Autonomic Neuroscience: Basic and Clinical 166 (2012) 15–21

Contents lists available at SciVerse ScienceDirect

Autonomic Neuroscience: Basic and Clinical

journal homepage: www.elsevier.com/locate/autneu

Chemical sympathectomy induces arterial accumulation of native and oxidized LDL in hypercholesterolemic rats

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ARTICLE INFO

Article history:
Received 16 February 2011
Received in revised form 17 August 2011
Accepted 18 August 2011

Keywords:
Sympathectomy
Cholesterol
Native LDL
OxLDL
Native LDL receptor
Scavenger LDL receptor
Aorta

ABSTRACT

The aim of the present study was to examine the effect of sympathectomy on plasmatic and arterial native and oxLDL levels, as well as arterial LDL receptors (LDLR) and scavenger receptors in hypercholesterolemic rats, which are normally protected against atherosclerosis.
Neonatal Wistar rats received subcutaneous injections of either guanethidine for sympathectomy (Gua + HC) or vehicle (HC), then were fed 1% cholesterol for three months. Intact normocholesterolemic rats were used as control of the HC group. Total cholesterol (TC) and LDL-cholesterol were evaluated in the plasma and the abdominal aorta by an auto-analyzer. Plasmatic and aortic oxLDL and native LDL-apo B100 were assessed by a sandwich ELISA. Aortic and hepatic native LDLR and aortic scavenger receptors (CD36 and SR-A) were quantified at mRNA and protein levels by real time PCR and western immunoblot. The effect of hypercholesterolemia was limited to an increase in plasmatic TC and LDL-cholesterol and a decrease in aortic apoB100 and aortic and hepatic LDLR. Hypercholesterolemia and sympathectomy in combination increased markedly plasmatic and aortic TC, LDL-cholesterol, apo B100 and oxLDL together with aortic scavenger receptors, but reduced markedly aortic and hepatic LDLR.
Sympathectomy broke down the rat's protection against hypercholesterolemia by promoting accumulation of native and oxLDL in the aorta via scavenger receptors.

1. Introduction

An early event in the progression of atherosclerosis is the accumulation of cholesterol-rich low density lipoprotein (LDL) in the subintima of the arterial wall. LDL uptake is mediated by the LDL receptor that plays a crucial role in the removal of native LDL from circulation via apolipoprotein B100 which serves as a ligand for the native LDL receptor (Havel and Kane, 1995).

LDL may become oxidized by multiple mechanisms in the circulation or after internalization in blood vessels and can be transported into the cells via cell-surface transmembrane scavenger receptors. Unlike the native LDL receptor, scavenger receptors bind to oxidized LDL (oxLDL) and mediate their uninhibited uptake leading to abnormal accumulation of cholesterol in blood vessels (Moore and Freeman, 2006). OxLDL accumulation through scavenger receptors promotes the transformation of macrophages and SMCs into foam cells and causes atherosclerotic plaque formation (Yla-Herttuala et al., 1989). The two major types of scavenger receptors CD36 and SR-A have been recognized as the most important receptors that bind and internalize oxLDL and contribute both to early foam cell formation and to the progression of disease (Kunjathoor et al., 2002).

The sympathetic nervous system may influence the amount of cholesterol in the arterial wall as demonstrated by Fronek and Turner (1980), who showed that sympathectomy in hypercholesterolemic animals induces abnormal accumulation of cholesterol in the arterial wall and increases the risk of atherosclerosis. However, sympathectomy is unable to induce atheromatous plaque formation when performed in normocholesterolemic animals. We have previously shown that sympathectomy can aggravate the progression of atherosclerosis in rabbits which develop spontaneously such disease when they are fed a diet incorporating cholesterol 7(Kacem et al., 2006). Interestingly, the rat is an animal that is protected against atherosclerosis under hypercholesterolemia by an unknown mechanism (Clowes et al., 1977). We recently showed that sympathectomy, when combined with hypercholesterolemia, induces atheromatous plaque formation in these animals (Hachani et al., 2010). We hypothesized that sympathectomy exacerbated the oxidation of

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doi:10.1016/j.autneu.2011.08.008

cholesterol-LDL to be accumulated in the blood vessels, since the increase in plasmatic native LDL itself is unable to induce atherosclerosis (Horio et al., 1991). To verify this hypothesis, and to examine the effect of sympathectomy on the arterial cholesterol load in the hypercholesterolemic model, total cholesterol, LDL-cholesterol, native and oxidized LDL concentrations were determined in plasma and aorta by means of automated analyses and sandwich ELISA. The aortic and hepatic native LDL receptors (LDLR) and aortic scavenger receptors (CD36 and SR-A) were examined at the mRNA and protein levels by real-time PCR and western blotting methods. These analyses were performed in hypercholesterolemic rats without (HC) and with (Gua + HC) sympathectomy with guanethidine. Measurements on intact normocholesterolemic rats (NC) were also performed to enable a better understanding of the changes leading to atherosclerosis in the sympathectomized hypercholesterolemic rat. The efficacy of the sympathectomy made with guanethidine was evaluated by analysis of perivascular sympathetic fibers and systolic blood pressure.

2. Materials and methods

2.1. Animals

The animal protocols used in this study were approved by the University Animal Care and Use Committee of the University of Paris VII (France) and of the Faculty of Sciences of Bizerte (Tunisia) and were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

One hundred twenty nine male Wistar rats were used and distributed in the different experimentations as shown in Table 1. We used the same method described previously to obtain sympathectomy with guanethidine (Gua + HC group) and sham sympathectomy with a physiological solution (HC group) that were then fed 1% cholesterol for three months after weaning at age one month (Hachani et al., 2010). Intact rats fed standard rat pellets without cholesterol (NC group) were used as control of the HC group. Systolic blood pressure (SBP) was measured in the NC, HC and Gua + HC groups just prior to sacrifice by the computerized, non-invasive tail-cuff BP-2000 Blood Pressure Analysis System on conscious rats without operator intervention. At the age of 4 months (end of treatment), the rats were weighed and blood was collected under pentobarbital anesthesia before sacrifice by an overdose of pentobarbital.

2.2. Plasma sampling and LDL extraction

The plasma recovered from blood after centrifugation was used for determination of total cholesterol (TC) concentration and for extraction of the plasmatic LDL fraction. To prevent oxidation and degradation of lipoproteins, a preservation cocktail containing 5 μL/mL aprotinin, 80 μg/mL gentamycin sulfate, 0.1% EDTA and 1 μL/mL PMSF (Sigma Chemical Co) was added to the plasma. The LDL fraction with a density ranging between 1.019 and 1.063 was extracted by sequential ultracentrifugation from three pooled plasmas as previously described (Itabe et al., 1996) and used to measure LDL-cholesterol (LDL-C), LDL-apo B100 (native LDL) and oxLDL concentrations.

2.3. Sampling: fluorescence labeling of catecholamine containing fibers, extraction of lipids and LDL, preparation of solubilized membrane proteins

The abdominal aorta was rapidly removed from the renal artery level to the bifurcation of the iliac arteries and used either for the visualization of sympathetic fibers by the glyoxylic acid method as described previously (Hachani et al., 2010) or for lipid and membrane protein extractions and mRNA assays. Simultaneously, the livers were immediately removed and placed in ice-cold 0.15 M saline for membrane protein extractions and mRNA assays. In three separate experiments per group (NC, HC and Gua + HC), lipids (Folch et al., 1957) and the LDL fraction with a density between 1.019 and 1.063 (Nishi et al., 2002) were extracted from three pooled aortae weighing approximately 50 mg for the determination of TC or LDL-C, native LDL (apo B100) and oxLDL concentrations.

Solubilized membrane proteins were prepared from three separate experiments in each group (NC, HC and Gua + HC), where membranes were prepared from five pooled aortae or from 1 g of liver taken from the same animals in each experiment as described previously (Schneider et al., 1981). Protein concentrations in the cellular membranes were determined by the Lowry method (Lowry et al., 1951) using BSA as a standard protein. Soluble membrane proteins were used for the quantification of aortic and hepatic native LDL receptors (LDLR) and aortic scavenger LDL receptors (CD36 and SR-AI) by western blot.

Table 1
Number of animals and measurements used in different experiments.

Experiments	Samples	Number of animals/group		
		NC	HC	Gua + HC
Visualization of catecholamine fibers 1 observation/animal	Aorta	–	6	6
Weight SBP (3 measurements/animal)	Animal	9	9	9
TC (2 measurements/animal)	Plasma			
TC (2 measurements/3 pooled aortae)	Aorta lipid extraction			
LDL-C, apo B100, oxLDL (2 measurements/3 pooled plasmas)	Plasma: extracted LDL fraction	9	9	9
LDL-C, apo B100, oxLDL (2 measurements/3 pooled aortae)	Aorta: extracted LDL fraction			
LDLR, CD36, SR-AI proteins (3 measurements/5 pooled aortae)	Solubilized membrane of aorta	15	15	15
LDLR, CD36, SR-AI mRNA (3 measurements/aorta)	Aorta homogenisation	6	6	6
LDLR protein LDLR mRNA (3 measurements/liver)	Solubilized membrane of liver Liver homogenisation			
Total animals		39	45	45

NC: normocholesterolemic intact group; HC: hypercholesterolemic group; GUA + HC: hypercholesterolemic sympathectomy group.