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Hyperlipidemia induced by a cholesterol-rich diet aggravates necrotizing pancreatitis in rats

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Abstract

The aim of the present study was to investigate whether hyperlipidemia can cause acute pancreatitis or alter its severity. Male Wistar rats were fed a 3% cholesterol-enriched diet or a normal diet for 16 weeks. Edematous and necrotizing pancreatitis was induced with 3×75 μg/kg body weight of cholecystokinin s.c. and 2×2 g/kg body weight of L-arginine i.p., respectively, in separate groups of normal and hyperlipidemic rats. The severity of the pancreatitis was assessed. We studied the influence of hyperlipidemia on the formation of oxygen-derived free radicals, endogenous scavengers, nitric oxide synthases (NOS), peroxynitrite ($ONOO^-$), heat shock protein 72 (HSP72) and nuclear factor-kappa B (NF-κB) activation in the pancreas during acute edematous and necrotizing pancreatitis. Hyperlipidemia did not worsen edematous, but aggravated necrotizing pancreatitis. The cholesterol-enriched diet significantly reduced the catalase and Mn-superoxide dismutase (SOD) and constitutive NOS (cNOS) activities and increased the inducible NOS (iNOS) in the pancreas relative to those in the rats on the normal diet. The pancreatic nitrotyrosine level, as a marker of $ONOO^-$, and the NF-κB DNA-binding activity in the pancreas, were significantly elevated in the cholesterol-fed rats. The pancreatic HSP72 expression during necrotizing pancreatitis was not influenced by the hyperlipidemia. The pancreatic Mn-SOD, Cu, Zn-SOD, glutathione peroxidase, total glutathione and cNOS activities were significantly reduced, while the catalase, iNOS and NF-κB DNA-binding activities were significantly increased in the animals with necrotizing pancreatitis on the cholesterol diet as compared with those with pancreatitis and receiving the normal diet. Hyperlipidemia induced with this cholesterol-enriched diet leads to decreases in endogenous scavenger and cNOS activities, results in iNOS and NF-κB activation and stimulates $ONOO^-$ generation in the pancreas, which may be responsible for the aggravation of acute necrotizing pancreatitis.

Keywords: Hyperlipidemia; Acute edematous pancreatitis; Acute necrotizing pancreatitis; Peroxynitrite; NF-κB; Heat shock protein; Nitric oxide synthases; Endogenous scavengers

1. Introduction

Hyperlipidemia is reported to be associated with acute pancreatitis in 12–38% of the cases. Hyperlipidemia, which may lead to acute pancreatitis, may be seen as an epiphenomenon of pancreatitis. Lipid levels increase above the normal in up to

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50% of patients with acute pancreatitis of any cause. The relationship between the two and the role of hyperlipidemia in the pathogenesis of acute pancreatitis is uncertain (Dominguez-Munoz et al., 1991; Toskes, 1990; Yadav and Pitchumoni, 2003).

Hyperlipidemia may be primary in origin or secondary to other clinical conditions, such as alcohol abuse, diabetes mellitus, pregnancy and the use of oral contraceptives. Consequently, most clinical reports have a high proportion of patients with alcoholism, which can itself induce acute pancreatitis. For ethical reasons, an experimental design that convincingly demonstrates causative or contributory effects of hyperlipidemia on acute pancreatitis is difficult to apply clinically. The role of hyperlipidemia in the pathogenesis of pancreatitis might therefore, not be deduced from clinical studies. It has been suggested that animal experiments should be resorted in order to assess the effect of hyperlipidemia on the course of acute pancreatitis (Zieve, 1968).

The mechanism of hyperlipidemic acute pancreatitis is not known. The increasing evidence that has accumulated in recent years indicates that a high-cholesterol diet impairs nitric oxide (NO)-cGMP signaling in both endothelial and nonendothelial cells (Ferdinandy et al., 1997; Deliconstantinos et al., 1995). In the normal pancreas, NO is synthesized from L-arginine (Arg) on the action of nitric oxide synthase (NOS), which exists in 3 isoforms: endothelial NOS (eNOS) and neuronal NOS (nNOS), which are constitutive (cNOS), and an inducible form (iNOS). NO appears to have a biphasic (protective and deleterious) role in acute pancreatitis (Vallance, 2003; Moncada and Higgs, 1993; Werner et al., 1998).

Experimental hypercholesterolemia is associated with an increased production of reactive oxygen species (ROS) (Parker et al., 1995), decreased activities of endogenous radical scavengers (Napoli et al., 1999), and a decreased bioavailability of NO (Ignarro et al., 1999). A reduced level of vascular NO release in hyperlipidemia has been revealed as a consequence of the enhanced formation of superoxide, which then reacts with NO to form the highly toxic peroxynitrite ion ($ONOO^-$) (White et al., 1994).

One of the most important transcription factors that control proinflammatory gene expression during acute pancreatitis is nuclear factor κB (NF-κB). In most cells, NF-κB is normally sequestered in the cytoplasm in an inactive form associated with a class of inhibitory proteins called IκBs. NF-κB is rapidly activated during acute pancreatitis, is translocated to the nucleus, binds to specific κB sequences in the promoter regions and transactivates the downstream genes, including interleukins, chemokines, adhesion molecules, receptors and enzymes (Barnes and Karin, 1997; Rakonczay et al., 2003a,b). Experimental hypercholesterinemia has been demonstrated to be associated with NF-κB activation in the coronary vasculature (Wilson et al., 2000). Moreover, NF-κB has been shown to play a critical role in the pathogenesis of acute experimental pancreatitis by regulating the expressions of many proinflammatory genes in the pancreas (Rakonczay et al., 2003a,b).

It is well known that the accumulation of the inducible member of the 70-kD heat shock protein family (HSP72) in response to a variety of stressors such as heat, mechanical stress, and ischemia confers long-lasting protection against further stress injury (Rakonczay et al., 2003a,b; Welch, 1993). Attenuation of HSP expression has been revealed in certain pathological conditions, such as aging, cardiac hypertrophy and hyperlipidemia (Csont et al., 2002; Locke and Tanguay, 1996; Tajima et al., 1997).

The aims of the present study were to investigate whether hyperlipidemia induced by a cholesterol-enriched diet can cause acute pancreatitis or alter its severity in rats and to analyze the possible pathomechanism. The effects of hyperlipidemia were examined on the levels of malondialdehyde (MDA), a marker of lipid peroxidation, endogenous scavengers and the various forms of NOS, on the generation of $ONOO^-$ and on the activation of NF-κB in the pancreas. A study was also made whether hyperlipidemia interacts with the pancreatic heat stress response.

2. Materials and methods

The experimental protocol followed the principles of Laboratory Animal Care of the National Institutes of Health, USA, and was approved by the ethics committee of the University of Szeged.

2.1. Animals and experimental protocol

80–100 g male Wistar rats were used. The animals were kept at a constant room temperature of 22±2 °C, under 12-h light–dark cycles, and were fed laboratory chow enriched with 3% cholesterol (cholesterol group) or standard chow (LATI, Gödöllő, Hungary) (control group) for 16 weeks. At the end of this 16-week controlled-diet period, acute edematous pancreatitis was induced with 3×75 μg/kg body weight of cholecystokinin (CCK) (Takács et al., 1996) s.c. (CCK and cholesterol+CCK groups), and acute necrotizing pancreatitis with 2×2 g/kg body weight of Arg i.p. (Czakó et al., 1998), in separate groups of normal and hyperlipidemic rats (Arg and cholesterol+Arg groups). The control rats received the same amount of 0.9% saline or an 8.6% solution of glycine in 0.9% saline at the same times instead of the CCK and Arg. At 6 h following the first CCK injection and at 24 h following the first Arg injection, the rats were sacrificed by aortic exsanguinations respectively, and the severity of the pancreatitis was assessed by measurement of the serum amylase and lipase concentrations, and the ratio pancreatic weight/body weight, and via the histology.

2.2. Serum assays

For serum assays, blood samples were centrifuged for 20 min at 2500×*g*. The serum amylase and lipase activities were determined by an Auto Analyzer (Prestige-24, Tokyo Boeki Medical System, Japan). Serum triglycerides and total cholesterol concentrations were measured in triplicates using commercially available colorimetric assay kits (Diagnosticum Rt, Budapest, Hungary) adapted to 96-well plates as described previously (Bjelik et al., 2006). The accuracy of the assays was monitored by using Standard Lipid Controls (Sentinel, Milan, Italy).