International Journal of Cardiology 171 (2014) 443–446

Contents lists available at ScienceDirect

International Journal of Cardiology

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

DNA aptamer raised against advanced glycation end products inhibits neointimal hyperplasia in balloon-injured rat carotid arteries ☆

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ARTICLE INFO

Article history:
Received 12 August 2013
Received in revised form 22 December 2013
Accepted 26 December 2013
Available online 8 January 2014

Keywords:
AGE-aptamer
Balloon injury
RAGE
Neointimal hyperplasia

ABSTRACT

Background: Advanced glycation end products (AGE) and their receptor (RAGE) interaction elicit inflammatory and proliferative reactions in arteries, thus playing a role in cardiovascular disease. We have recently found that high-affinity DNA aptamer directed against AGE (AGE-aptamer) prevents the progression of experimental diabetic nephropathy by blocking the harmful actions of AGEs in the kidney. However, effects of AGE-aptamer on vascular injury remain unknown. In this study, we examined whether and how AGE-aptamer inhibits neointimal hyperplasia in balloon-injured rat carotid arteries.

Methods: Male Wistar rats (weighting ca. 400 g at 11 weeks old) were anesthetized with sodium pentobarbital. The left common carotid artery was balloon-injured 3 times with 2F Fogaty catheter inserted through the femoral artery. Then the rats received continuous intraperitoneal infusion (3 μg/day) of either AGE-aptamer or control-aptamer by an osmotic mini pump for 2 weeks. 14 days after the procedure, the left common carotid arteries were excised for morphometric, immunohistochemical and western blot analyses.

Results: Compared with control-aptamer, AGE-aptamer significantly suppressed neointima formation after balloon injury and reduced AGE accumulation, oxidative stress generation, proliferation cell nuclear antigen-positive area, macrophage infiltration, RAGE and platelet-derived growth factor-BB (PDGF-BB) expression levels in balloon-injured carotid arteries.

Conclusion: The present study suggests that AGE-aptamer could prevent balloon injury-induced neointimal hyperplasia by reducing PDGF-BB and macrophage infiltration via suppression of the AGE-RAGE-mediated oxidative stress generation. AGE-aptamer might be a novel therapeutic strategy for suppressing neointima formation after balloon angioplasty.

1. Introduction

A non-enzymatic reaction between ketones or aldehydes and the amino groups of proteins, lipids and nucleic acids contributes to the aging of macromolecules [1,2]. Under hyperglycemic conditions, this process begins with the conversion of reversible Schiff base adducts, and then to more stable, covalently-bound Amadori rearrangement products [1,2]. Over a course of days to weeks, these early glycation products undergo further reactions and rearrangements to become irreversibly crossed-linked, fluorescent protein derivatives termed advanced glycation end products (AGE) [1,2]. There is accumulating evidence that AGE and their receptor (RAGE) interaction elicit oxidative stress generation and evokes inflammatory and thrombogenic reactions in vessels, thereby being involved in accelerated atherosclerosis [3,4]. Therefore, blockade of the AGE-RAGE axis may be a novel therapeutic target for cardiovascular disease.

In the 1990s, an in vitro-selection process called systematic evolution of ligands by exponential enrichment (SELEX) was developed to screen aptamers, single-stranded DNA or RNA molecules that can bind with high affinity and specificity to a wide range of target proteins [5]. Due to its small size, non-immunogenicity and ease of modification compared to conventional monoclonal antibodies, a couple of aptamers have been used in the clinical fields as a tool for modulating various protein function [6,7]. We have recently found that high-affinity DNA aptamer directed against AGE (AGE-aptamer) blocks the progression of nephropathy in an animal model of type 2 diabetes [8]. Therefore, in this study, we examined whether AGE-aptamer inhibits neointimal hyperplasia in balloon-injured rat carotid arteries.

2. Methods

2.1. Materials

Bovine serum albumin (BSA) (essentially fatty acid free and essentially globulin free, lyophilized powder) was purchased from Sigma (St. Louis, MO, USA). D-glyceraldehyde from Nakalai Tesque (Kyoto, Japan).

☆ These authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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http://dx.doi.org/10.1016/j.ijcard.2013.12.143

2.2. Preparation of AGE

AGE-BSA was prepared as described previously [8]. In brief, BSA was incubated under sterile conditions with D-glyceraldehyde for 7 days. Then, unbounded sugars were removed by dialysis against phosphate-buffered saline. Preparations were tested for endotoxin using Endospecy ES-20S system (Seikagaku Co., Tokyo, Japan); no endotoxin was detectable.

2.3. Preparation and selection of AGE-aptamer

Preparation and selection of DNA aptamers were performed as described previously [8]. Sequences of AGE-aptamer and control DNA aptamer (Control-aptamer) are below. AGE-aptamer; 5′-CCGAAACCAGACCACCCCACCAAGGCCACTCGGTCGAACCGCCAACACTCACCCCA-3′, Control-aptamer; 5′-GTTATCTGTCATAGGAACAGTCAGACTCAGCGTCGCAGTTCAGGGCACTTTAGCAC-3′. Since DNA aptamers are susceptible to degradation by nucleases, we modified aptamers with phosphorothioate as described previously [8].

2.4. Rat carotid artery balloon injury (BI) model

Male Wistar rats (weighting ca. 400 g at 11 weeks old; Charles River Breeding Laboratories, Yokohama, Japan) were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneal). The left common carotid artery was balloon-injured 3 times with 2 F Fogaty catheter (Edwards Lifesciences) inserted through the femoral artery as described previously [9]. Sham-operated rats (Sham) underwent the same operation, except that the balloon was not inserted. Then rats received continuous intraperitoneal infusion (3 μg/day) of either AGE-aptamer or control-aptamer by an osmotic mini pump (Alzet osmotic pumps, model 1004, Cupertino, CA, USA) as described previously [8]. 14 days after the procedure, the rats were perfused with phosphate-buffered saline intravenously in a pressure-unfixed manner. Then the rats were killed, and the left common carotid arteries were excised for morphometric, immunohistochemical and western blot analyses. Blood chemistries were measured with standard enzymatic methods as described previously [8]. All animal experiments were conducted according to the guidelines provided by the Kurume University Institutional Animal Care and Use Committee under an approved protocol.

2.5. Morphometric analysis

After fixation with 4% paraformaldehyde, the specimens of carotid arteries were embedded in paraffin, sectioned at 4-μm intervals and stained with hematoxylin and eosin. To evaluate the thickening of neointima, the areas encroached by the external elastic lamina (EEL area), the internal elastic lamina (IEL area), and the lumen area were measured with a computerized digital image analysis system (NIH image). The neointima to media area (I/M) ratio was calculated as following formula; I/M ratio (%) = [IEL area − lumen area] / [EEL area − IEL area] × 100 (%).

2.6. Immunohistochemical analysis

The specimens of carotid arteries were embedded in paraffin, sectioned at 4-μm intervals and mounted on glass slides. The sections were incubated in 0.3% hydrogen peroxide methanol for 30 min, and incubated overnight at 4 °C with antibodies raised against AGE, 8-hydroxy-2′-deoxyguanosine (8-OHdG) (Nikken Seil Co., Ltd. (Tokyo, Japan)), proliferation cell nuclear antigen (PCNA) (Abcam, Cambridge, United Kingdom), F4/80 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), RAGE (Santa Cruz Biotechnology Inc.) or platelet-derived growth factor-BB (PDGF-BB) (Abcam). Then the reactions were visualized with an avidin–biotin–alkaline phosphatase kit (Vectastain ABC-AP, Vector Laboratories, Inc. CA. USA) (for AGE and 8-OHdG staining) or with a Histofine Simple Stain MAX-PO(MULTI) kit (Nichirei Co., Tokyo, Japan) (for PCNA, F4/80, RAGE and PDGF-BB staining) as described previously [10,11]. Immunoreactivity was measured by microcomputer-assisted image J.

2.7. Western blot analysis

Ten micrograms of protein extracted from rat carotid arteries was subjected to SDS-PAGE and western blotting with specific primary Abs against RAGE (Santa Cruz Biotechnology), PDGF-BB (Abcam), or α-tubulin (Sigma) as described previously [9]. Detection was performed by enhanced chemiluminescence (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom).

2.8. Statistical blot analysis

All values were presented as mean ± standard errors. One-way analysis of variance (ANOVA) followed by the Tukey's test was performed for statistical comparisons; $p < 0.05$ was considered significant.

3. Results

Clinical characteristics of animals are shown in Table 1. There was no significant difference of body weight, total cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL-cholesterol), aspartate aminotransferase, alanine aminotransferase and creatinine levels between control-aptamer and AGE-aptamer-treated rats (Table 1).

Table 1
Clinical characteristics of animals.

	Control-aptamer	AGE-aptamer
Number	5	5
Body weight (g)	396 ± 8	396 ± 7
Total cholesterol (mg/dl)	59 ± 5	64 ± 2
Triglycerides (mg/dl)	126 ± 21	143 ± 18
HDL-cholesterol (mg/dl)	36 ± 4	42 ± 2
Asparate aminotransferase (IU/l)	47 ± 2	48 ± 3
Alanine aminotransferase (IU/l)	26 ± 2	22 ± 0.7
Creatinine (mg/dl)	0.26 ± 0.02	0.26 ± 0.02

As shown in Fig. 1A, histopathological examination of the injured arteries revealed a marked increase in neointimal thickening; the I/M ratio at 14 days after BI was increased to about 15-fold compared with that of non-injured artery. Compared with control-aptamer, AGE-aptamer significantly decreased the I/M ratio at 14 days after BI (Fig. 1A).

Furthermore, immunohistochemical analyses showed that expression levels of AGE, PCNA, 8-OHdG and F4/80, markers of oxidative stress and macrophages, respectively, were increased in the neointima of balloon-injured carotid arteries, all of which was significantly reduced by the treatment with AGE-aptamer (Fig. 1B–E). We also confirmed that AGE-aptamer treatment significantly decreased RAGE and PDGF-BB levels in balloon-injured carotid arteries (Fig. 2).

4. Discussion

Traumatic injury of the vessel wall elicits inflammatory and thrombogenic reactions, smooth muscle cell proliferation and migration, which could cause neointima formation in injured vessels [12–14]. Progressive neointimal hyperplasia can frequently complicate vascular angioplasty and surgery, contributes to restenosis after angioplasty, carotid endarterectomy restenosis, in-stent restenosis, cardiac allograft vasculopathy, and vascular bypass graft occlusion [12–14]. Even though there is no perfect animal model that completely replicates the various stages of neointimal hyperplasia after vascular injury [15], balloon-injured rat carotid artery model has been a widely used and validated model for evaluating the neointima formation [16,17]. This is a reason why this study used balloon-injured normal rat model and checked the neointimal thickness in the carotid arteries.

In the present study, we found for the first time that infusion of AGE-aptamer not only inhibited the neointima formation, but also reduced the expression levels of AGE, RAGE and an oxidative stress marker, 8-OHdG in balloon-injured carotid arteries, although it did not affect lipid parameters (data not shown). Engagement of RAGE with AGE stimulates RAGE expression and oxidative stress generation in vascular cells, which could in turn promote the formation and accumulation of AGE again [18,19]. Indeed, aortic AGE accumulation has been suppressed in RAGE-deficient diabetic apolipoprotein E knockout mice [20]. Since we have recently found that the AGE-aptamer used here actually binds to AGE and resultantly block the binding of AGE to RAGE [8], the present observations suggest that AGE-aptamer could decrease the AGE levels in balloon-injured arteries by blocking the positive feedback loop between RAGE-mediated oxidative stress generation and AGE accumulation. Given the fact that no toxicities related to AGE-aptamer were observed following the intraperitoneal injection, continuous infusion of AGE-aptamer might be a safe and effective therapeutic strategy for preventing the restenosis after angioplasty or carotid endarterectomy or the neointima formation following arterial bypass graft surgery. However, this will have to be affirmed before widespread clinical use of the AGE-aptamer is entertained.

PDGF-BB is highly expressed at the site of balloon injury, and there is a growing body of evidence that PDGF-BB is a potent mitogen and