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Chronic intermittent mental stress promotes atherosclerotic plaque vulnerability, myocardial infarction and sudden death in mice

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ABSTRACT

Vulnerable atherosclerotic plaques are prone to plaque rupture leading to acute cardiovascular syndromes and death. Elucidating the risk of plaque rupture is important to define better therapeutic or preventive strategies. In the present study, we investigated the effect of chronic intermittent mental stress on atherosclerotic plaque stability and cardiovascular mortality in apolipoprotein E-deficient ($ApoE^{-/-}$) mice with a heterozygous mutation in the fibrillin-1 gene ($Fbn1^{C1039G+/-}$). This mouse model displays exacerbated atherosclerosis with spontaneous plaque ruptures, myocardial infarction and sudden death, when fed a Western-type diet (WD).

Female $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice were fed a WD for up to 25 weeks. After 10 weeks WD, mice were divided in a control (n = 27) and mental stress (n = 29) group. The chronic intermittent mental stress protocol consisted of 3 triggers: water avoidance, damp bedding and restraint stress, in a randomly assigned order lasting 6 h every weekday for 15 weeks.

Chronic intermittent mental stress resulted in a significant increase in the amount of macrophages in atherosclerotic plaques of the proximal ascending aorta, whereas type I collagen and fibrous cap thickness were decreased. The coronary arteries of mental stress-treated mice showed larger plaques, more stenosis, and an increased degree of perivascular fibrosis. Moreover, myocardial infarctions occurred more frequently in the mental stress group. As compared to the control group, the survival of stressed $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice decreased from 67% to 52% at 25 weeks WD, presumably due to myocardial infarctions.

In conclusion, chronic intermittent mental stress promotes plaque instability, myocardial infarctions, and mortality of $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice.

1. Introduction

Atherosclerosis is a progressive inflammatory disease of the large and medium-sized arteries, characterized by the formation of plaques in the vessel wall. During the development of the disease, the stability of the atherosclerotic plaque plays a major role. Features of plaque instability include a large necrotic core, a high infiltration of inflammatory macrophages and a thin fibrous cap, composed of few smooth muscle cells (SMCs) and collagen fibers. When a plaque develops such an unstable phenotype, it may easily rupture, leading to thrombosis and subsequent myocardial infarction, stroke or even sudden death [16,17,34]. Despite the significant therapeutic advances in cardiology over the past decades, atherosclerotic plaque rupture remains a leading cause of acute cardiovascular death. Therefore, investigating risk factors of atherosclerosis is very important because it may lead to new therapeutic targets or prevention methods.

Recent evidence suggests that mental stress is an important trigger for atherosclerosis and its complications [29,31]. For instance, grieving over the death of a loved-one, the recession of the stock market but also major sporting events, can increase the risk of an acute myocardial infarction [6,15,21]. Moreover, marital stress and job insecurity can have a negative influence on coronary health [5,26].

The aim of this study was to determine the impact of chronic

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intermittent mental stress on atherosclerotic plaque stability and cardiovascular mortality. To this end, apolipoprotein E deficient mice ($ApoE^{-/-}$) with a heterozygous mutation in the fibrillin-1 gene ($Fbn1^{C1039G+/-}$) were used. Recently, we reported that this unique mouse model shows an accelerated plaque progression, spontaneous plaque ruptures, myocardial infarction and sudden death [32,33]. Therefore, it is an adequate model to study the effects of mental stress on plaque vulnerability and the occurrence of myocardial infarctions.

2. Methods

2.1. Mice

Female $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice were fed a Western-type diet (WD; TD88137, Harlan Teklad) starting at an age of 6 weeks. The animals were housed in a temperature-controlled room with a 12-h light/dark cycle and had free access to water and food. Cases of sudden death were documented. At the end of the experiment (25 weeks WD), plasma samples were obtained from the retro-orbital plexus of anesthetized mice (sodium pentobarbital 75 mg/kg, i.p.). Subsequently, the mice were sacrificed with sodium pentobarbital (250 mg/kg, i.p.). Analysis of total plasma cholesterol was performed via a commercially available kit (Randox, Crumlin, UK). The animal procedures were performed conform the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and all experiments were approved by the ethics committee of the University of Antwerp.

2.2. Mental stress protocol

$ApoE^{-/-}Fbn1^{C1039G+/-}$ mice were divided in a control (n = 27) and mental stress (n = 29) group. At 10 weeks WD, a mental stress protocol adapted from Marcondes et al. [18], was initiated and consisted of 3 different triggers: water avoidance, damp bedding and restraint stress. To induce water avoidance stress, mice were placed in an empty cage filled with 0.3 cm of water. A platform (diameter of 3 cm) was placed in the center of the cage as a dry environment. During restraint stress, mice were placed in Plexiglas restrainers that prevented them from inverting their position. Damp bedding was created by adding sufficient water to wet the bedding of the cage. These 3 triggers were randomly assigned over time to avoid habituation. The control group remained in the home cage during the experiment, while the mental stress group was subjected to daily stress triggers that lasted 6 h for 5 consecutive days, followed by 2 days of rest. The protocol was executed for 15 weeks and, at the end, all mice had received the same number of each stress trigger. To document the efficacy of the protocol, body weight was measured before and after the 5-days mental stress period. Moreover, plasma corticosterone and aldosterone were measured using ELISA (EIA kit, Enzo Life Sciences, Farmingdale, NY).

2.3. Echocardiography

Transthoracic echocardiograms were performed at the start of the stress protocol (10 weeks WD), at 17 weeks WD and at the end of the experiment (n = 9–11 per group). The procedure was performed on anesthetized mice (sevoflurane; 8% for induction and 4.5% for maintenance, SevoFlo®, Penlon vaporizer) using a Toshiba diagnostic ultrasound system (SSA-700A), equipped with a 15 MHz transducer. End-diastolic diameter (EDD) and end-systolic diameter (ESD) were measured and fractional shortening (FS) was calculated.

2.4. Histology

After sudden death or sacrifice of $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice, the proximal ascending aorta and the heart were collected. Tissues were fixed in 4% formaldehyde (pH 7.4) for 24 h, dehydrated overnight in 60% isopropanol and embedded in paraffin. Serial cross sections (5 μm) of the proximal ascending aorta and heart were prepared for histological analysis. Atherosclerotic plaque size, necrotic core and the occurrence and size of plaque calcifications of the proximal ascending aorta were analyzed on haematoxylin–eosin (H–E) stained sections. Immunohistochemical staining with a primary antibody against Mac-3 (Pharmingen, San Diego, CA) was used to determine the percentage of macrophages in the plaques. Collagen type I content was measured in Sirius red stained sections under polarized light. Fibrous cap thickness was determined as the median value of 10 measurements per atherosclerotic plaque on α-SMC actin (Sigma, St Louis, MO) stained sections. The occurrence of myocardial infarctions and perivascular fibrosis, measured as the perivascular collagen area divided by the luminal area (PVCA/LA) of 10 coronary arteries per mouse, was analyzed on Masson's trichrome stained sections (cut from the middle of the heart to the apex). Septal wall thickness (median value of 3 measurements per heart) was determined on H-E stained sections. If plaques were present in the coronary arteries, plaque size and percentage stenosis were measured on Masson's trichrome stained sections.

2.5. Statistical analysis

All data are expressed as mean ± SEM. Statistical analyses were performed using SPSS software (version 20, SPSS Inc., Chicago, IL). Statistical tests are specified in the figure legends. Histological data of the proximal ascending aorta only include mice that survived the mental stress protocol (25 weeks WD). Data on the heart include all mice. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Plasma corticosterone and aldosterone, body weight and total plasma cholesterol

Both plasma corticosterone and aldosterone levels were significantly higher (2–3 times) in mice subjected to chronic intermittent mental stress as compared to controls (Table 1).

Control and stress $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice were weighed at the beginning and end of each 5-day stress period. Control mice did

Table 1
Plasma, atherosclerotic plaque and heart characteristics.

	Control	Stress
Plasma:		
Corticosterone (ng/ml)[a]	47 ± 9	116 ± 21*
Aldosterone (pg/ml)[b]	216 ± 33	652 ± 139*
Total cholesterol (mg/dl)[c]	442 ± 31	544 ± 45
Atherosclerotic plaque:		
Plaque size ($x10^3$ μm^2)[d]	988 ± 94	1082 ± 125
Necrotic core (%)[e]	13 ± 2	13 ± 2
Heart and coronary arteries:		
Myocardial infarctions[f]	8/27 (30%)	16/29 (55%)
Coronary plaques[g]	7/27 (26%)	13/29 (45%)

[a] Control n = 6 and stress n = 8, Independent samples t test; *p < 0.05.
[b] Control n = 6 and stress n = 8, Independent samples t test; *p < 0.05.
[c] Control n = 16 and stress n = 12.
[d] Proximal ascending aorta, control n = 18 and stress n = 15.
[e] Proximal ascending aorta, control n = 18 and stress n = 15.
[f] Number of mice showing myocardial infarctions, Pearson's chi-squared test; p = 0.05.
[g] Number of mice showing coronary plaques, Pearson's chi-squared test; p = 0.14.