Digestive Diseases and Sciences, Vol. 51, No. 2 (February 2006), pp. 298–302 (© 2006)
DOI: 10.1007/s10620-006-3128-9

Resveratrol, a Red Wine Constituent Polyphenol, Protects Gastric Tissue Against the Oxidative Stress in Cholestatic Rats

VEDAT KIRIMLIOGLU, MD,* CENGIZ ARA, MD,* MEHMET YILMAZ, MD,* DINCER OZGOR, MD,* BURAK ISIK, MD,* GOKHAN SOGUTLU, MD,* HALE KIRIMLIOGLU, MD,† AYSUN BAY KARABULUT, PhD,‡ SEZAI YILMAZ, MD,* CUNEYT KAYAALP, MD,* and SAIM YOLOGLU, MD,§

This experimental study was designed to determine the effects of resveratrol on the level of malondialdehyde (MDA), reduced glutathione (GSH), and nitric oxide (NO) in gastric tissue after bile duct ligation (BDL). Swiss albino rats were divided into three groups: Group 1, sham ($n = 7$); Group 2, BDL (BDL only group; $n = 7$); and Group 3, BDL plus resveratrol ($n = 7$). Animals in the resveratrol group were treated with 10 mg/kg resveratrol (i.p.) once a day throughout 28 days. In the resveratrol group, levels of MDA and NO in gastric tissue were significantly lower than in the BDL-only group ($P < 0.001$). The level of GSH in the resveratrol group was significantly higher than in the BDL-only group ($P < 0.001$). The present study demonstrates that intraperitoneal administration of resveratrol maintains antioxidant defenses and reduces oxidative gastric damage. This effect of resveratrol may be useful to preserve gastric tissue under oxidative stress due to cholestasis.

KEY WORDS: resveratrol; biliary cirrhosis; malondialdehyde; reduced glutathione; nitric oxide.

Cirrhotic patients display increased susceptibilitity to gastric mucasal damage, as characterized by hemorrhagic gastropaty and peptic ulceration. In recent years, it has become clear that the entity which was termed congestive gastropaty or portal hypertensive gastropathy may be responsible for 30–50% of upper gastrointestinal tract bleeding in cirrhotic patients (1, 2).

Fatal bleeding in upper gastrointestinal tractus often occurs in critical illness or postoperative patients with obstructive jaundice (3), and the frequency of gastrointestinal ulcerations is higher in jaundiced patients than in the normal population (4). A previous experimental study has shown that the gastric mucosa of cholestatic rats is more vulnerable than that of normal animals (5). In rats with biliary obstruction, excessive nitric oxide (NO) production, increased generation of oxygen free radicals, and lipid peroxidation in the portal hypertensive gastric mucosa have also been implicated in its increased susceptibility to gastric injury (6–8). Resveratrol (3,5,4′-*trans*-trihydroxystilbene) is a natural phytoalexin present in grapes and red wine which possesses a variety of biological activities including anti-inflammatory, anticarcinogenic, and antioxidative activities (9–11).

Although resveratrol has antioxidant features, its effect against gastric oxidative stress in rats with biliary obstruction has not been investigated. The aim of this study was to evaluate whether resveratrol administration can protect gastric tissue against oxidative stress in rats with BDLs. To assess the ability of resveratrol function as an antioxidant in gastric tissue of rats with BDLs, we measured the levels of malondialdehyde (MDA), reduced glutathione (GSH),

Manuscript received April 9, 2005; accepted May 24, 2005.

From the Departments of *General Surgery, †Pathology, ‡Biochemistry, and §Biostatistics, Inonu University School of Medicine, Malatya, Turkey.

Address for reprint requests: Cengiz Ara, MD, Inonu University Turgut Ozal Medical Center, Department of General Surgery, 44069 Malatya, Turkey; cara@inonu.edu.tr.

and NO in gastric tissue of these rats. Gastric injury and effect of resveratrol administration were also evaluated by histological examination.

MATERIALS AND METHODS

A total of 21 3-month-old male Swiss-Albino rats weighing 300–350 g were included in the study. Rats were obtained from Firat University, Animal Laboratory, Elazı Turkey. The study was approved by the Inonu University Ethics Committee.

Rats were kept in stainless-steel cages and allowed free access to food and water ad libitum. Food was withheld for 8 hr prior to surgery, but free access to water was allowed. Rats were subjected to a controlled environment regarding temperature and humidity and a 12-hr light–dark cycle.

Surgical Technique. All surgical procedures were performed under intraperitoneal ketamine (50 mg/kg) and xylazine HCl (10 mg/kg) anesthesia. The 21 rats were divided into three groups: Group 1, sham-operated group ($n = 7$); Group 2, BDL-only group ($n = 7$); and Group 3, BDL plus resveratrol group ($n = 7$).

The sham group received only laparotomy. Secondary biliary cirrhosis was induced by double ligation and division of the common bile duct (12). Abdominal layers were closed with appropriate suture materials. All animals were maintained under the same conditions after surgery. Resveratrol was given intraperitoneally (i.p.) at a dose of 10 mg /kg day, which is reported to cause a marked antioxidative effect (13). The BDL plus resveratrol group received 10 mg/kg resveratrol (Sigma Chemical Co., St. Louis, MO, USA) i.p. once a day throughout 28 days. To eliminate complications arising from the diurnal effects, all rats were sacrificed under anesthesia at the same time of day. The blood samples were taken by vena cava inferior puncture. A part of the liver and the stomach were removed. The stomachs were excised along the greater curvature to inspect macroscopic damages and each stomach was then cut into grips obliquely across the entire corpus. Liver and stomach were fixed in formaldehyde solution for routine histopathologic examinations. The stomach was placed in liquid nitrogen and stored at -85°C for analyses of MDA, GSH, and NO levels.

Biochemical Analyses. The plasma was used to measure total bilirubin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) as indicative parameters of hepatic function (Olympus Diagnostica Gmbit, Ireland). The gastric tissues were homogenized and the MDA contents of homogenates were determined spectrophotometrically (14). The amounts of lipid peroxides were calculated as thiobarbituric acid-reactive substances of lipid peroxidation and results are given as nanomoles per gram of tissue. As tissue nitrite (NO_2–) and nitrate (NO_3–) levels can be used to estimate NO production, we measured the concentration of these stable NO oxidative metabolites. Quantitation of NO_2^- and NO_3– was based on the Griess reaction, in which a chromophore with a strong absorbance at 545 nm is formed by reaction of $NO_2^-$with a mixture of naphthylethylenediamine and sulfanilamide (15). Results are expressed as micromoles per gram of tissue.

Glutathione was determined by the spectrophotometric method, which was based on the use of Elman's reagent (16). Results are expressed as nanomoles per gram of tissue.

Histopatologic Evaluation. Liver and gastric tissues were stained with hematoxylin and eosin and Masson trichrome dye. Gastric histological changes were assessed under the light microscope by a liver pathologist uninformed about the groups. For assessment of inflammation and necrosis, the histologic activity index of Ishak *et al.* (17) was applied.

Statistical Analysis. The Statistical Package for Social Sciences (SPSS) version 10.0 was used for statistical analysis. Individual group parameters were assessed using the Mann-Whitney U test. The results are given in the text as means ± SD for all comparisons; statistical significance was defined as $P < 0.05$.

RESULTS

Visual evaluation of the liver demonstrated cholestatic changes such as congestion and edema of the liver and dilation of the extrahepatic part of the bile duct in all rats undergoing BDL.

The results for MDA, GSH, and NO levels in the three groups are listed in Table 1. In resveratrol-treated rats, levels of MDA and NO were significantly lower than those of in the BDL-only group ($P < 0.001$). The levels of GSH in resveratrol-treated rats were significantly higher than in the BDL-only group ($P < 0.001$).

Apparently, resveratrol administration reduced lipid peroxidation and ameliorated GSH and NO status. Total bilirubin, AST, and ALT levels in rats with BDLs were higher than in the sham and resveratrol groups (Table 2). In other words, in resveratrol-treated rats, the levels of total bilirubin, ALT, and AST were lower than in the BDL group.

TABLE 1. GASTRIC TISSUE LEVELS OF GSH, NO, AND MDA ACTIVITIES IN THE GROUPS

	MDA (nmol/g tissue)	*GSH (nmol/g tissue)*	*NO (μmol/g tissue)*
Groups			
1. Sham ($n = 7$)	68.65 ± 4.75	1.84 ± 0.18	77.66 ± 9.03
2. Bile duct ligation ($n = 7$)	121.75 ± 27.82	0.71 ± 0.25	98.37 ± 5.52
3. Resveratrol ($n = 7$)	60.19 ± 7.15	1.98 ± 0.19	60.11 ± 5.34
P values*			
1 vs 2	0.001	0.001	0.001
2 vs 3	0.001	0.001	0.001

Note. GSH, reduced glutathione; NO, nitric oxide; MDA, malondialdehyde.
*$P < 0.05$ considered statistically significant.

TABLE 2. PLASMA TOTAL BILIRUBIN, AST, AND ALT LEVELS IN THE GROUPS

	Total bilirubin (mg/dl)	*AST (U/L)*	*ALT (U/L)*
Groups			
1. Sham	1.8 ± 0.2	39 ± 4	96 ± 4
2. Bile duct ligation	2.7 ± 0.7	53 ± 18	140 ± 12
3. Resveratrol	2.3 ± 0.3	45 ± 13	110 ± 9
P values			
1 vs 2	0.001	0.001	0.001
2 vs 3	0.001	0.004	0.001

Note. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

In the sham-operated mouse livers, mild lymphoid portal inflammation was the only histopathologic change. There was no ductular proliferation or fibrosis. In the bile duct ligated rats, there was marked bile duct proliferation with incomplete nodule formation (score 3–4) and mild to moderate portal lymphocytic inflammation (Figure 1). Pathologic examination of the stomach in resveratrol-treated rats did not reveal any significant differences from the BDL-only group (Figures 2 and 3).

DISCUSSION

Various experimental models have been used for gastric injury. Calibrated stenosis of the portal vein, carbon tetrachloride-induced cirrhosis, and BDL-induced secondary biliary cirrhosis are frequently used for this purpose (18–20). Ferraz *et al.* (19) revealed that the rat with chronic BDL, which develops consistent biliary cirrhosis, is an excellent experimental model to investigate hemodynamic and gastric mucosal disturbances associated with cirrhosis. The present study indicates that intraperitoneal administration of resveratrol at a dose of 10 mg/kg day reduced MDA and NO and increased GSH levels in gastric tissue after BDL in rats. A recent study showed that plasma levels of nitrite and nitrate are higher in cholestatic rats than in normal animals and this finding confirms the assumption of increased production of NO in cholestasis (21). In another study Chen *et al.* (22) reported that endogenous NO produced by constitutive NO synthase regulates mucosal perfusion and has been suggested to protect the gastrointestinal mucosa from a variety of stimuli. This is the reason why the histopathologic findings are normal in BDL-only rats. On the other hand, although the levels of NO are decreased in the resveratrol group, the histopathologic signs of gastric mucosa are normal. This depends on the protective effect of resveratrol in gastric mucosa by increasing levels of GSH and decreasing levels of MDA.

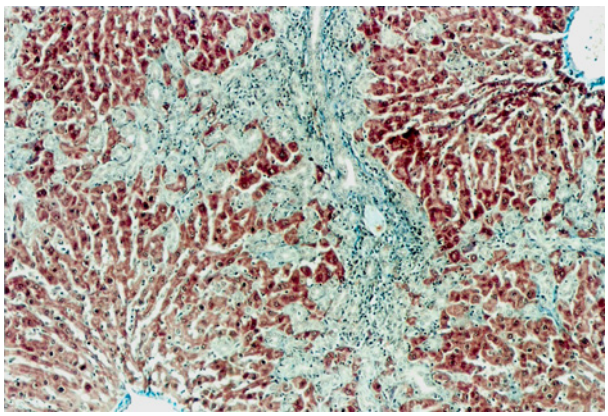

Fig 1. Marked ductuler proliferation and moderate lymphocytic infiltration in the portal area in the BDL-only group. (Masson trichrome; original magnification, 100×.)

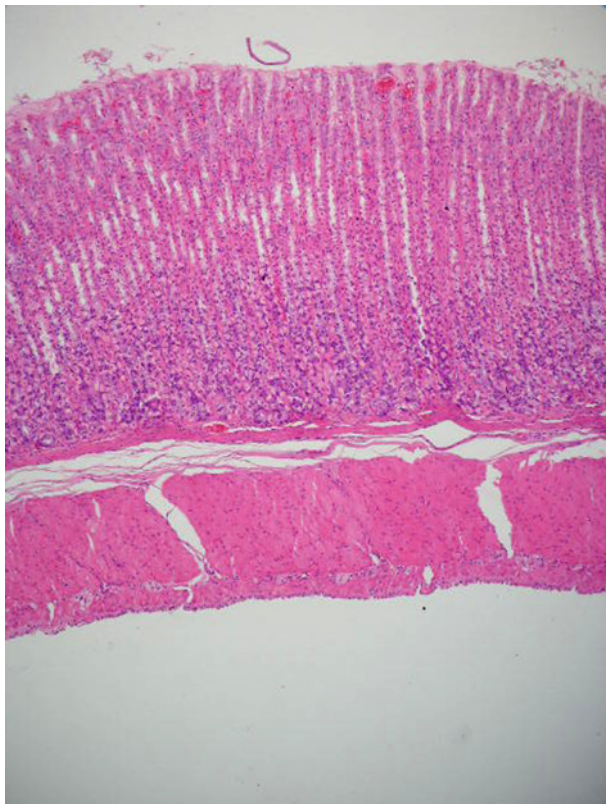

Fig 2. There is no marked inflammation, fibrosis, or epithelial change in the gastric corpus in the BDL-only group. (Hematoxylin and eosin; original magnification, 100×.)

In most tissues, MDA is formed as a result of the peroxidative decomposition of polyunsaturated fatty acids. Therefore, measurements of the amount of MDA provide an index of oxidative stress and lipid peroxidation (23). There are several reports indicating increased levels of MDA in rats with BDL (7, 8). Our results are similar to these previous works reporting high levels of MDA. In

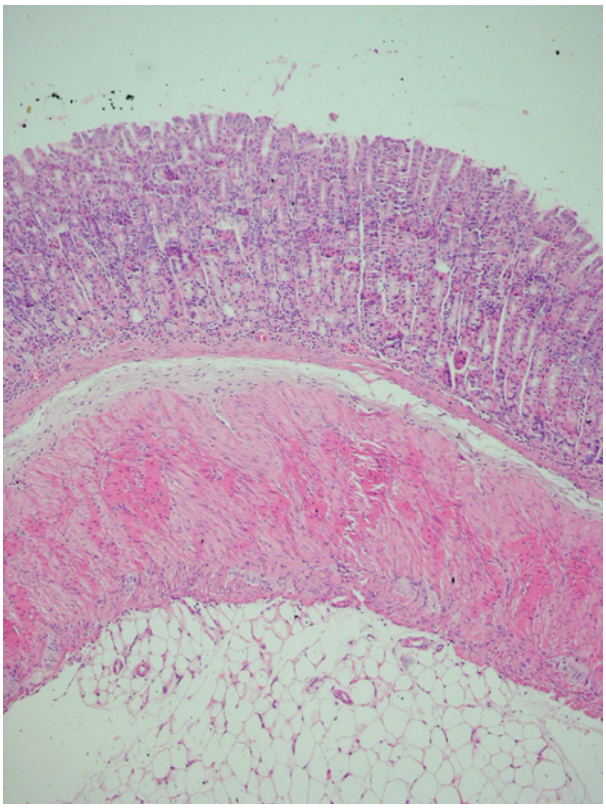

Fig 3. There is no marked inflammation, fibrosis, or epithelial change in the gastric corpus in the resveratrol-treated group. (Hematoxylin and eosin; original magnification, 100×.)

the present study, levels of MDA in resveratrol-treated rats were significantly lower than in the BDL-only group. Although tissue MDA levels were clearly decreased by resveratrol, its exact mechanism is not known. Reductions in MDA levels in resveratrol-treated rats are probably due to its antioxidant and free radical-scavenging effect (24). Chanvitayapongs *et al.* (25) showed that resveratrol not only possessed antioxidant properties, but also reduced the cell death induced by oxidized lipoproteins.

GSH, the most abundant nonenzymatic antioxidant agent present in cells, plays an important role in defense against oxidative-stress induced cell injury (26). Several studies experimental liver cirrhosis (7, 8, 27) have shown decreased levels of GSH in gastric tissue and plasma. Our results are in agreement with these previous works reporting low levels of GSH. In the present study, the levels of GSH in the gastric mucosa of the BDL-only group were significantly lower than in resveratrol-treated BDL rats. Different mechanisms may contribute to the reduced activity of antioxidant enzymes (GSH) in BDL-only rats. For instance, GSH is inhibited by superoxide anion (28) and excessive lipid peroxidation can cause increased GSH consumption (9). The increased GSH levels in the resveratrol group may be related to the antioxidant and free-radical scavenging effect of resveratrol. Another explanation of this significant increase in GSH levels in resveratrol-treated rats could depend on resveratrol stimulation of γ-glutamylcysteine synthetase activity (29). This increased GSH is consistent with the protective effects of resveratrol against oxidative damage in gastric tissue in cholestasis.

Several studies have demonstrated increased levels of NO in cholestasis (6, 21). In the present study, levels of NO in BDL only group were significantly higher than those of resveratrol-treated rats. These results concur with previous works. However, the results of some studies in BDL-only rats do not support our study (30, 31). The gastric wall blood response to exogenous NO/cyclic guanosine-3′,5′-monophosphate (cGMP)-dependent vasodilators is impaired in bile duct-ligated rats (32). Cause of NO overproduction in the BDL-only group may be due to an elevated incidence of endotoxemia after BDL (33). On the other hand, the low levels of NO in resveratrol-treated rats may be due to inhibition of NO and peroxynitrite ($ONOO^-$) by resveratrol (34, 35).

In conclusion, gastric oxidative stress occurs in BDL-only rats. The present study demonstrates that intraperitoneal administration of resveratrol maintains antioxidant defenses and reduces gastric oxidative damage. This effect of resveratrol may be useful to preserve antioxidant defenses against gastric oxidative stress in cholestatic in rats. Nevertheless, more investigations are required to evaluate antioxidant effects of resveratrol on gastric tissue in cholestasis.

REFERENCES

1. Triger DR, Hosking SW: The gastric mucosa in portal hypertension. J Hepatol 8:267–272, 1989
2. Sarfeh IJ, Tarnawski A: Increased susceptibility of the portal hypertensive gastric mucosa to damage. J Clin Gastroenterol 13:18–21, 1991
3. Moritomo H, Nagahata Y, Urakawa T, Saitoh Y: The mechanism of development of acute gastric ulceration following biliary drainage in obstructive jaundice. Nippon Geka Gakkai Zasshi 96:1–9, 1995
4. Bastid C, Tellechea J, Sahel J: Does jaundice increase the frequency of gastroduodenal ulcerations? Hepatogastroenterology 37:612–614, 1990
5. Sasaki I, Konno Y, Kamiyama Y, Sato T: Acute gastric ulceration in rats with obstructive jaundice with special reference to gastric mucosal blood flow. Tohoku J Exp Med 151:351–358, 1987
6. Nahavandi A, Mani AR, Homayounfar H, Akbari MR, Dehpour AR: The role of the interaction between endogenous opioids and nitric oxide in the pathophysiology of ethanol-induced gastric damage in cholestatic rats. Fundam Clin Pharmacol 15:181–187, 2001
7. Kaur S, Kaur U, Tandon C, Dhawan V, Ganguly NK, Majumdar S: Gastropathy and defense mechanisms in common bile duct ligated portal hypertensive rats. Mol Cell Biochem 203:79–85, 2000

8. Canturk NZ, Canturk Z, Ozbilim G, Yenisey C: Protective effect of vitamin E on gastric mucosal injury in rats with biliary obstruction. Can J Gastroenterol 14:499–503, 2000
9. Sgambato A, Ardito R, Faraglia B, Boninsegna A, Wolf FI, Cittadini A: Resveratrol, a natural phenolic compound, inhibits cell proliferation and prevents oxidative DNA damage. Mutat Res 496:171–180, 2001
10. Surh YJ, Hurh YJ, Kang JY, Lee E, Kong G, Lee SJ: Resveratrol, an antioxidant present in red wine, induces apoptosis in human promyelocytic leukemia (HL-60) cells. Cancer Lett 140:1–10, 1999
11. Burkitt MJ, Duncan J: Effects of trans-resveratrol on copper-dependent hydroxyl-radical formation and DNA damage: evidence for hydroxyl-radical scavenging and a novel, glutathione-sparing mechanism of action. Arch Biochem Biophys 381:253–263, 2000
12. Pastor A, Collado PS, Almar M, Gonzalez-Gallego J: Microsomal function in biliary obstructed rats: effects of S-adenosylmethionine. J Hepatol 24:353–359, 1996
13. Hascalik S, Celik O, Turkoz Y, Hascalik M, Cigremis Y, Mizrak B, Yologlu S: Resveratrol, a red wine constituent polyphenol, protects from ischemia-reperfusion damage of the ovaries. Gynecol Obstet Invest 57:218–223, 2004
14. Mihara M, Uchiyama M: Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86:271–278, 1978
15. Cortas NK, Wakid NW: Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin Chem 36:1440–1443, 1990
16. Sahna E, Parlakpinar H, Ozturk F, Cigremis Y, Acet A: The protective effects of physiological and pharmacological concentrations of melatonin on renal ischemia-reperfusion injury in rats. Urol Res 31:188–193, 2003
17. Ishak K, Baptista A, Bianchi L, *et al.*: Histological grading and staging of chronic hepatitis. J Hepatol 22:696–699, 1995
18. Nishizaki Y, Kaunitz J, Oda M, Guth PH: Impairment of gastric mucosal defenses measured in vivo in cirrhotic rats. Hepatology 20:445–452, 1994
19. Ferraz JGP, McKnight GW, Sharkey KA, Wallace JL: Impaired vasodilatory responses in the gastric microcirculation of anesthetized rats with secondary biliary cirrhosis. Gastroenterology 108:1183–1191, 1995
20. Casadevall M, Tabasco-Minguillan J, Panes J, Cirera I, Pique JM, Teres J: Time-course changes in gastric mucosal blood perfusion following portal vein constriction in rats. J Hepatol 23:193–198, 1995
21. Nahavandi A, Dehpour AR, Mani AR, Homayounfar H, Abdoli A: N(G)-Nitro-L-arginine methylester is protective against ethanol-induced gastric damage in cholestatic rats. Eur J Pharmacol 370:283–286, 1999
22. Chen K, Inoue M, Wasa M, Fukuzawa M, Kamata S, Okada A: Expression of endothelial constitutive nitric oxide synthase mRNA in gastrointestinal mucosa and its downregulation by endotoxin. Life Sci 61:1323–1329, 1997
23. Halliwell B, Chirico S: Lipid peroxidation: its mechanism, measurement, and significance. Am J Clin Nutr 57:715–724, 1993
24. Cai YJ, Fang JG, Ma LP, Yang L, Liu ZL: Inhibition of free radical-induced peroxidation of rat liver microsomes by resveratrol and its analogues. Biochim Biophys Acta 1637:31–38, 2003
25. Chanvitayapongs S, Draczynska-Lusiak B, Sun AY: Amelioration of oxidative stress by antioxidants and resveratrol in PC12 cells. Neuroreport 8:1499–1502, 1997
26. Meister A, Anderson ME: Glutathione. Annu Rev Biochem 52:711–760, 1983
27. Hosoda A, Yamada S, Kawasaki H: Effects of liver damage induced by carbon tetrachloride on glutathione and glutathione-dependent enzymes in rat gastric mucosa. Res Commun Chem Pathol Pharmacol 79:141–150, 1993
28. Blum J, Fridovich I: Inactivation of glutathione peroxidase by superoxide radical. Arch Biochem Biophys 240:500–508, 1985
29. Sekhar KR, Spitz DR, Harris S, Nguyen TT, Meredith MJ, Holt JT, Gius D, Marnett LJ, Summar ML, Freeman ML: Redox-sensitive interaction between KIAA0132 and Nrf2 mediates indomethacin-induced expression of gamma-glutamylcysteine synthetase. Free Radic Biol Med 32:650–662, 2002
30. Fernandez M, Garcia-Pagan JC, Casadevall M, Bernadich C, Piera C, Whittle BJ, Pique JM, Bosch J, Rodes J: Evidence against a role for inducible nitric oxide synthase in the hyperdynamic circulation of portal-hypertensive rats. Gastroenterology 108:1487–1495, 1995
31. Kanwar S, Kubes P, Tepperman BL, Lee SS: Nitric oxide synthase activity in portal-hypertensive and cirrhotic rats. J Hepatol 25:85–89, 1996
32. Geraldo J, Ferraz P, Wallace JL: Prostaglandins modulate the responsiveness of the gastric microcirculation of sodium nitroprusside in cirrhotic rats. Hepatology 23:123–129, 1996
33. Inan M, Sayek I, Tel BC, Sahin-Erdemli I: Role of endotoxin and nitric oxide in the pathogenesis of renal failure in obstructive jaundice. Br J Surg 84:943–947, 1997
34. Cho DI, Koo NY, Chung WJ, Kim TS, Ryu SY, Im SY, Kim KM: Effects of resveratrol-related hydroxystilbenes on the nitric oxide production in macrophage cells:structural requirements and mechanism of action. Life Sci 71:2071–2082, 2002
35. Brito P, Almeida LM, Dinis TC: The interaction of resveratrol with ferrylmyoglobin and peroxynitrite; protection against LDL oxidation. Free Radic Res 36:621–631, 2002