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Prevention of neointima formation by taurine ingestion after carotid balloon injury

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ABSTRACT

The sulfur-containing amino acid, taurine, has been shown to ameliorate the vascular disorders. We examined the effects of taurine ingestion on intimal thickening following balloon injury. Balloon injury was induced in the left common carotid artery of Wistar rats. Taurine (3% (w/v)) was mixed in the drinking water from 2 days prior to, until 14 days after the induction of balloon injury. The ratio of intima-to-media was significantly reduced by 26% in the taurine-treated rats at 14 days after the induction of injury, which was associated with reduced proliferation of the vascular smooth muscle cells (SMCs) in both the media and the intima. Attenuation of arterial superoxide production by taurine ingestion was evident from the results of both the lucigenin chemiluminescence method and in situ detection by dihydroethidium (DHE) staining. Moreover, LPS-stimulated TNF-α production in the blood cells was decreased in the taurine-treated rats. The results of the study showed that taurine suppresses neointimal formation in balloon-injured arteries, associated with reduced proliferation of the vascular SMCs, which is attributable to the anti-oxidative effects of taurine. In addition, the anti-inflammatory effects of taurine chloramine produced by neutrophils may be related to reduction in SMC proliferation in part.

1. Introduction

Reactive oxygen species (ROS) which can be released from NAD(P)H oxidase, xanthine oxidase, lipoxygenase, mitochondria, or the uncoupling nitric oxide synthase plays a central role in the upregulation of oxidative stress in the coronary and carotid arteries after balloon injury (Shi et al., 2001; Szöcs et al., 2002). ROS generation has been implicated in growth signaling pathways and in smooth muscle proliferation and migration (Napoli et al., 2001). Proliferating smooth muscle cells, myocytes and fibroblasts (Szöcs et al., 2002), as well as the infiltrating macrophages and neutrophils (Okamoto et al., 2001) produce ROS in the vessel wall (Szöcs et al., 2002). Indeed, pharmacological antioxidant intervention with probucol (Ishizaka et al., 1995) or N-acetylcysteine (Mass et al., 1995) has been shown to reduce neointima formation.

Taurine, a sulfur-containing amino acid, is abundantly present in animal tissues and plays roles in several essential biological processes (Huxtable, 1992). Pharmacological intervention in animal models has demonstrated the beneficial effects of this amino acid against cardiovascular diseases, including hypertension (Nara et al., 1978), atherosclerosis (Petty et al., 1990), and hyperlipidemia (Murakami et al., 1996). Epidemiological studies worldwide have suggested the beneficial effects of taurine ingestion in reducing the incidence of cardiovascular diseases (Yamori et al., 2001). Conversely, taurine deficiency has been shown to be associated with various disorders such as leukopenia, blindness, movement disorders, retinal degeneration, liver disease and cardiomyopathy (Warskulat et al., 2004, 2006; Ito et al., 2008). Taurine has been shown to have anti-oxidative actions and to ameliorate oxidative stress in various organs and tissues including the heart, liver, kidney, lung, and blood vessels (Kingston et al., 2004; Waters et al., 2001; Trachtman et al. 1995). In humans, taurine reduces exercise-induced oxidative stress (Zhang et al., 2004). Taurine was also shown to protect the biomembrane against oxygen radicals in vitro (Nakamura et al., 1993). We have previously shown that dietary taurine supplementation reduces atherosclerotic lesions in several animal models, including apoE-deficient mice (Kondo et al., 2001), WHHL rabbits (Murakami et al., 2002), and spontaneously hyperlipidemic mice (Matsushima et al., 2003), independent of the serum lipid levels. Based on the results of determination of lipid peroxide levels, it appears that attenuation of vascular lesions by taurine might be attributable to its anti-oxidative effects in part.

Activation of neutrophils results in the generation of hydrogen peroxide (H_2O_2) and superoxide through a respiratory burst, and release of the heme enzyme, myeloperoxidase (Babior, 2000). This

Abbreviations: BrdU, 5-bromo-2′-deoxyuridine; DHE, dihydroethidium; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein-1 MPO, myeloperoxidase; ROS, reactive oxygen species; SMC, smooth muscle cell.

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enzyme catalyses the reaction of H_2O_2 with chloride ions at physiological concentrations, to yield the potent oxidant, hypochlorous acid (HOCl). HOCl plays a major role in host defenses against bacteria and other invading pathogens in vivo. However, excessive or misplaced generation of HOCl can cause tissue damage and has been implicated in the development of a number of diseases. Taurine is the most abundant free amino acid in leukocytes, especially in neutrophils (20–50 mM) (Fukuda et al., 1982). It reacts with HOCl to form taurine chloramines, a less reactive and less toxic substance which serves as a scavenger for HOCl. Under physiological conditions, HOCl toxicity is reduced *via* this process. It is of interest to note that the produced taurine chloramine inhibits the generation of TNF-α, IL-6, NO, PGE_2 and MCP-1 in activated macrophages (Marcinkiewicz et al., 1995). Similarly, taurine chloramine has also been shown to inhibit the generation of superoxide and proinflammatory mediators in leukocytes or neutrophils prepared from humans or mice (Kim et al., 1996). These results suggest that taurine chloramines can be produced at the sites of inflammation to function as a modulator of inflammation (Schuller-Levis and Park, 2004). The pathogenesis of vascular diseases is thought to be closely related to inflammation, which plays a critical role in the vascular SMC proliferation, migration and matrix production in response to vascular injury (Hay et al., 2001). Infiltration by neutrophils, monocytes/macrophages and T lymphocytes is observed in the adventitia of the vessel wall after vessel injury (Xing et al., 2004). Thus, perivascular inflammatory cells participate in the recruitment and activation of adventitial cells through the release of cytokines and chemokines, and contribute to vascular remodeling. We examined the effects of taurine on the intimal hyperplasia in rats using a balloon catheter injury model, and investigated the possible involvement of the anti-oxidative and anti-inflammatory actions of taurine in the attenuation of neointimal proliferation in the balloon-injured arteries.

2. Materials and methods

2.1. Arterial injury model

Balloon injury was induced in the left common carotid artery of male Wistar rats weighing 290–330 g (SLC, Shizuoka, Japan). The animals were anesthetized by intraperitoneal injection of pentobarbital. A 2F Fogarty balloon catheter (Baxter) was introduced into the common carotid artery through an arteriotomy in the external carotid artery, as described in a previous report (Murakami et al., 2001). Taurine was dissolved at 3% (w/v) in the drinking water for the rats, which they were provided *ad libitum*, from 2 days prior to, until 14 days after the induction of balloon injury. The rats were sacrificed on Day 1, 7 or 14 after the balloon injury, and the left common carotid arteries were perfusion-fixed, and embedded in paraffin. Five cross sections (3-μm thick) were stained with hematoxylin–eosin and elastica von Gieson. Areas of the media and neointima were determined with an image analyzer (LEICA Q500MC, Leica, Cambridge, UK), and the intima-to-media area ratio was determined. All the animal experiments were conducted in accordance with the regulations of the Animal Ethical Committee of Taisho Pharmaceutical Co.

2.2. Cellular proliferation in vivo

To detect proliferative cells, 5-bromo-2′- deoxyuridine (BrdU) (Sigma Chemical Co) was administered intraperitoneally to the rats at a dose of 25 mg/kg, 17, 9 and 1 h before the rats were sacrificed on Day 7 after the injury (Zeymer et al., 1992). Three cross sections per artery were prepared and immunostained for BrdU, using an anti-BrdU monoclonal antibody (Dako Co). Sections were lightly counterstained with hematoxylin and eosin and the number of positive nuclei per section was counted to determine the labeling index ([stained nuclei/total nuclei] × 100).

2.3. Serum levels of lipids and taurine

Blood samples were collected prior to the start of the taurine treatment and on Day 14 after the balloon injury, and serum was separated by low-speed centrifugation. The serum levels of the total cholesterol and HDL cholesterol were determined enzymatically, using the commercially available kits, Autosera CHO-2 and Cholestest HDL (Daiichi Kagaku Yakuhin, Tokyo, Japan), respectively. Serum triglyceride was also determined enzymatically, using a commercial kit, Clinimate TG-2 (Daiichi Kagaku Yakuhin). The plasma taurine levels were determined on Day 14 after the induction of balloon injury using HPLC and an Eicompak MA-5ODS column (Eicom, Kyoto, Japan) (Turnell and Cooper, 1982).

2.4. Measurement of vascular superoxide production

The production of $\cdot O_2^-$ in the vessel wall was examined by the lucigenin chemiluminescence assay, as described previously (Rajagopalan et al., 1996). The injured and noninjured carotid arteries were carefully dissected, placed in a solution of modified Krebs–Hepes buffer containing 99.01 mM NaCl, 4.69 mM KCl, 1.87 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.03 mM K_2HPO_4, 25 mM $NaHCO_3$, 20 mM Na-Hepes, and 11.1 mM glucose, pH 7.4., and cut into 5-mm ring segments. The artery specimens were equilibrated for 30 min at 37 °C. Scintillation vials containing 2 ml of Krebs–Hepes buffer with 250 μM lucigenin (bis-N-methylacridinium nitrate) were placed in the scintillation counter. After allowing dark adaptation, the background counts were recorded in out-of-coincidence mode and a vascular specimen was added to the vial. The scintillation counts were then recorded every 2 min for 15 min, followed by subtraction of the respective background counts. The arteries were then dried at 90 °C for 24 h, and the dry weights were determined.

2.5. Assay of TNF-α production

TNF-α production was determined as previously described (Martinez et al., 2004). Briefly, blood specimens were collected from the anesthetized rats by cardiac puncture and incubated with LPS (Sigma-Aldrich, St. Louis, MO) at 37 °C for 18 h in an atmosphere of 5% carbon dioxide/95% air. The cell-free supernatants obtained by centrifugation at 3000 × *g* for 10 min were stored at −80 °C until the TNF-α measurement. TNF-α was determined using a commercially available ELISA kit (BD Biosciences, San Jose, CA).

2.6. Histological study

The carotid artery was excised and used for the following studies. In situ detection of superoxide production was evaluated with the fluorescent dye, dihydroethidium (DHE). Five micrometer-thick snap-frozen sections were incubated with 2×10^{-6} mol/l DHE (Sigma-Aldrich, St. Louis, MO) for 30 min at 37 °C in a light-protected humidified chamber, rinsed with phosphate buffered saline (PBS), and then examined under a fluorescence microscope (Carl Zeiss Axioplan 2, Jena, Germany) using filter set 15 (emission maximum: 570 nm). For the immunohistochemical studies, the carotid artery specimens were immersed with 2.5% glutaraldehyde in 0.1 M phosphate buffer or neutralized 10% formalin. Thereafter, cryosections were prepared from the glutaraldehyde-fixed samples or paraffin sections were made from the formalin-fixed samples. Immnunohistochemical analysis was performed using anti-taurine antibody (1:100 dilution, Chemicon AB137, Temecula, CA, USA), as previously described (Sakurai et al., 2003), using cryosections. The other antibodies used for the studies on the paraffin sections were as