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EXTRACT OF GRAPEFRUIT-SEED REDUCES ACUTE PANCREATITIS INDUCED BY ISCHEMIA/REPERFUSION IN RATS; POSSIBLE IMPLICATION OF TISSUE ANTIOXIDANTS

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Grapefruit seed extract (GSE) has been shown to exert antibacterial, antifungal and antioxidant activity possibly due to the presence of naringenin, the flavonoid with cytoprotective action on the gastric mucosa. No study so far has been undertaken to determine whether this GSE is also capable of preventing acute pancreatic damage induced by ischemia/reperfusion (I/R), which is known to result from reduction of anti-oxidative capability of pancreatic tissue, and whether its possible preventive effect involves an antioxidative action of this biocomponent. In this study carried out on rats with acute hemorrhagic pancreatitis induced by 30 min partial pancreatic ischemia followed by 6 h of reperfusion, the GSE or vehicle (vegetable glycerin) was applied intragastrically in gradually increasing amounts (50-500 µl) 30 min before I/R. Pretreatment with GSE decreased the extent of pancreatitis with maximal protective effect of GSE at the dose 250 µl. GSE reduced the pancreatitis-evoked increase in serum lipase and poly-C specific ribonuclease activity, and attenuated the marked fall in pancreatic blood flow and pancreatic DNA synthesis. GSE administered alone increased significantly pancreatic tissue content of lipid peroxidation products, malondialdehyde and 4-hydroxyalkens, and when administered before I/R, GSE reduced the pancreatitis-induced lipid peroxidation. We conclude that GSE exerts protective activity against I/R-induced pancreatitis probably due to the activation of antioxidative mechanisms in the pancreas and the improvement of pancreatic blood flow.

Key words: *Ischemia/reperfusion, pancreatitis, grapefruit-seed extract, pancreatic blood flow, pancreatoprotection.*

INTRODUCTION

Grapefruit-seed extract (GSE) containing bioactive flavonoids, has been shown to possess antibacterial, antiviral and antifungal properties (1-3). This beneficial action of GSE has been attributed to the antioxidative activity of citrous flavonoids such as naringenin, that was found in grapefruit juice and seeds and shown to exhibit cytoprotective activity against injury induced by algal toxins in isolated hepatocytes (4). In addition, naringenin was reported to exhibit anti-cancer activity against human breast cancers (5).

Therapeutic efficacy of citrous fruits such as grapefruits or red grapes is supported by the fact that they contain different classes of polyphenolic flavonoids, that have been shown to inhibit platelet aggregation (6), thus decreasing the risk of coronary thrombosis and myocardial infarction.

The involvement of GSE flavonoids in the mechanism of pancreatic integrity and in the protection of pancreas against the damage induced by acute inflammation has not been so far studied. On the other hand, it was recently revealed that the flavonoid attenuates gastric mucosal lesions produced by various ulcerogens (7). In another report, naringenin was reported to exhibit the protective effects against the gastric injury induced by absolute ethanol predominantly due to the increase in the mucus secretion (8). It might be of interest that this gastroprotective effect of naringenin and accompanying increase in the mucus secretion were, in part, attenuated by indomethacin suggesting the involvement of endogenous prostaglandins in the mechanism of this flavonoid-induced gastroprotection (8). However, it remains to establish whether GSE influences the development of acute pancreatic injury induced by I/R and accompanying stress and if so what might be the mechanism of pancreatic protection induced by GSE.

The purpose of this study carried out on rats with acute pancreatitis induced by I/R was to determine: 1) whether GSE can exert the pancreatoprotective effect against the damage induced the I/R ; 2) whether it affects the blood flow in the pancreas and 3) whether anti-oxidant system of the pancreatic tissue is involved in the GSE-induced pancreatoprotection.

MATERIALS AND METHODS

Animals and treatment

Studies were performed on male Wistar rats weighing 200-250 g according to the experimental protocol approved by the Committee for Research and Animal Ethics of Jagiellonian University.

Animals were housed in cages with wire mesh bottoms, with normal room temperature and a 12-hour light-dark cycle. Thirty min before induction of acute pancreatitis, rats were treated with grapefruit-seed extract (CITRO kapky/kvapky), purchased from Herb-Pharma s.r.o., Velke Ludnice, Slovakia. Grapefruit-seed extract was applied intragastrically (i.g.) in a volume of 2.0 ml of saline at the doses of 50, 100, 250 or 500 μ l. Control group received i.g. the same dose of vehicle

(vegetable glycerin) in 2.0 ml of saline. Acute pancreatitis was induced by limitation of pancreatic blood flow in inferior splenic artery followed by reperfusion as described previously (9). Briefly, after fasting for 24 h with free access to water, rats were anesthetized with ketamine (50 mg/kg intraperitoneally, Bioketan, Biowet, Gorzów, Poland). After longitudinal laparotomy, ischemia in the splenic region of the pancreas was induced by clamping of inferior splenic artery using microvascular clips. Thirty min later, microvascular clips were removed to obtain pancreatic reperfusion and the abdominal cavity was closed by suture. In sham-operated control animals, longitudinal laparotomy and mobilization of pancreas without clamping of any arteries was performed. Animals were anesthetized again and sacrificed after 6 h of reperfusion (N=8 animals in each experimental group).

Determination of pancreatic blood flow

At the time of experiment cessation animals were anesthetized with ketamine (50 mg/kg i.p., Bioketan, Biowet, Gorzów, Poland) and the abdominal cavity was opened. The pancreas was exposed for the measurement of the blood flow in the pancreatic tissue by laser Doppler flowmeter using PeriFlux 4001 Master monitor (Perimed AB, Järfälla, Sweden), as described previously (10). The pancreatic blood flow was presented as percent change from control value obtained in sham-operated rats.

Determination of serum lipase activity

Immediately after measurement of pancreatic blood flow, the abdominal aorta was exposed, the blood was taken and serum separated from the blood cells. Serum lipase activity was determined, as described previously (11), using a Kodak Ectachem DT II System analyzer (Eastman Kodak Company, Rochester, NY, USA) and Lipa DT Slides (Vitros DT Chemistry System, Johnson & Johnson Clinical Diagnostic, Inc., Rochester, NY, USA). The values of serum lipase activity were expressed as units/liter.

Determination of pancreatic DNA synthesis

After the blood withdrawal, the pancreas was carefully dissected out from its attachment to the stomach, the duodenum, and the spleen. Fat and peripancreatic tissue were trimmed away. Samples of pancreatic tissue were taken for study of DNA synthesis and lipid peroxidation. The rate of DNA synthesis in the portion of minced pancreatic tissue was determined by incubating the tissue at 37°C for 45 min in 2 ml of medium containing 8 μ Ci /ml of [³H]thymidine ([6-³H]thymidine, 20-30 Ci/mmol; Institute for Research, Production and Application of Radioisotopes, Prague, Czech Republic), as described previously (12). The incorporation of [³H]thymidine into DNA was determined by counting 0.5 ml DNA-containing supernatant in a liquid scintillation system. DNA synthesis was expressed as [³H]thymidine disintegrations per minute per microgram DNA (dpm/ μ g DNA).

Measurement of pancreatic lipid peroxidation

Lipid peroxidation was determined by measurement of malondialdehyde (MDA) and 4-hydroxyalkens (HAE) using commercial kit Bioxytech ®LPO-586™ (OxisResearch™, OXIS Health Products, Inc., Portland, OR, USA), as described previously (13). The Bioxytech ®LPO-586™ is a colorimetric assay based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole, with MDA and HAE at 45°C. One molecule of either MDA or HAE reacts with 2

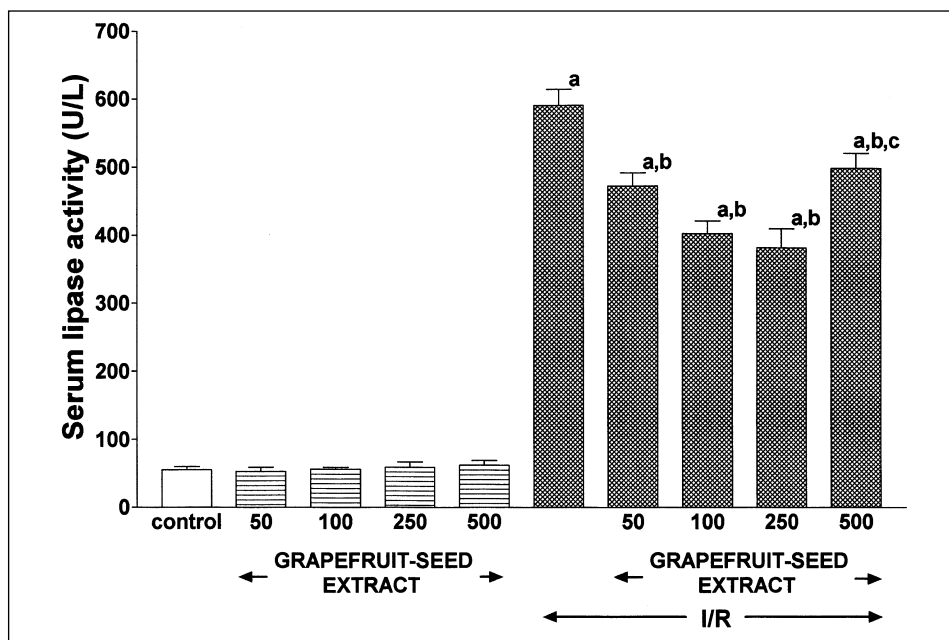


Fig. 1. Effect of grapefruit-seed extract (GSE), given intragastrically at the dose 50, 100, 250 or 500 μ l, on serum lipase activity in rats with or without ischemia/reperfusion (I/R)-induced pancreatitis. Mean \pm S.E.M. N=8 animals in each experimental group. ^aP<0.05 compared with control; ^bP<0.05 compared with I/R alone; ^cP<0.05 compared with 250 μ l of GES + I/R.

molecules of N-methyl-2-phenylindole to yield a stable chromophore with maximal absorbance at 586 nm. The results were expressed in nmol per g of pancreatic tissue.

Determination of serum poly-C specific ribonuclease

Poly-C specific ribonuclease activity was determined using Warsaw & Lee procedure (14), employing polycyidylic acid (poly-C) as ribonuclease substrate, as described previously in details (15). Briefly, serum samples (50 μ l) were placed to small centrifuge glass tubes with 400 μ l of 100 mM sodium phosphate buffer pH 7.8. Fifty μ l of 1 g% buffer Poly-C solution was added to each prepared sample and the mixtures were immediately transferred to a water bath with temperature 37 $^{\circ}$ C. After 15 minutes of incubation, the samples were placed on ice and 1 ml of cold ice lanthanum nitrate (20 mM) in perchloric acid (420 mM) solution (precipitating agent) was added. The samples were left on ice for 30 minutes to allow precipitated Poly-C to coagulate, and subsequently were centrifuged at 4 $^{\circ}$ C for 15 min. Samples of clear supernatant (100 μ l) were taken and diluted in 900 μ l of distilled water. In the diluted supernatant light absorption at 278 nm was determined. Each test was performed in duplicate, and final P-RNase activity was calculated as a mean of two values and expressed in units per liter (u/l).

Statistical analysis

Comparison of the differences between the mean values of various groups of experiments was made by analysis of variance and the Student's T test for unpaired data. A difference with a P value of less than 0.05 was considered statistically significant. Results are expressed as means (\pm S.E.M.).

RESULTS

The pancreas of sham-operated animals showed macroscopically no tissue alterations. In contrast, pancreatic ischemia followed by reperfusion (I/R) produced acute necrotizing pancreatitis in all rats tested. The pancreas was swollen with visible foci of hemorrhages. Serum lipase activity was increased by 537 u/l (*Fig. 1*), whereas pancreatic DNA synthesis (*Fig. 2*) and pancreatic blood flow (*Fig. 3*) were decreased by 41 and 62%, respectively. Additionally, the I/R-induced pancreatitis caused a twofold increase in serum poly-C ribonuclease activity (*Fig. 4*) and eightfold increase in pancreatic lipid peroxidation (pancreatic content of malondialdehyde (MDA) and 4-hydroxyalkens (HAE) (*Fig. 5*).

In sham-operated animals, i.g. administration of grapefruit-seed extract at gradually increasing doses of 50-500 μ l was without significant effect on serum lipase (*Fig. 1*) and poly-C ribonuclease (*Fig. 4*) activity. Pancreatic DNA synthesis (*Fig. 2*), and pancreatic blood flow (*Fig. 3*) were also unchanged. In contrast, grapefruit-seed extract caused the significant increase in pancreatic lipid peroxidation (*Fig. 5*). In animals treated with the highest dose of grapefruit-seed extract (500 μ l), the pancreatic content of MDA + HAE reached 23.6 ± 1.9

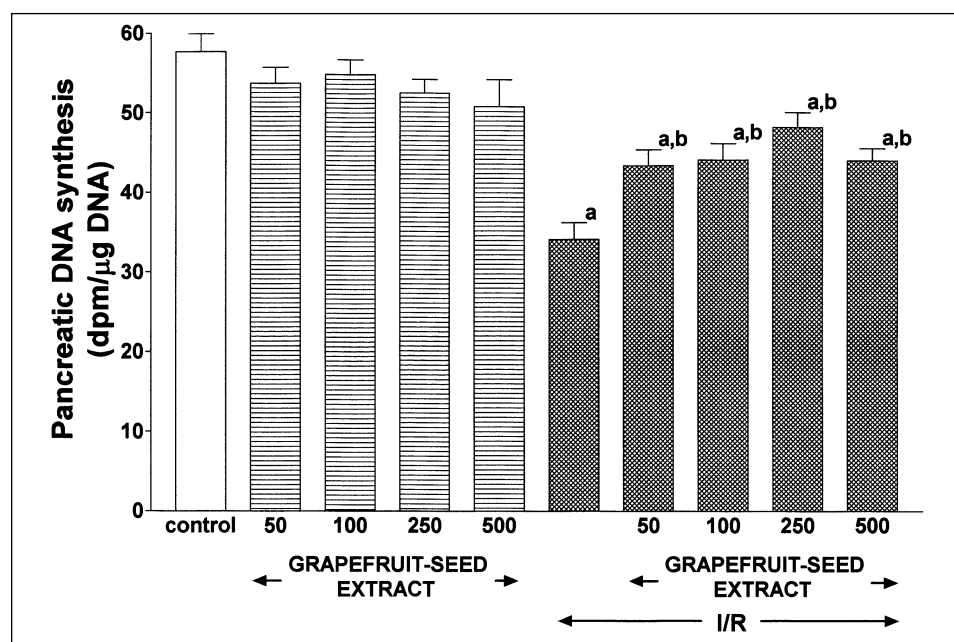


Fig. 2. Effect of grapefruit-seed extract (given intragastrically at the dose 50, 100, 250 or 500 μ l) on pancreatic DNA synthesis in rats with or without ischemia/reperfusion (I/R)-induced pancreatitis. Mean \pm S.E.M. N=8 animals in each experimental group. ^aP<0.05 compared with control. ^bP<0.05 compared with I/R alone.

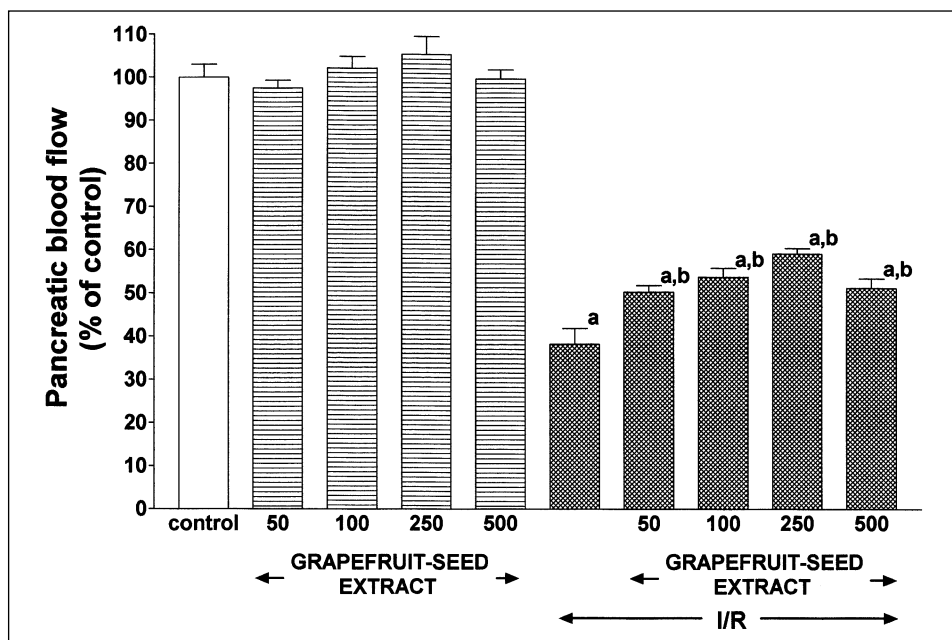


Fig. 3. Effect of grapefruit-seed extract (given intragastrically at the dose 50, 100, 250 or 500 μ l) on pancreatic blood flow in rats with or without ischemia/reperfusion (I/R)-induced pancreatitis. Mean \pm S.E.M. N=8 animals in each experimental group. ^aP<0.05 compared with control. ^bP<0.05 compared with I/R alone.

nmol/g tissue and this value was significantly higher than that observed in control group treated with saline (6.2 ± 0.5 nmol/g tissue) or animals treated with the smallest dose of GSE (14.8 ± 1.3 nmol/g tissue).

In animals with I/R-induced pancreatitis, the treatment with grapefruit-seed extract at the doses ranging from 50 to 250 μ l caused the significant decrease in serum lipase activity (Fig. 1). Administration of grapefruit-seed extract at the dose 250 μ l caused maximal reduction in serum lipase activity. This effect was attenuated, when grapefruit-seed extract was used at the highest dose - 500 μ l of GSE.

Treatment with grapefruit-seed extract partly reversed the pancreatitis-evoked decrease in pancreatic DNA synthesis (Fig. 2) and pancreatic blood flow (Fig. 3). This effect was maximally expressed, when grapefruit-seed extract was administered at the dose 250 μ l. The effect of highest dose of grapefruit-seed extract (500 μ l) was similar to that observed after 50 μ l of GSE.

Administration of grapefruit-seed extract at the dose 50 μ l did not significantly affect the pancreatic content of MDA + HAE in animals with I/R-induced pancreatitis (Fig. 5), whereas the dose 100-500 μ l of grapefruit-seed extract significantly reduced the pancreatitis-evoked increase in pancreatic lipid peroxidation. Maximal reduction of pancreatic content of MDA + HAE in

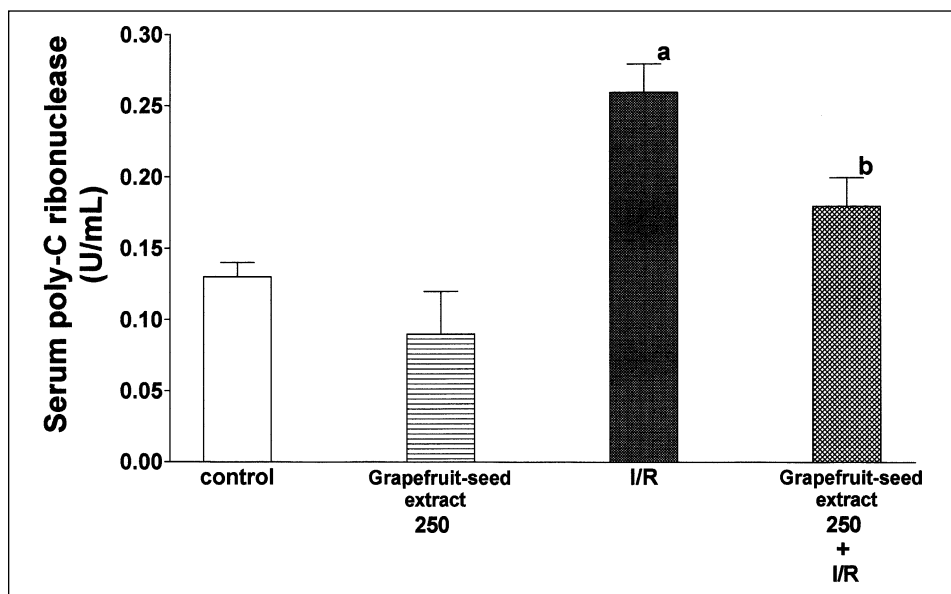


Fig. 4. Effect of grapefruit-seed extract, given intragastrically at the dose 250 μ l on serum poly-C ribonuclease activity in rats with or without ischemia/reperfusion (I/R)-induced pancreatitis. Mean \pm S.E.M. N=8 animals in each experimental group. ^aP<0.05 compared with control. ^bP<0.05 compared with I/R alone.

animals with I/R-induced pancreatitis was observed, when grapefruit-seed extract was administered at the dose 250 μ l.

Grapefruit-seed extract given alone at the dose 250 μ l did not affect the serum poly-C ribonuclease activity but pretreatment with grapefruit-seed extract significantly reduced the pancreatitis-evoked increase in serum poly-C ribonuclease activity (by 27%) (Fig. 4).

DISCUSSION

This study carried out on rats with acute pancreatitis induced by I/R confirms our previous reports that partial reduction in pancreatic blood flow in the splenic region of the pancreas results in the development of acute hemorrhagic and necrotizing pancreatitis resembling those occurring under clinical conditions (9). Ischemia itself produce pancreatic slight tissue damage but the major damage occurred after ischemia followed by subsequent reperfusion, as it happens in other tissues including the stomach (16), intestine (17) and cardiac (18) or skeletal muscle (19). This extensive tissue damage observed after I/R is accompanied by the fall in pancreatic blood flow (9), noticed also in this report, and by the excessive production of reactive oxygen species (ROS) (20, 21) due

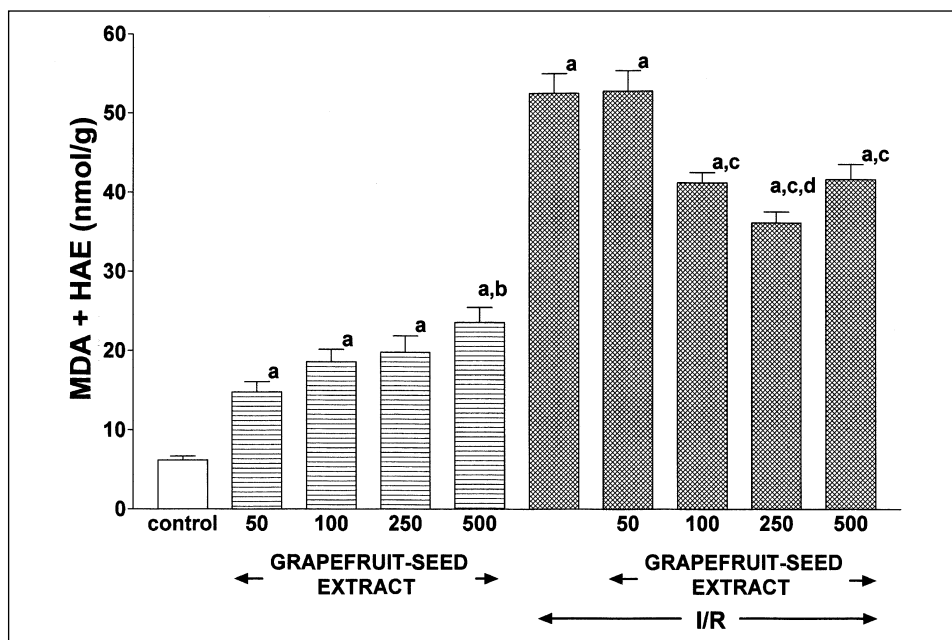


Fig. 5. Effect of grapefruit-seed extract (GSE), given intragastrically at the dose 50, 100, 250 or 500 μ l, on pancreatic content of malondialdehyde (MDA) and 4-hydroxyalkens (HAE) in rats with or without ischemia/reperfusion (I/R)-induced pancreatitis. Mean \pm S.E.M. N=8 animals in each experimental group. ^aP<0.05 compared with control; ^bP<0.05 compared with 50 μ l of GSE alone; ^cP<0.05 compared with I/R alone; ^dP<0.05 compared with 50 μ l of GSE + I/R.

to the activation of leukocytes and the formation of pro-inflammatory interleukins (9, 22).

The major finding of the present study is the demonstration for the first time that grapefruit-seed extract applied intragastrically is capable of reducing significantly the extent of pancreatitis induced by I/R, and that this was accompanied by attenuation of the I/R-induced severe fall in pancreatic blood flow. This effect was combined with the significant reduction in tissue contents of malondialdehyde (MDA) and 4-hydroxyalkens (HAE), the products of lipid peroxidation possibly caused by ROS in the pancreas subjected to I/R. As we outlined previously (23), the accumulation of products of ROS-mediated lipid peroxidation in pancreatic acinar cells is a good evidence of the oxidative stress, documented in this report by the marked increase in tissue contents of MDA and HAE in rats subjected to I/R-induced pancreatitis.

The anti-oxidative influence of grapefruit-seed extract occurred despite of the fact that the agent was applied intragastrically, suggesting that the active principle, flavonoids present in the grape seed products, possibly reached the pancreatic tissue *via* circulation to provide pancreatic protection. Further studies are required to determine whether the favorable action of grapefruit-seed extract

on the pancreas subjected to I/R is accompanied by the rise the in expression of antioxidants and antioxidative enzymes that have been shown to prevent acute pancreatitis induced by various means (23).

It is of interest that, in our present study, grapefruit-seed extract applied alone, without subsequent I/R and induction of acute pancreatitis, cause small but significant increase in pancreatic lipid peroxidation. This observation may suggest that inhibitory effect of grapefruit-seed extract on pancreatic lipid peroxidation observed in I/R-induced pancreatic is a secondary effect dependent on the stimulation of antioxidative mechanism in the pancreas. It looks that grapefruit-seed extract acts as some kind of preconditioning factor that adapts the pancreas to the subsequent ischemia with reperfusion. The support for this hypothesis is an observation that ischemic preconditioning by brief exposure to ischemia protects the pancreas and renders the pancreatic tissue more resistant against the damage evoked by subsequent severe ischemia (24).

Pancreatic-type poly-C specific ribonuclease in one of the few direct markers of pancreatic injury (14) and plasma activity of this enzyme is increased in patients with acute pancreatitis who develop the pancreatic necrosis and the severe course of disease (14, 15). In our present study, I/R-induced pancreatitis increased serum activity of poly-C ribonuclease and this effect was reversed by pretreatment with GSE. This observation, apart the reduction in serum lipase activity and an increase in pancreatic DNA synthesis, represents an additional evidence of protective effect of GSE administration on the pancreas.

An alternative explication for the observed protective activity of GSE could be its antimicrobial effects. As reported by Cunliffe and Mahida (25), the lining of GI tract is exposed to a wide range of microorganisms and it is also capable of expressing and releasing its own antimicrobial peptides such as alpha and beta-defensins these are powerful antibacterial agents acting as chemoattractants for cells of the innate and adaptive-immune system. The grapefruit-seed extract was found to exert powerful antibacterial and antifungal effects (1, 2). Grapefruit-seed extract was found to disrupt the bacterial membrane and liberate the cytoplasmic content almost immediately after exposure of bacteria to the extract even at highly diluted concentrations. According to our experience (unpublished observations), the grapefruit-seed extract used in this study has extremely powerful antimicrobial and antifungal action *in vitro*, therefore, it is likely that this extract, in addition to exerts by itself the anti-microbial activity, may release the antimicrobial peptides from gastric lining following its application into the stomach. The question remains whether antimicrobial agents, present in grapefruit-seed extract or released from gastric mucosa after its intragastric application, reach the pancreas in the concentrations sufficient to exhibit the anti-inflammatory effect. It is most likely that antimicrobial activity of GSE indirectly affects the development of acute pancreatitis. Probably, intragastric administration of GSE, causing local antibacterial and antifungal effects, can reduce the production of pro-inflammatory mediators and this effect may reduce

the systemic inflammatory response in the course of acute pancreatitis. This hypothesis is supported by our present finding that administration of GSE reduces serum lipase activity in animals with I/R-induced pancreatitis. In agreement with this hypothesis is also recent observation that presence of persistent gastric infection with *Helicobacter pylori* aggravates severity of I/R-induced pancreatitis (26) and mechanism of this harmful effect of gastric *Helicobacter pylori* infection on the pancreas involves the increase in production of pro-inflammatory cytokines. Also clinical data indicate the relationship between antibacterial treatment and the course of acute pancreatitis (27, 28).

In summary, our results provide the evidence that grapefruit-seed extract, containing citrus flavonoids, exerts a potent pancreatoprotective activity against the I/R-induced pancreatitis and this protective effect on the pancreas involves the stimulation of antioxidative mechanisms, and the preservation of pancreatic blood flow.

REFERENCES

1. Hegggers JP, Cottingham J, Gusman J, et al. The effectiveness of processed grapefruit-seed extract as an antibacterial agent: II. Mechanism of action and in vitro toxicity. *J Altern Complement Med* 2002; 8: 333-340.
2. Reagor L, Gusman J, McCoy L, Carino E, Hegggers JP. The effectiveness of processed grapefruit-seed extract as an antibacterial agent: I. An in vitro agar assay. *J Altern Complement Med* 2002; 8:325-332.
3. Proteggente AR, Pannala AS, Paganga G, et al. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radic Res* 2002; 36: 217-233.
4. Blakson H, Grotterod EM, Seglen PO. Prevention of toxin-induced cytoskeletal disruption and apoptotic liver cell death by grapefruit flavonoid, naringin. *Cell Death Differ* 2000; 7: 739-746.
5. So FV, Guthrie N, Chambers AF, Moussa M, Carroll KK. Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus juices. *Nutr Cancer*. 1996; 26: 167-181.
6. Keevil JG, Osman HE, Reed JD, Folts JD. Grape juice, but not orange juice or grapefruit juice, inhibits human platelet aggregation. *J Nutr* 2000; 130: 53-56.
7. Konturek SJ, Dozdowicz D, Brzozowski T, et al. Importance of lipid peroxidation, sensory nerves and nitric oxide pathway in gastroprotective action of grapefruit-seed extract. *Gastroenterology* 2004; 126(suppl 2):A544.
8. Motilva V, Alarcon de la Lastra C, Martin MJ. Ulcer-protecting effects of naringenin on gastric lesions induced by ethanol in rat: role of endogenous prostaglandins. *J Pharm Pharmacol* 1994; 46: 91-94.
9. Dembinski A, Warzecha Z, Ceranowicz P, et al. Pancreatic damage and regeneration in the course of ischemia-induced acute pancreatitis in rats. *J Physiol Pharmacol* 2001; 52: 221-236.
10. Konturek SJ, Szlachcic A, Dembinski A, Warzecha Z, Jaworek J, Stachura J. Nitric oxide in pancreatic secretion and hormone-induced pancreatitis in rats. *Int J Pancreatol* 1994; 15: 19-28.
11. Dembinski A, Warzecha Z, Ceranowicz P, et al. Ghrelin attenuates the development of acute pancreatitis in rat. *J Physiol Pharmacol* 2003; 54: 561-573.

12. Warzecha Z, Dembinski A, Ceranowicz P, et al. IGF-1 stimulates production of interleukin-10 and inhibits development of caerulein-induced pancreatitis. *J Physiol Pharmacol* 2003; 54: 575-90.
13. Kwiecien S, Brzozowski T, Konturek PC, et al. The role of reactive oxygen species and capsaicin-sensitive sensory nerves in the pathomechanisms of gastric ulcers induced by stress. *J Physiol Pharmacol* 2003;54:423-437.
14. Warshaw AL, Lee KH. Serum ribonuclease elevations and pancreatic necrosis in acute pancreatitis. *Surgery* 1979; 86: 227-234.
15. Naskalski JW, Kusnierz-Cabala B, Panek J, Kedra B. Poly-C specific ribonuclease activity correlates with increased concentrations of IL-6, IL-8 and sTNFR55/sTNFR75 in plasma of patients with acute pancreatitis. *J Physiol Pharmacol* 2003; 54: 439-448.
16. Andrews FJ, Malcontenti-Wilson C, O'Brien PE. Protection against gastric ischemia-reperfusion injury by nitric oxide generators. *Dis Dis Sci* 1994; 39: 366-373.
17. Kubes P. Ischemia-reperfusion in feline small intestine: a role for nitric oxide. *Am J Physiol* 1993; 264: G143-G149.
18. Weyrich AS, Ma X, Lefer DJ, Albertine KH, Lefer AM. In vivo neutralization of P-selectin protects feline heart and endothelium in myocardial ischemia and reperfusion injury. *J Clin Invest* 1993; 91: 2620-2629.
19. Pang CH, Forrest CR, Mounsey R. Pharmacological intervention in ischemia-induced reperfusion injury in the skeletal muscle. *Microsurgery* 1993; 14: 176-182.
20. Grandner DN, Korthuis RJ. Physiologic mechanism of posts ischemic tissue injury. *Annu Rev Physiol* 1995; 57: 311-332.
21. Toyama MT, Lewis MPN, Kusske AM, Reber PU, Ashley SW, Reber HA. Ischemia-reperfusion mechanism in acute pancreatitis. *Scand J Gastroenterol* 1996; 31 (suppl 219): 20-23.
22. Warzecha Z, Dembinski A, Ceranowicz P, Influence of leptin administration on the course of acute ischemic pancreatitis. *J Physiol Pharmacol* 2002; 53: 775-790.
23. Dabrowski A, Konturek SJ, Konturek JW, Gabryelewicz A. Role of oxidative stress in the pathogenesis of caerulein-induced acute pancreatitis. *Eur J Pharmacol* 1999; 377: 1-11.
24. Dembinski A, Warzecha Z, Ceranowicz P, et al. Ischemic preconditioning reduces the severity of ischemia/reperfusion-induced pancreatitis. *Eur J Pharmacol* 2003; 473: 207-216.
25. Cunliffe RN, Mahida YR. Expression and regulation of antimicrobial peptides in the gastrointestinal tract. *J Leukoc Biol* 2004; 75: 49-58.
26. Warzecha Z, Dembinski A, Ceranowicz P, et al. Deleterious effect of Helicobacter pylori infection on the course of acute pancreatitis in rats. *Pancreatology* 2002; 2: 386-395.
27. Asanuma Y, Furuya T, Tamaka J, Sato T, Shibata S, Koyama K. The application of immobilized polymyxin B fiber in the treatment of septic shock associated with severe acute pancreatitis: report of two cases. *Surg Today* 1999; 29: 1177-1182.
28. Wilcox M, Kumani P, Fitzgerald P, Keer V, Leeder P. Staphylococcus epidermidis and acute necrotising pancreatitis. *Lancet* 1996; 347: 480-481.

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