving glycometabolic control in type 1 diabetic patients.[1,15] However, the procedures during the isolation process may cause cellular dysfunction. It has been previously reported that oxidative stress plays a major role in triggering the death of islets.[16] On the other hand; the endogenous antioxidant enzyme systems fail to prevent the oxidative stress sufficiently. It has been indicated that excessive reactive oxygen species (ROS) cause β-cell dysfunction, such as defects in insulin synthesis and secretion, and apoptosis.[17] Also, it has been found that β cells are very susceptible to oxidative stress because the expression levels of antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), are relatively low in β cells.[18] The experimental studies have shown that the overexpression of antioxidant enzymes protects β cells from oxidative stress-induced dysfunction.[19] Chung et al. showed that the expression of catalase was increased by a synthetic ligand of peroxisome proliferator-activated receptor-γ (PPARγ), and that it mediated protection of β cells from oxidative injury. Our results showed that oxidative stress was not prevented without any antioxidant addiction. High oxidative stress produced loss of cellular viability in islets. We performed two different doses of H_2O_2 to induce oxidative stress. Both doses of H_2O_2 (150 and 300 μM) caused a significant rise in protein oxidation and lipid peroxidation as well as increased DNA fragmentation in islets in vitro. The injury induced by oxidative stress was prevented by carvacrol treatment applied to rats before removal of pancreas. The prevention of oxidative stress results in protection of islets from cellular injury.[16,20]

The carvacrol doses also are important for induction of antioxidant and protective effects. We investigated whether carvacrol treatment prevented oxidative stress successfully. Three different doses of carvacrol were initially applied before the isolation process. High dose carvacrol did not prevent the tissue injury in islets. The carcavrol dose with 80 mg caused a significant decrease in islet viability in comparison with the control group. The other

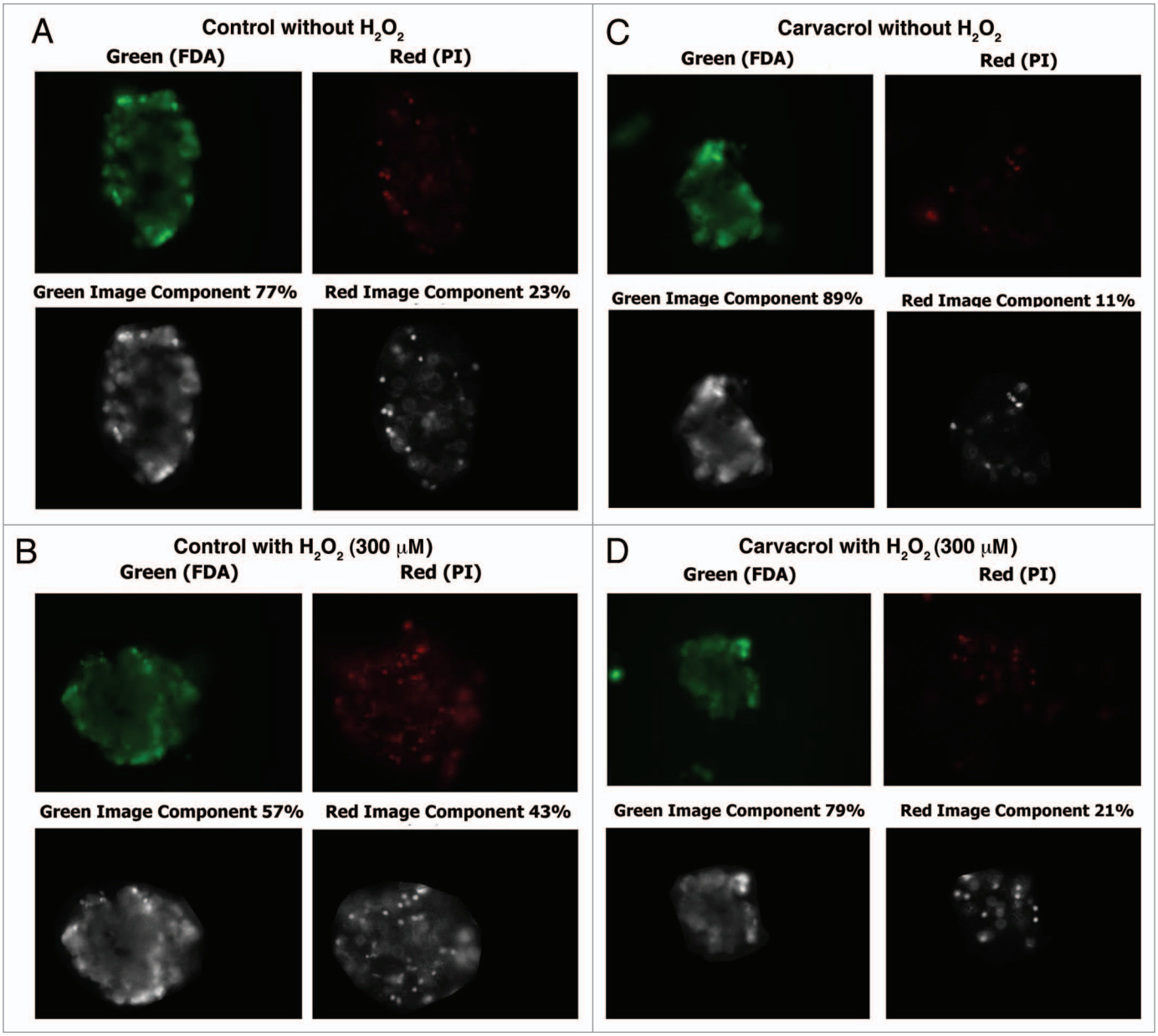

Figure 1. Cellular viability of islets. Florescein diacetate (FDA) and propidium iodine (PI) staining show viability. Uncolored pictures are Matlab analysis and the green color percentages to whole islet give the viability. Green color pictures are FDA stained and red color pictures are PI stained in each group of pictures. (**A**) The example of control islet without H_2O_2. The islet has 77% viability according to green and red color analysis of the picture. (**B**) The example of control islet with H_2O_2 (300 μM). The islet has a decrease in viability of 57% according to green and red color analysis of the picture. (**C**) The example of carvacrol (20 mg/kg/day) islet without H_2O_2. The islet has 89% viability according to green and red color analysis of the picture. (**D**) The example of carvacrol (20 mg/kg/day) islet with H_2O_2 (300 μM) has a viability of 79% according to green and red color analysis of the picture.

experiments were designed with 20 mg carvacrol dose to analyze the protective effect against H_2O_2 induced cell injury. Significant decreases in the levels of protein oxidation and lipid peroxidation were observed on carvacrol administration. Carvacrol has been reported to scavenge superoxide radicals and hydrogen peroxide.[21] Aristatile et al. treated rats with doses of 20, 40, and 80 mg/kg carvacrol to prevent D-galactosamine induced oxidative injury and measured oxidant/antioxidant systems in plasma, kidney and liver tissue. Their findings were similar to ours in the way that 20 mg/kg dose caused more decrease in lipid peroxidation.[21] They have also found high lipid peroxidation level in 80 mg carvacrol dose. In the light of our results and those pertaining to Aristatile et al.[21] results, high dose carvacrol causes tissue injury in cells and 20 mg/kg (for rats) seems to be a better dosage to prevent oxidative injury.

It has been shown that Carvacrol exhibited a strong inhibitory activity against cyclooxygenase-2 in vitro.[22] The isolation of islets from a donor induces an inflammatory process in the early phase. One of the recent studies indicated that inflammation-related genes' mRNA levels were significantly upregulated in the 24 h

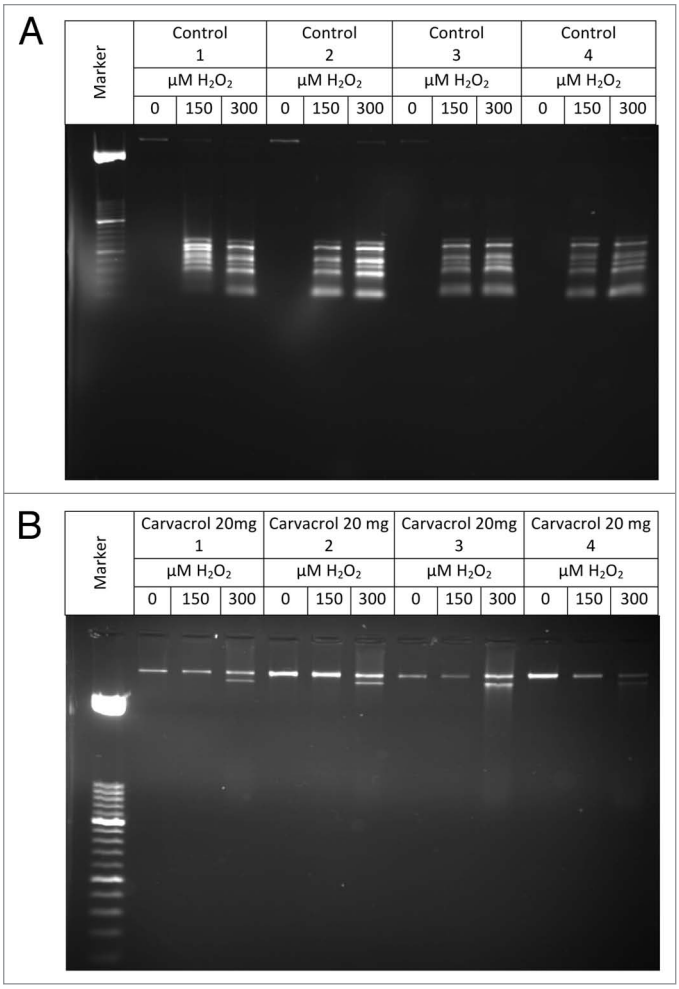

Figure 2. DNA fragmentations. (**A**) The DNA fragmentation of control samples is seen in photograph. The band numbers are more than two in H_2O_2 treated islets. (**B**) The DNA fragmentation of carvacrol (20 mg/kg) group is seen. There are only two bands in 300 μmol H_2O_2 treated samples. There is no DNA fragmentation after H_2O_2 treatment in carvacrol group.

preserved pancreata.[23] Carvacrol may exhibit an anti-inflammatory action on islet cells.

In the DNA fragmentation test, high apoptotic appearance was observed without carvacrol treatment. Slamenova et al.[14] showed that replacement of drinking water by carvacrol supplemented water led to a significant reduction in DNA strand breaks induced by H_2O_2 in both hepatocytes and testicular cells. The present study demonstrated that Carvacrol treatment successfully prevented the DNA fragmentation induced by H_2O_2 in islets. The effect of carvacrol on DNA fragmentation was mostly due to the prevention of cellular injury induced by ROS. The formation of high ROS caused the DNA fragmentation that showed the injury on genetic material in the present study. The treatment with carvacrol restored the imbalance between ROS and antioxidant system and by the way prevented the fragmentation.[24]

In conclusion, the viability of donor pancreas islets is protected with carvacrol treatment and carvacrol effectively protects pancreatic islets cells from induced injury, lipid peroxidation, protein oxidation, and DNA fragmentation.

Material and Methods

Animals

Forty male Wistar Albino rats (280 ± 30 g) were placed in a quiet and temperature- (~22°C) and humidity-controlled (~60%) room in which a 12:12 h light-dark cycle was maintained. All experiments in this study were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) revised 1996 and approved by the Local Ethical Committee of Laboratory Animals at Ankara Numune Training and Research Hospital. The animal studies were done in Pancreas Islet Research Center, Ankara. Rats were randomly selected and grouped.

First group of experiments were done for dose determination of carvacrol (Sigma-Aldrich). The groups were designed with three different doses 20, 40 and 80 mg/kg/day of carvacrol beside the control and dimethyl sulphoxide (DMSO, AppliChem) implemented groups for the viability analyses. The carvacrol was dissolved in 5% DMSO and applied orally by orogastric tube for 9 d. The same volume of 5% DMSO or serum physiologic NaCl solutions was also given orally to DMSO or control groups for 9 d. These five groups contained equal number of rats in each one (n = 8). The oxidative parameters and DNA fragmentation analysis were done from only one carvacrol group.

The decision on carvacrol dose was given after the analysis of three different carvacrol groups that were exposed to oxidative damage with H_2O_2. The rest of the analyses was performed with the group that had the highest viability ratio at 20 mg/kg/day of carvacrol treatment. The group number of oxidative damage and DNA fragmentation analysis were three.

Islet isolation and H_2O_2 treatment

Each islet isolation process was done on two rats from the same group at the 9th day of the treatments. After the rats were anesthetized with ketamine hydrochloride (75 mg/kg) and xylazine (10 mg/kg), the abdomen was opened and the pancreas was gently placed outside the abdomen.

The removal of pancreas and islet isolation was done according to methods modified from other studies.[25,26] After placing pancreas outside the abdomen, the ampulla vater was clamped at duodenum. The cannulation of pancreatic duct was done with PE50 catheter starting from liver side toward to pancreas. The collagenase V (Sigma) solution was prepared in Hanks' Balanced Salt Solution (HBSS, Lonza). The collagenase solution was gently given from the pancreatic duct and the pancreas was blown up. Then, the pancreas was removed and excised from close intestine, fat tissues softly. The removed pancreas was put into a falcon tube to start enzymatic digestion process for 19 min at 37°C. The HBSS with fetal bovine serum (HBSS/FBS) was prepared by 500 ml HBSS with 50 ml FBS- fetal bovine serum, 5 mL Penicillin/streptomycin and 5 mL L-glutamine (Lonza). At the end of incubation in water bath, HBSS/FBS was added to a falcon tube. The pellet suspension was filtered by 450 μm steal sieve to provide mechanical digestion.

The filtered pancreas tissue was thus mostly islets. The pellet was washed and suspended with RPMI/FBS solution (500 ml RPMI-1640 with 50 mL FBS solution, 5 mL Penisilin/streptomisin and 5 mL L-glutamin) (Lonza). After the tubes were centrifuged for 3 min at 1300 rpm with slow brake, the supernatant part was thrown away gently. Then, the RPMI/FPS was added to pellet up to 50 mL in falcon tube. The washing with HBSS/FBS was repeated three times with the same procedure. The purification of islets was done by ficoll-1077 (Biochrom). After purification, the islets were washed with RPMI/FBS before use for experiments. The purity of islets was tested by microscopic examination of samples with dithizone dye (DTZ).[27]

Islets were isolated and purified from each group, they were counted with DTZ and exposed to oxidative damage by placing them into mediums without H_2O_2 or that contain 150 or 300 μM H_2O_2 for 15 min on +4°C in dark. At the end of this procedure, the islets were taken into 15 mL tubes for washing. The tubes were centrifuged for 3 min.

After this period, the islets were taken into 15 mL tubes and RPMI/FBS was added to wash them. Then, they were centrifuged for 3 min at slow-brake. The islet suspensions were taken and divided for florescence microscopic examination, oxidative parameters and DNA fragmentation analysis. The samples for oxidative parameters and DNA fragmentation analysis were kept at –80°C.

The evaluation of pancreatic islet's viability by using florescence dyes florescein diacetate (FDA) and propidium iodine (PI)

Florescein Diacetate (FDA, Sigma) and Propidium ıodine (PI, Sigma) were used to analyze living and non-living islets. PI is a florescence dye that interacts with nucleic acids of non-living cells because it enters cell when there is cell membrane injury. PI gives an orange red colored florescence (excitement wavelength = 536 nm, emission wavelength = 617 nm) when it reaches to cell DNA. FDA dye stains cytoplasm of living cells. It gives green florescence under blue light excitation (490 nm) by staining. The FDA stock solution was prepared with 1 mg FDA in 100 ml acetone and kept at –20°C until usage. The PI stock solution was prepared with 6.346 mg PI in 100 ml phosphate buffer solution (PBS) and kept at +4°C. Then FDA/PI solution was prepared with 28 μL FDA and 42 μL PI stock solution added to 930 μL PBS solution. The mixture was kept at +4°C in a dark until usage.

In order to stain the islets with florescence FDA/PI, 1 mL of cell suspension was placed in a 60 mm petridish and 50 μL of FDA/PI solution was added to it. The Petri was kept at room temperature in a dark and moist place for 90 min. The analysis was done under invert florescence microscope (Leica). The living cells were observed in green color with FDA dye under blue excitation light, whereas the dead cells were observed at orange-red color with PI dye under green excitation light.[28] The photos of the islets were taken and analyzed. The percentages of the living cells were determined by a color analysis code written with the Matlab program. The viability of percentages was taken from 5–10 different islets in one sample.

The analysis of DNA fragmentation

The DNA fragmentation is a hallmark of apoptosis in mammalian cells. We used a sensitive kit (BioVision's Apoptotic DNA Ladder Extraction Kit) to identify DNA strand breaks, a marker of apoptosis. All of the steps were applied according to the manufacturer's instructions. Only fragmented DNA was amplified via the procedure. 30 μl of final volume from each sample was loaded onto a 1.2% agarose gel containing 0.5 μg/ml ethidium bromide in both gel and running buffer. The samples were run with a DNA ladder (DNA molecular weight marker XIII, Roche) at 80 V electrophoreses for 1.5–2 h, and visualized onto a gel documentation system (Syngene).

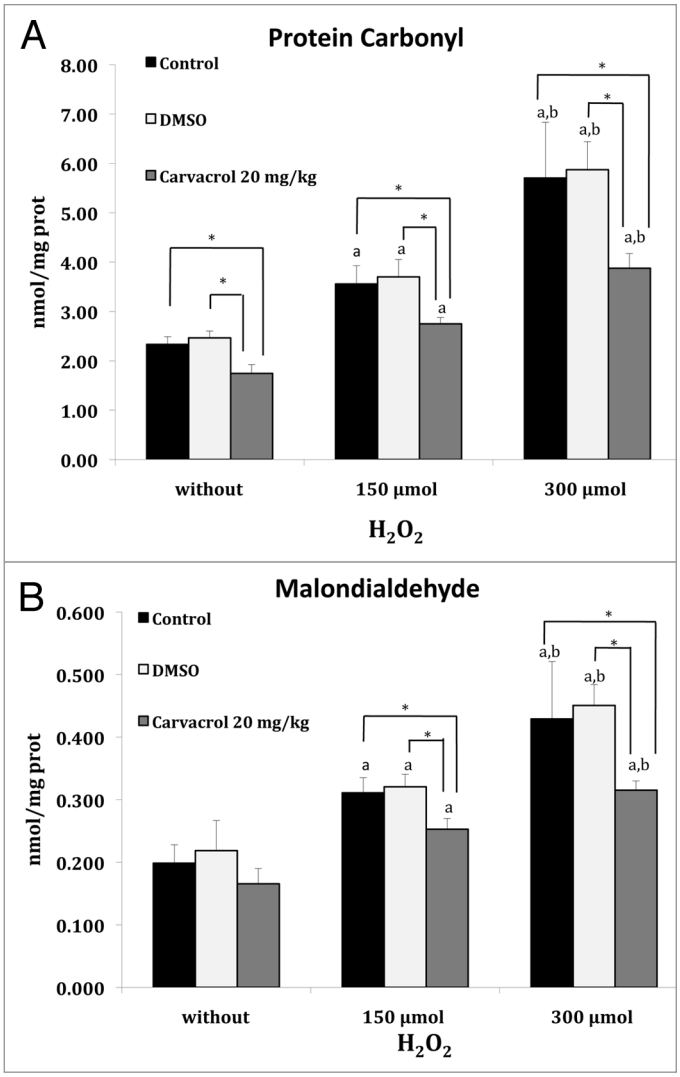

Figure 3. The oxidative stress parameters. The oxidative parameters, lipid peroxidation and protein oxidation, were measured spectrophotometrically after homogenization of islets. (**A**) The graph represents protein carbonyl contents of control, DMSO and carvacrol (20 mg/kg) groups. The carvacrol group has significantly lower protein carbonyl content after H_2O_2 treatment in comparison with control and DMSO. (**B**) The lipid peroxidation index, malondialdehyde (MDA), is presented. The MDA level of carvacrol group with H_2O_2 treatments is significantly lower than control and DMSO groups with H_2O_2 treatments. [a], $p < 0.05$ in comparison with "Without H_2O_2 group"; [b], $p < 0.05$ in comparison with "H_2O_2 (150 μM) group"; *, $p < 0.05$.

Oxidative parameters

The lipid peroxidation index, MDA, and protein oxidation index, protein carbonyl content, were measured from islets

package. First, the samples were homogenized (IKA) and the homogenated samples were used for MDA, protein carbonyl and Lowry protein analysis. The protein measurement was done according to the method of Lowry et al.[29]

The MDA determination was done by a method based on the reaction with thiobarbituric acid (TBA) at 90°–100°C.[30] The 10% Trichloroacetic acid (TCA) was added the same volume of the homogenated sample and mixed. Then, the mixture was incubated at 90°–100°C for 15 min. After centrifugation, the 0.675% TBA was added to the supernatant to start the reaction at at 90–100°C. The measurement was done at 532 nm.

The protein carbonyl content was analyzed by the method based on the reaction of the carbonyl group with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone.[31] The 20% TCA was added the same volume of homogenated sample and mixed. The mixture was incubated and centrifuged to remove supernatant. The pellet was incubated with 2,4-dinitrophenylhydrazine and washed with ethanol-ethyl acetate mixture. The NaOH solution was used to dissolve formed 2,4-dinitrophenylhydrazone. The measurement was done at 360 nm.

Statistical analysis. The results were analyzed by using SPSS for Windows. The distributions of the groups were analyzed with Shapiro Wilk test. Because the parameters in all groups showed normal distribution, one-way ANOVA test was used for analysis. Post Hoc multiple comparisons were done with LSD. The results were presented as mean ± StdDev. P values < 0.05 were regarded as statistically significant.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This study was supported by "Republic of Turkey Prime Ministry State Planning Organization" with the project no. of 2008K120370. Ersin Fadillioglu and Tuncay Delibasi were partly supported by The Turkish Academy of Science (TUBA).

Supplemental Material

Supplemental material may be downloaded here:
http://www.landesbioscience.com/journals/islets/article/25519/

References

1. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 2000; 343:230-8; PMID:10911004; http://dx.doi.org/10.1056/NEJM200007273430401.
2. Ribeiro MM, Klein D, Pileggi A, Molano RD, Fraker C, Ricordi C, et al. Heme oxygenase-1 fused to a TAT peptide transduces and protects pancreatic beta-cells. Biochem Biophys Res Commun 2003; 305:876-81; PMID:12767912; http://dx.doi.org/10.1016/S0006-291X(03)00856-8.
3. Lenzen S, Drinkgern J, Tiedge M. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. Free Radic Biol Med 1996; 20:463-6; PMID:8720919; http://dx.doi.org/10.1016/0891-5849(96)02051-5.
4. Cattan P, Berney T, Schena S, Molano RD, Pileggi A, Vizzardelli C, et al. Early assessment of apoptosis in isolated islets of Langerhans. Transplantation 2001; 71:857-62; PMID:11349716; http://dx.doi.org/10.1097/00007890-200104150-00006.
5. Hosseini A, Baeeri M, Rahimifard M, Navaei-Nigjeh M, Mohammadirad A, Pourkhalili N, et al. Antiapoptotic effects of cerium oxide and yttrium oxide nanoparticles in isolated rat pancreatic islets. Hum Exp Toxicol 2013; 32:544-53; PMID:23696423; http://dx.doi.org/10.1177/0960327112468175.
6. De Vincenzi M, Stammati A, De Vincenzi A, Silano M. Constituents of aromatic plants: carvacrol. Fitoterapia 2004; 75:801-4; PMID:15567271; http://dx.doi.org/10.1016/j.fitote.2004.05.002.
7. Yu H, Zhang ZL, Chen J, Pei A, Hua F, Qian X, et al. Carvacrol, a food-additive, provides neuroprotection on focal cerebral ischemia/reperfusion injury in mice. PLoS One 2012; 7:e33584; PMID:22438954; http://dx.doi.org/10.1371/journal.pone.0033584.
8. Hajhashemi V, Ghannadi A, Pezeshkian SK. Antinociceptive and anti-inflammatory effects of Satureja hortensis L. extracts and essential oil. J Ethnopharmacol 2002; 82:83-7; PMID:12241981; http://dx.doi.org/10.1016/S0378-8741(02)00137-X.
9. Yanishlieva NV, Marinova EM, Gordon MH, Reneva VG. Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chem 1999; 64:59-66; http://dx.doi.org/10.1016/S0308-8146(98)00086-7.
10. Ultee A, Kets EP, Smid EJ. Mechanisms of action of carvacrol on the food-borne pathogen Bacillus cereus. Appl Environ Microbiol 1999; 65:4606-10; PMID:10508096.
11. Aeschbach R, Löliger J, Scott BC, Murcia A, Butler J, Halliwell B, et al. Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. Food Chem Toxicol 1994; 32:31-6; PMID:7510659; http://dx.doi.org/10.1016/0278-6915(84)90033-4.
12. Aydın E, Türkez H, Keles MS. The effect of carvacrol on healthy neurons and N2a cancer cells: some biochemical, anticancerogenicity and genotoxicity studies. Cytotechnology 2013; •••; PMID:23553016; http://dx.doi.org/10.1007/s10616-013-9547-5.
13. Landa P, Kokoska L, Pribylova M, Vanek T, Marsik P. In vitro anti-inflammatory activity of carvacrol: ınhibitory effect on COX-2 catalyzed prostaglandin E(2) biosynthesis. Arch Pharm Res 2009; 32:75-8; PMID:19183879; http://dx.doi.org/10.1007/s12272-009-1120-6.
14. Slamenova D, Horvathova E, Marsalkova L, Wsolova L. Carvacrol given to rats in drinking water reduces the level of DNA lesions induced in freshly isolated hepatocytes and testicular cells by H(2)O(2). Neoplasma 2008; 55:394-9; PMID:18665749.
15. Merani S, Shapiro AM. Current status of pancreatic islet transplantation. Clin Sci (Lond) 2006; 110:611-25; PMID:16689680; http://dx.doi.org/10.1042/CS20050342.
16. Bottino R, Balamurugan AN, Tse H, Thirunavukkarasu C, Ge X, Profozich J, et al. Response of human islets to isolation stress and the effect of antioxidant treatment. Diabetes 2004; 53:2559-68; PMID:15448084; http://dx.doi.org/10.2337/diabetes.53.10.2559.
17. Li N, Brun T, Cnop M, Cunha DA, Eizirik DL, Maechler P. Transient oxidative stress damages mitochondrial machinery inducing persistent beta-cell dysfunction. J Biol Chem 2009; 284:23602-12; PMID:19546218; http://dx.doi.org/10.1074/jbc.M109.024323.
18. Grankvist K, Marklund SL, Täljedal ıB. CuZn-superoxide dismutase, Mn-superoxide dismutase, catalase and glutathione peroxidase in pancreatic islets and other tissues in the mouse. Biochem J 1981; 199:393-8; PMID:7041886.
19. Chung SS, Kim M, Lee JS, Ahn BY, Jung HS, Lee HM, et al. Mechanism for antioxidative effects of thiazolidinediones in pancreatic β-cells. Am J Physiol Endocrinol Metab 2011; 301:E912-21; PMID:21846907; http://dx.doi.org/10.1152/ajpendo.00120.2011.
20. Park L, Min D, Kim H, Park J, Choi S, Park Y. The combination of metallothionein and superoxide dismutase protects pancreatic β cells from oxidative damage. Diabetes Metab Res Rev 2011; 27:802-8; PMID:22069263; http://dx.doi.org/10.1002/dmrr.1254.
21. Aristatile B, Al-Numair KS, Veeramani C, Pugalendi KV. Effect of carvacrol on hepatic marker enzymes and antioxidant status in D-galactosamine-induced hepatotoxicity in rats. Fundam Clin Pharmacol 2009; 23:757-65; PMID:19650854; http://dx.doi.org/10.1111/j.1472-8206.2009.00721.x.
22. Lima Mda S, Quintans-Júnior LJ, de Santana WA, Martins Kaneto C, Pereira Soares MB, Villarreal CF. Anti-inflammatory effects of carvacrol: evidence for a key role of interleukin-10. Eur J Pharmacol. 2013; 699:112-7; PMID: 23220159; http://dx.doi.org/10.1016/j.ejphar.2012.11.040.
23. Itoh T, Sugimoto K, Shimoda M, Chujo D, Takita M, ıwahashi S, et al. Establishment of a prolonged pancreas preservation model for islet isolation research in mice. islets 2011; 3:376-80; PMID:22045261; http://dx.doi.org/10.4161/isl.3.6.18159.
24. Frankic T, Levart A, Salobir J. The effect of vitamin E and plant extract mixture composed of carvacrol, cinnamaldehyde and capsaicin on oxidative stress induced by high PUFA load in young pigs. Animal 2010; 4:572-8; PMID:22444044; http://dx.doi.org/10.1017/S1751731109991339.
25. Carter JD, Dula SB, Corbin KL, Wu R, Nunemaker CS. A practical guide to rodent islet isolation and assessment. Biol Proced Online 2009; 11:3-31; PMID:19957062; http://dx.doi.org/10.1007/s12575-009-9021-0.
26. Morgan NG, Diakogiannaki E, Russell MA. The incubation and monitoring of cell viability in primary rat islets of Langerhans and pancreatic beta-cell lines. Methods Mol Biol 2009; 560:53-64; PMID:19504243; http://dx.doi.org/10.1007/978-1-59745-448-3_5.
27. Ricordi C. Quantitative and qualitative standards for islet isolation assessment in humans and large mammals. Pancreas 1991; 6:242-4; PMID:1679542; http://dx.doi.org/10.1097/00006676-199103000-00018.

28. London NJ, Contractor H, Lake SP, Aucott GC, Bell PR, James RF. A fluorometric viability assay for single human and rat islets. Horm Metab Res Suppl 1990; 25:82-7; PMID:2088991.
29. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265-75; PMID:14907713.
30. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. Oxygen radicals in biological systems. In: Packer L, Glazer AN, ed. Methods in Enzymology. 186. California: Academic Press, 1990:407-21.
31. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. In: Packer L, Glazer AN, editors. Methods in Enzymology, Oxygen radicals in biological systems. 186. California: Academic Press, 1990:464-78.