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Short communication

Association of an oral formulation of angiotensin-(1–7) with atenolol improves lipid metabolism in hypertensive rats

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ABSTRACT

The β-adrenergic blockers and antagonists of the renin-angiotensin system (RAS) are among the drugs that present better results in the control of cardio-metabolic diseases. The aim of the present study was to evaluate the effect of the association of the β-blocker, atenolol, and an oral formulation of Ang-(1–7) on lipid metabolism in spontaneously hypertensive rats (SHR). The main results showed that SHR treated with oral formulation of Ang-(1–7) in combination to atenolol have an improvement of lipid metabolism with a reduction of total plasma cholesterol, improvement of oral fat load tolerance and an increase in the lipolytic response stimulated by the β-adrenergic agonist, isoproterenol, without modification of resting glucose or insulin sensitivity in adipocytes. In conclusion, we showed that administration of an Ang-(1–7) oral formulation in association with a β-blocker induces beneficial effects on dyslipidemia treatment associated with hypertension.

1. Introduction

Dyslipidemia is well recognized as one of the most pernicious metabolic disorders, consisting in an important risk factor of cardiovascular disease, the most prevalent worldwide [5,11]. Studies have shown a potential role for antihypertensive drugs on lipid regulation [15]. β-Adrenergic blockers and antagonists of the renin-angiotensin system (RAS) are among the drugs that present better results in the control of metabolic syndrome and dyslipidemia [4,15,25,27,29].

Recent studies point out for a role of ACE2/Angiotensin-(1–7)/Mas axis as an important counterregulatory arm of the RAS, opposing several angiotensin (Ang) II actions in obesity [17,20,21]. Transgenic animals that present a life time increase in plasma Ang-(1–7) showed an improved lipid and glucose metabolism indicating an important metabolic effect for Ang-(1–7) [20]. On the other hand, mice that lack the Ang-(1–7) receptor, Mas [21], present a metabolic-like syndrome [21].

β-Blockers were also shown to present direct action on metabolic tissues such as muscle, liver and adipose tissue [10,18,26], inhibiting hormone-sensitive lipase activity in the early weeks of treatment and modulating cholesterol biosynthesis and/or catabolism [26]. In this context, the aim of the present study was to evaluate the effect of the association of a β-blocker, atenolol, and an oral formulation of Ang-(1–7) [12] on lipid metabolism in spontaneously hypertensive rats (SHR).

2. Materials and methods

2.1. Animals

Experiments were performed in male SHR (20 ± 2 weeks old) obtained from the animal facilities Biological Science Institute (CEBIO, UFMG, Belo Horizonte, MG, Brazil) kept in 12 h light/dark cycle room.

2.2. Treatment

Four group of animals received orogastric gavage (1 mL/kg, daily) for 14 weeks of: (a) Ang-(1–7)/hydroxypropyl-β-cyclodextrin [CD-Ang-(1–7), 30 μg/kg/day of the peptide; $n = 8$];

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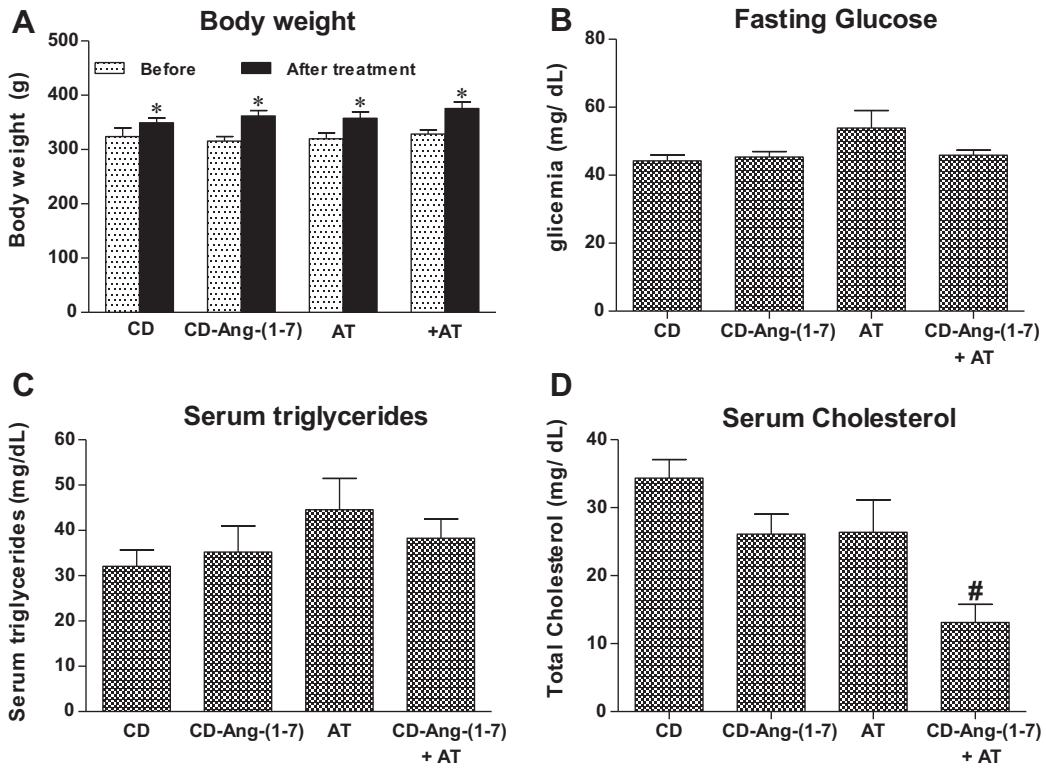

Fig. 1. Body weight (g; A) before and after treatment, fasting glucose levels (mg/dL; B) in the blood of animals treated for 8 weeks with: empty HP-β-CD (CD; $n = 16$); or CD-Ang-(1–7) ($n = 8$); or atenolol (AT; $n = 8$); or CD-Ang-(1–7) associated with atenolol (CD-Ang-(1–7) + AT; $n = 9$). Triglycerides (mg/dL; C) and total cholesterol (mg/dL; D) in the serum of animals treated for 14 weeks with: CD-Ang-(1–7) ($n = 7$); or atenolol (AT; $n = 8$); or CD-Ang-(1–7) associated with atenolol (CD-Ang-(1–7) + AT; $n = 8$); or HP-β-CD (CD; $n = 8$). $^{*}p < 0.05$ in comparison to before (Student's t-test for paired observations); $^{\#}p < 0.05$ in comparison to control-CD (one-way ANOVA followed by Newman–Keuls test).

(b) β-blocker (atenolol, 3 mg/kg/day; $n = 8$); (c) the association of CD-Ang-(1–7) and atenolol ($n = 9$) at same doses; and (d) vehicle, hydroxypropyl-β-cyclodextrin (CD, 50 μg/kg/day; $n = 9$). The proportion of Ang-(1–7) and hydroxypropyl-β-cyclodextrin in the oral formulation was 43% and 57%, respectively. Blood pressure was measured in a group of animals by telemetry for 8 weeks, as previously described [3].

2.3. Lipid profile

After 14 weeks of treatment, total serum cholesterol and triglycerides were measured by enzymatic method (Kit KATAL Biotecnológica Ind. Com. Ltd., Brazil) in fasted animals. In addition, oral fat tolerance was evaluated by the changes in serum triglycerides 60, 120, and 210 min after oral administration of a fat load (10 mL/kg), as described by Mori et al. [14].

2.4. Lipolysis

Lipolysis was measured by the rate of glycerol released from adipocytes. To isolate adipocytes, the fat pads of epididymus were digested with collagenase at 37 °C in a shaking water bath for 45 min. Next, cells were filtered through nylon mesh and washed three times with a HEPES buffer (pH 7.4) containing: 137 mM NaCl, 5 mM KCl, 4.2 mM $NaHCO_3$, 1.3 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.5 mM $MgSO_4$, 0.5 mM KH_2PO_4, 20 mM HEPES and 1% BSA. After a period of incubation, an aliquot of the infranatant was removed for enzymatic determination of glycerol released into the medium. Glycerol levels were measured before and after isoproterenol (0.1 μmol/L) or isoproterenol followed by insulin (12.5 ng/mL) incubation.

2.5. Fasting glucose

At eighth week of treatment, fasting glucose was evaluated in tail blood sample using an Accu-Check glucometer (Roche Diagnostics, Indianapolis, IN).

2.6. Glucose uptake

Glucose uptake was also measured in isolated adipocytes from epididymal fat tissue. The isolated adipocytes were incubated for 45 min at 37 °C in the presence or absence of insulin (50 ng/mL). The uptake of 2-deoxy-[3H]glucose (2DOG) was used to determine the insulin sensitivity in glucose uptake, as described by Green [7]. The assay was initiated by the addition of 2DOG (0.2 μCi/tube) for 3 min. Next, cells were separated by centrifugation through silicone oil and cell-associated radioactivity was determined by scintillation counting. Nonspecific association of 2DOG was determined by performing parallel incubations in the presence of 15 mmol/l phloretin, and this value was subtracted from glucose transport activity in each condition.

2.7. Lipoprotein lipase (LPL) activity

Epididymal fat pads were homogenized in buffer Tris–HCl (pH 8.3) containing detergents. LPL activity was measured using a [9,10-3H]triolein containing substrate with lecithin [16] and 24 h fasted rat plasma as a source of apo CII. The reaction was stopped with a mixture of extraction [1], and the released 3H-free fatty acids (FFAs) were quantified by liquid scintillation. The enzyme activity was expressed as nanomoles of FFA released per minute.