Rosiglitazone and Vascular Injury in Hypercholesterolemic Rabbits: Neointimal Formation Assessment

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Abstract

Background: Rosiglitazone has been the focus of extensive discussion.

Objective: To evaluate the effects of rosiglitazone on iliac arteries, both at the injury site and the contralateral artery, of hypercholesterolemic rabbits undergoing balloon catheter injury.

Methods: White male rabbits were fed a hypercholesterolemic diet by oral gavage for 6 weeks and divided into two groups as follows: rosiglitazone group (14 rabbits treated with rosiglitazone during 6 weeks) and the control group (18 rabbits without rosiglitazone). Animals underwent balloon catheter injury of the right iliac artery on the 14th day.

Results: In the contralateral iliac artery, there was no significant difference in the intima/media layer area ratio (IMR) between the control and rosiglitazone groups. Rosiglitazone did not reduce the probability of type I, II, or III lesions (72.73% *vs* 92.31%; p=0.30) and type IV or V lesions (27.27% *vs* 7.69%; p=0.30). As for the homolateral iliac artery, the intimal area was significantly lower in the rosiglitazone group, as compared to the control group (p = 0.024). The luminal layer area was higher in the rosiglitazone group *vs* the control group (p < 0.0001). There was a significant reduction of 65% in the IMR in the rosiglitazone group vs. the control group (p = 0.021). None of the histological criteria for type I-V atherosclerotic lesions (American Heart Association) were found in the homolateral iliac artery.

Conclusion: These findings demonstrate that rosiglitazone given for 6 weeks prevents atherogenesis at the injury site, but not in a vessel distant from the injury site. (Arq Bras Cardiol 2010; 95(3): 283-288)

Key words: Rosiglitazone; rabbits; hypercholesterolemia; atherosclerosis/prevention & control.

Introduction

The peroxisome proliferator-activated receptor-γ (PPARγ), mainly rosiglitazone (RGZ), has been the focus of extensive discussion in recent publications[1-6]. There are apparent increases in the risk of myocardial infarctions and cardiovascular-related deaths associated with RGZ of which mechanisms are uncertain[1,3,5]. Therefore, a more thorough understanding of all mechanisms implicated in the metabolism of RGZ is essential. Local and systemic changes in the vascular bed after catheter balloon injury have been widely described in the literature[7,8]. There have been reports that vascular injury can cause changes in healthy tissues at locations distant from the injury site. We conducted experiments to analyze the effects of RGZ on local neointimal formation and the contralateral uninjured iliac artery in hypercholesterolemic rabbits.

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Methods

Animals

Thirty-two white adult male rabbits (New Zealand), weighing 2.474 ± 0.348 kg, were studied. Animals were handled in compliance with the Guiding Principles in the Care and Use of Animals. Protocol approval was obtained from the Pontificia Universidade Catolica Animal Research Committee. During the first 14 days, the animals were fed a hypercholesterolemic diet (1% cholesterol-Sigma Aldrich™). Subsequently, they were fed a 0.5% cholesterol diet until sacrifice (42 days). The animals were divided into two groups as follows: control group (CG), consisting of 18 rabbits that did not receive RGZ, and rosiglitazone group (RG), consisting of 14 rabbits treated with RGZ throughout the entire experiment (42 days). Rosiglitazone was administered by oral gavage (3mg/kg body weight/day).

Vascular injury

The rabbits underwent balloon catheter (20 x 3 mm/5 atm/5 min) injury of the right iliac artery on the fourteenth day of the experiment. Anesthesia was induced with ketamine (Vetanarcol™-König - 3.5 mg/kg) and intramuscular xylazine

(Coopazine™-Coopers - 5 mg/kg). After the procedure, the animals received intramuscular analgesics for 3 days (25 mg/day of flunixin - Banamine™ - Schering-Plough) and intramuscular antibiotics for 4 days (100 mg/day of oxitetracyclin - TormicinaP™ - Toruga). The rabbits were sacrificed by a lethal dose of barbiturate on day 42, and their aorta and iliac arteries were retrieved for immunohistochemical and histological analysis.

Quantitative histopathology

Histological analysis was performed by an experienced pathologist (LN) unaware of the RGZ treatment. The analyses were performed microscopically in conjunction with Image Pro-plus™ 4.5 Software (Media Cybernetics Inc., Silver Spring, MD, USA). Histomorphometric parameters were obtained by the calculation of the intima/media layer area ratio (the area of the intimal layer divided by the area of the medial layer) according to the method described by Phillips, et al[9]. The quantification of total collagen was performed by the Sirius red polarization method[10]. Atherosclerotic lesions were analyzed and classified according to Stary, et al[11-13].

Immunohistochemistry

Tissue preparation and immunohistological techniques were performed according to the manufacturer's instructions included in the kits (Dako Corporation, Carpinteria, CA, USA). Sections were stained for macrophage cells using primary monoclonal antibody, RAM-11(Dako™, Carpinteria, CA), and for alpha-actin smooth muscle cells with primary polyclonal antibody HHF-35 (Dako™, Carpinteria, CA). For the qualitative immunohistochemical comparisons of macrophage and smooth muscle cell presence in the intimal area, sections were computed and scored in percentages of animals with cells in both iliac arteries. For the quantitative immunohistochemical comparisons of macrophage or smooth muscle cell content in the intimal area, sections were computed and scored in percentages of cells in the intima.

Blood chemistry

Blood samples were obtained on the first day of the experiment, immediately before balloon catheter injury, and also immediately before sacrifice by cardiac puncture. Clinical laboratory assessment included fasting serum glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TGC). Measurements were obtained using an automated system (Abbott Architect ci8200; Abbott Laboratories, Abbott Park, IL).

Statistical analysis

The sample size calculation was based on the study of Wang Zhao-hui et al[14]. The main variable of interest was considered to be the ratio between the intimal layer and the medial layer. To detect a minimum difference of 0.15 between the averages of groups, with a significance level of 5% and power of the test by 80%, the minimum number of animals in each group was defined as 12. Categorical variables were expressed as percentages and continuous variables were expressed as mean ± SD and medians. The Shapiro-Wilks test was used for testing sample normality. For quantitative parameters, the Student *t*-test and Mann-Whitney nonparametric test were used for the comparison between CG and RG. Fisher's exact test was used for qualitative or categorical variables. Statistical significance was indicated by a value of $p < 0.05$. Analyses were performed using Statistica/W version 5.1 (StatSoft, Tulsa, OK).

Results

A - Metabolic and lipid profiles

Animal weights did not differ between groups (data not shown). Baseline glucose, total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TGC) levels were relatively equal in all groups before initiation of the diet. On day 14, two weeks after administering the cholesterol-rich diet, fasting glucose levels were higher in CG. At the time of sacrifice, glucose levels did not differ between the two groups. Graded elevations in TC and TGC levels were observed from the initial phase through the vascular lesion until sacrifice, with no significant differences between groups. A graded elevation in HDL-C was observed in both groups. Higher levels of HDL-C were observed in RG vs. CG at the time of vascular injury, as well as the time of sacrifice (Table 1).

B - Histomorphometry

Homolateral iliac artery

Intimal area was significantly lower in RG *vs* CG ($p = 0.024$), while luminal layer area was higher in RG *vs* CG ($p < 0.0001$). There was a significant reduction of 65% in intima/media layer area ratio (IMR) in RG *vs* CG ($p = 0.021$). (Table 2; Figure 1). According to the histological analysis proposed by Stary et al, none of the criteria for type I-V lesions were found in RG. There was no collagen deposit in the intimal or medial layers in RG.

Contralateral iliac artery

There was no significant difference in the intima/media

Table 1 - Metabolic and lipid profiles (mean ± SD)

		CG	RG	P value
Baseline	TC (mg/dl	58.62±25.08	43.54±14.82	NS
	HDL-C (mg/dl)	24.23±5.31	22.69±6.26	NS
	TGC (mg/dl	79.62±26.64	79.85±34.31	NS
	Glucose (mg/dl)	121.38±17.43	117.92±11.44	NS
Vascular injury		524.38±258.94	318.46±212.86	NS
		32.46±19.66	50.69±21.91	NS
		72.77±34.95	86.92±56.34	NS
		250.23±93.02	166.62±38.2	0.001
Sacrifice	TC (mg/dl)	852.46±308.48	593.54±219.86	NS
	HDL-C (mg/dl)	42.62±38.23	69.08±19.7	0.001
	TGC (mg/dl)	126.77±85.66	277.31±248.14	NS

NS - non significant. CG - control group; RG - rosiglitazone group.

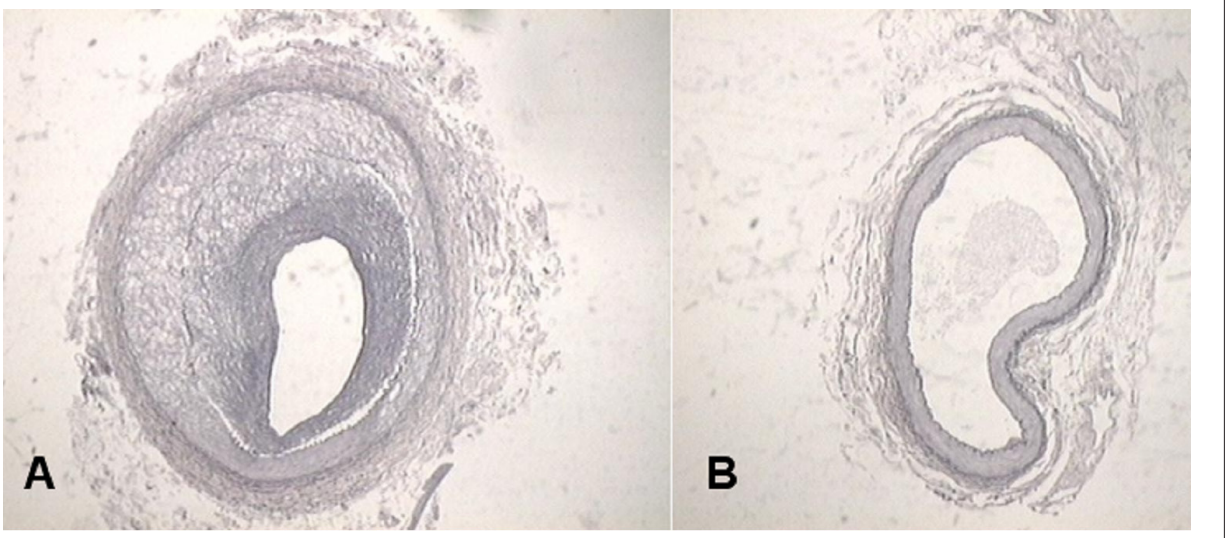

Figure 1 - Representative histological sections demonstrating neointimal formation on the homolateral iliac artery (orcein staining). Panel A - control group. Panel B - RG. NI represents neointima.

Table 2 - **Quantitative histopathological analysis of the homolateral iliac artery**

Area	Group	Mean	DP	Minimum	Maximum	P
Intimal area	CG	320,340.22	392,880.74	14,720.20	1,512,612.11	0.024
	RG	83,115.01	65,440.66	16,187.50	269,226.60	
Luminal area	CG	458,711.01	363,013.82	1,853.54	1,773,080.00	<0.0001
	RG	861,255.24	303,153.71	222,741.70	1,586,336.00	
IMR	CG	0.50	0.41	0.04	1.13	0.021
	RG	0.18	0.14	0.03	0.49	

CG - control group; RG - rosiglitazone group. IMR represents intima/media area ratio. Area was estimated in square micrometers.

layer area ratio between CG and RG. According to the histological classification proposed by Stary et al, rosiglitazone did not reduce the probability of type I, II, or III lesions (72.73% *vs* 92.31%; p=0.30) and type IV or V lesions (27.27% *vs* 7.69%; p=0.30) when compared to CG. Moreover, there were no differences in the extent of collagen deposition (types I and III) between CG and RG (data not shown).

C - Immunohistochemistry

Homolateral iliac artery

The rosiglitazone group did not present any intimal cell markers when compared to CG (see below).

Contralateral iliac artery

There was no statistically significant difference in the percentage of animals with macrophages in the intimal layer between CG and RG (33.4% *vs* 71.5%; p = 0.07). The percentage of animals with smooth muscle cells in the intimal layer was higher in RG when compared to CG (22.3% *vs* 71.5%; p = 0.011; Table 3).

Discussion

To investigate the effects of rosiglitazone, a PPARγ ligand, on atherogenesis in an animal model, we used rabbits with six-fold increased cholesterol levels at the time of vascular injury and fourteen-fold increased levels at the time of euthanasia. The animal model used here was based on previous studies in which rabbits rapidly develop hypercholesterolemia after excessive cholesterol feeding[15,16,22,23].

There are some advantages in using rabbits as animal models, mainly the low cost of the experiment. The metabolic effects of a high cholesterol diet on rabbits have been extensively explained in our previous study[24]. Briefly, in this model, a graded and significant elevation in glucose levels also occurs, and we believe that it could be secondary to the development of some degree of insulin resistance, although this was not evaluated in the present study.

Table 3 - Percentage of macrophages and smooth muscle cells (SMC) in the intimal layer of the contralateral iliac artery

	CG (N / %)	RG (N / %)
Animals with macrophages (p=0.07)	6 (33.4%)	10 (71.5%)
≤ 10% cells	3 (16.6%)	5 (35.7%)
10 to 25% cells	1 (5.5%)	0
25 to 50% cells	2 (11.1%)	5 (35.7%)
50 to 75% cells	0	0
> 75% cells	0	0
Animals with SMC (p=0.01)	4 (22.3%)	10 (71.5%)
≤ 10% cells	1 (5.5%)	3 (21.4%)
10 to 25% cells	0	0
25 to 50% cells	2 (11.1%)	5 (35.7%)
50 to 75% cells	1 (5.5%)	2 (14.2%)
> 75% cells	0	0

CG - control group; RG - rosiglitazone group.

We also observed a significant elevation in the levels of triglycerides and HDL-C, but these effects on triglycerides have been somewhat variable in the literature[14,25,26]. The rabbits underwent balloon catheter injury and the subsequent effects of RGZ were investigated locally and on a vessel distant from the injury site. On the homolateral iliac artery, the RG group did not exhibit any atherosclerotic lesions or show any collagen deposition or macrophage and smooth muscle cell markers in their intimal layer. The most significant findings were identified in the upper luminal area and the lower intimal area in vessels of RGZ-treated rabbits. Additionally, the immunohistochemical analysis demonstrated a reduced macrophage and smooth muscle cell recruitment into the vascular arterial wall when RGZ was used. Surprisingly, RGZ did not exert any effect on the contralateral iliac artery. Regarding the contralateral iliac artery, our data showed that RGZ had no significant effect on the percentage of animals with intimal macrophages, initial and advanced atherosclerotic lesions, and intima/media layer ratio. In addition, we found a significant increase in animals exhibiting smooth muscle cells in the intimal layer of unballooned iliac arteries. While other studies have shown evidence of the antiatherogenic effects of PPARγ ligand in different animal models and in diabetic patients[16-21], the present study reports a lack of antiatherogenic effects of a PPARγ agonist on a vessel distant from the injury site. We cannot rule out the possibility that our histological analysis reflected a short period of exposure to RGZ. Moreover, we did not evaluate artery vasodilation, peroxynitrite ($ONOO^-$) formation, endothelial nitric oxide (NO), or the expression of vasodilator-stimulated phosphoprotein VASP (P-VASP), which should be assessed in future studies and could certainly explain some of our findings.

Recently, the RECORD study demonstrated that the addition of rosiglitazone to glucose-lowering therapy in individuals with type 2 diabetes increases the risk of heart failure, but not the risk of overall cardiovascular morbidity or mortality, when compared with standard glucose-lowering drugs[27]. To compare the risk of acute myocardial infarction, heart failure and death in patients with type 2 diabetes, 39,736 patients received pioglitazone or rosiglitazone. Pioglitazone was associated with a significantly lower risk of heart failure and death, when compared to rosiglitazone, in older patients with no clinical advantage for rosiglitazone. In contrast, when rosiglitazone was administered to individuals with impaired glucose tolerance and/or impaired fasting glucose without cardiovascular disease or diabetes, it modestly reduced carotid intima-media thickness[28]. These controversial and opposing effects of rosiglitazone in injured vessels raise some questions about the protective and non-protective effects of these drugs when administered to diabetic patients or when attempting to avoid the systemic effects of a balloon coronary angioplasty[27-35].

Conclusions

The current study demonstrates that in the animal model with hypercholesterolemic rabbits, rosiglitazone given for 6 weeks prevents atherogenesis at the injury site, but not at a vessel distant from the catheter balloon injury.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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