Co-administration of retinoic acid and atorvastatin mitigates high-fat diet induced renal damage in rats

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Abstract

Obesity causes many problems such as cardiovascular and chronic kidney diseases. The aim of this study was to evaluate the efficacy of retinoic acid and atorvastatin co-administration in kidneys protection against high-fat diet induced damage. Twenty-five male Wistar rats (20.00 ± 2.00 g) were divided into five groups: 1) Control (standard diet), 2) High-fat diet (cholesterol 1.00%, 75 days), 3) High-fat diet + atorvastatin (20.00 mg kg⁻¹ per day, orally, on the 30th day, for 45 consecutive days), 4) High-fat diet + retinoic acid (5 mg kg⁻¹ per day, orally, on the 30th day, for 45 consecutive days), and 5) High-fat diet + atorvastatin and retinoic acid. At the end, blood and tissue samples were collected for biochemical and histological analyses. The results showed that atorvastatin and retinoic acid alone and in combination decreased cholesterol and low-density lipoprotein and increased high-density lipoprotein in high-fat diet. Also, atorvastatin – caused total antioxidant capacity increase and protein carbonyl content decrease the in the renal tissue. Atorvastatin also prevented high-fat diet-induced renal histological injury. Treatment with atorvastatin significantly mitigates high-fat diet-induced renal changes probably due to its potent antioxidant and lipid-lowering effects. The effect of retinoic acid in renal protection in a high-fat diet is far less than that of atorvastatin. The protective effect of the combination of these two agents in the high-fat diet on the kidneys seems to be due to the effect of atorvastatin.

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چکیده

تجویز هم‌مان اسید ریتونیک و آتوروستاتین، آسیب کلیوی ناشی از رژیم غذایی پرچرب را در موش های صحرایی کاهش می‌دهد.

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Introduction

Obesity is a health problem for industrialized countries potentially leading to a decreased life expectancy. Obesity is currently a medical problem and among the complications associated with the pathological aspects of this disease, renal disease is a significant issue whose pathophysiological mechanisms are not fully known yet. For example, hypertension, hyperlipidemia and insulin resistance affect renal function in different ways.

Obesity is a condition in which kidney's demonstrate functional and morphological changes. Some studies have demonstrated that patients with the metabolic syndrome are at risk for chronic kidney disease (CKD). Few therapies are available for CKD. Previous studies have suggested that statins may slow the CKD progression. More recent meta-analyses, not including cardiovascular studies, have found that statins provide no kidney function protection. Based on other reports, atorvastatin and pravastatin have beneficial effects on kidney function.

Atorvastatin is a member of the drug class known as statins. It is used to reduce cholesterol biosynthesis. Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early limiting step in cholesterol biosynthesis.

Retinoic acids (RAs), a group of natural and synthetic derivatives of vitamin A, play important regulatory roles in cellular proliferation, differentiation and apoptosis. Retinoid can have an anti-oxidant effect through blocking lipid peroxidation in streptozotocin (STZ)-induced diabetes in rats. Retinoid have been shown to protect against renal injury in several experimental animal models of kidney diseases. However, the long-term clinical use of retinoids in patients with kidney disease is problematic due to its side-effect profile including differentiation syndrome, depression, psychosis, severe acne, skin and mucosal membranes dryness, inflammatory bowel diseases and teratogenicity. The treatment of kidney glomerular disease is challenging. Therefore, it is important to identify new treatment targets or regimes to protect the kidneys under different conditions. The aim of this study was to examine the effectiveness of using a lipid-lowering agent on the contradictory effects of retinoic acid on kidney damages.

Materials and Methods

Chemicals. Atorvastatin (Poursina, Tehran, Iran), retinoic acid (Sigma, Munich, Germany), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL) and cholesterol measurements kit (Pars Biochemical, Tehran, Iran), total anti-oxidant capacity (TAC) kit (ZellBio, Ulm, Germany), protein carbonyl (PCO) kit (Cayman, Hamburg, Germany) were used in this study.

Study design. Twenty-five male Wistar rats (200.00 ± 20.00 g) were purchased from Pasteur Institute, Tehran, Iran. All procedures for animals were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised in 1985) and approved by the Ethical Committee of Urmia University of Medical Sciences, Urmia, Iran (Ethical code: Ir.umsu.rec.1395.406). Animals were maintained under controlled conditions of temperature (21.00 ± 2.00 °C) and a 12/12 hr light/dark cycle. Animals were divided into five groups (five animals each): 1) control (standard diet), 2) high-fat diet (HFD; cholesterol 1.00% of food weight for 75 days), 3) HFD + atorvastatin (20.00 mg kg⁻¹ per day, orally, on the 30th day, for 45 consecutive days), 4) HFD + RA (5.00 mg kg⁻¹ per day, orally, on the 30th day, for 45 consecutive days), and 5) HFD + atorvastatin + RA. The dose of atorvastatin was equivalent to doses used in previous studies reporting that the doses of 10.00 to 80.00 mg kg⁻¹ of atorvastatin in humans are comparable to doses of 5.00 to 18.00 mg kg⁻¹ in rats via oral administration. At the end of the study, the animals were anesthetized with ether and sacrificed. Blood sample was collected by cardiac puncture and centrifuged for biochemical including HDL, LDL, cholesterol, blood urea nitrogen (BUN) and creatinine analysis. Also, tissue samples were collected for biochemical and histological analyses.

Biochemical analyses. At the end of the study, the animals were fasted for 12 hr. Afterwards, blood sample was collected for the measurement of BUN, creatinine and serum lipid profile. Serum BUN and creatinine were measured using an auto-analyzer (BT3000; Biotecnica Instruments, Rome, Italy) and serum sodium and potassium concentrations were measured by flame photometry. After blood collection, serum was separated and stored at –20 °C until analysis. Blood total cholesterol, TG, HDL and LDL concentrations were measured via a blood analysis kit using an enzymatic colorimetric method. Left kidneys were cut into suitable pieces and homogenized with a Potter-Elvehjem tissue homogenizer (Thomas Scientific, Swedesboro, USA) in 10.00 mM potassium phosphate buffer with the pH of 7.40. The homogenate was centrifuged for 15 min at 1000 rpm at 4 °C and the resultant supernatant was used for PCO and TAC determinations.

Protein carbonyl measurement. The tissue PCO content was determined spectrophotometrically by a method based on the formation of a Schiff base from the reaction between 2,4-dinitrophenylhydrazine (DNPH) and PCOs. The DNPH reacts with PCOs forming a Schiff base to produce the corresponding hydrazone which can be analyzed spectrophotometrically. Briefly, after the precipitation of protein with an equal volume of 1.00% trichloroacetic acid, the pellet was re-suspended in 10 mmol L⁻¹ DNPH plus 2N hydrochloric acid or in 2N HCl as a control blank. After the washing procedure with 1:1 ethanol-ethyl acetate, the final pellet was dissolved in
The carbonyl group was determined from the absorbance at 370 nm. The carbonyl content was calculated in terms of nmol mg⁻¹ protein.

**Total antioxidant capacity measurement.** Kidney homogenate TAC was measured using a commercial kit following the manufacturer’s protocol on the basis of the ferric-reducing antioxidant power (FRAP) assay. The FRAP assay measures the change in absorbance at the wavelength of 593 nm due to the formation of a blue-colored Fe²⁺-tripyridyltriazine compound from the colorless oxidized Fe³⁺ by the action of electron-donating antioxidants.²⁴ Concisely, 50.00 μL of kidney homogenate was added to 1.50 mL of FRAP reagent in a test tube and incubated at 37 °C for 10 min. The absorbance of the blue-colored complex was read against the reagent blank (1.50 mL of FRAP reagent plus 50.00 μL of distilled water) at 593 nm. The data were expressed as μmol ferric ions reduced to ferrous form per mg protein.

**Protein measurement** The protein concentration tested in 1 mL of homogenate used for TAC was estimated by the method of Lowry et al. using bovine serum as a standard.²⁵ Results of TAC were adjusted by protein content in each sample as μmol mg⁻¹ protein.

**Histological study** Following blood sample collection, to assess the extent of renal tissue damage, the right kidney was removed and stored in 10% formaldehyde. Tissue sections were stained with hematoxylin and eosin (H & E) and Masson’s trichrome (MTC) stains and observed under the light microscope.

**Statistical analysis.** Results are expressed as mean ± SD. Data were analyzed using one-way ANOVA followed by Tukey’s test in SPSS (version 22; SPSS Inc., Chicago, USA). The p values less than 0.05 were considered as statistically significant.

**Results**

**Serum concentration of BUN, creatinine, Na and K.** As shown in Table 1, a slight increase could be noted in the BUN and creatinine levels of the HFD rats in comparison with the control, although these increases were not significant. There was no significant difference regarding the serum concentration of sodium and potassium.

**Table 1. Serum levels of creatinine, blood urea nitrogen, sodium and potassium and lipid profile in experimental groups.**

| Parameters | Control | HFD | HFD + AT | HFD + RA | HFD + RA + AT |
|------------|---------|-----|---------|---------|---------------|
| Cr (mg dL⁻¹) | 0.79 ± 0.04 | 1.06 ± 0.11 | 0.87 ± 0.09 | 0.98 ± 0.17 | 0.94 ± 0.23 |
| BUN (mg dL⁻¹) | 31.45 ± 2.24 | 36.62 ± 5.43 | 32.65 ± 4.71 | 28.25 ± 3.42 | 24.73 ± 5.31 |
| Na (meq L⁻¹) | 136.00 ± 2.13 | 139.77 ± 1.85 | 145.74 ± 4.56 | 145.00 ± 2.82 | 139.37 ± 1.58 |
| K (meq L⁻¹) | 3.43 ± 0.44 | 4.41 ± 0.91 | 3.22 ± 0.13 | 3.74 ± 0.21 | 3.53 ± 0.42 |
| TG (mg dL⁻¹) | 136.25 ± 26.85 | 188.57 ± 27.15 | 129.75 ± 19.33† | 161.33 ± 25.35 | 170.25 ± 22.81 |
| Chol (mg dL⁻¹) | 74.00 ± 21.72 | 1266.25 ± 161.93* | 245.00 ± 81.35† | 718.00 ± 85.18† | 378.33 ± 95.34† |
| HDL (mg dL⁻¹) | 15.56 ± 4.25 | 32.00 ± 9.26* | 66.75 ± 16.82† | 82.59 ± 20.43† | 73.33 ± 20.57† |
| LDL (mg dL⁻¹) | 26.00 ± 4.71 | 576.00 ± 108.85* | 1527.25 ± 29.4† | 322.53 ± 57.27† | 306.33 ± 51.57† |

HFD: High fat diet; AT: Atorvastatin; RA: Retinoic Acid; Cr: Creatinine; Chol: Cholesterol; TG: triglyceride; LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

* and † show the significance in comparison with control and HFD groups, respectively (p < 0.05).
The HFD can alter the function and structure of rat kidney. In the present study, the effects of HFD on rat kidneys were studied. Serum BUN and creatinine increased in HFD indicating a change in renal function, although these changes were not significant compared to the controls. Although the glomerular filtration rate (GFR) was not measured in this study, the results of previous studies have shown that obesity induces hemodynamic change and increases GFR. Since the serum level of BUN and creatinine is strongly dependent on the GFR, the lack of any significant increase in these parameters may be due to the increased GFR. Because of its beneficial effects on lipid profile and antioxidant status, atorvastatin restores renal function changes. This finding is in agreement with previous studies showing that atorvastatin improves renal function in patients with chronic renal failure.

In the present study, HFD increased lipid profile (TG, cholesterol, LDL and HDL). The increase in cholesterol and LDL was significant compared to the controls. With regard to atorvastatin, its effects are clear and proven. This agent has long been used as a hydrophobic statin for the treatment of dyslipidemia, especially hypercholesterolemia. In the current study, the effect of atorvastatin in the modulation of lipid profile is similar to previous reports. However, the significant finding of the present study is the effects of RA on cholesterol and LDL reduction and HDL increase in HFD. According to the previous studies, RA must be
used with caution, especially in renal patients. The long-term use of retinoids in renal patients is a challenge due to their side-effect profile, although RA has been shown to protect kidney in various laboratory models of renal disease.17

Oxidative stress can play an important role in kidney disease. One of the mechanisms is the oxidation of LDL resulting in damaged endothelial cells losing their normal performance.31,32 The results of the present study indicated that the HFD group had a significant decrease in TAC compared to the control group that can increase the tissue damage of the kidney. In line with previous studies,32,33 the results of the present study showed that atorvastatin can increase the antioxidant capacity of the tissue and reduce tissue damage in the kidney. In the present study, RA did not increase the antioxidant capacity in HFD. Nevertheless, the results of other studies suggest that retinoids can delay renal damage and have antioxidant effects by stopping fat peroxidation in STZ-induced diabetes in rats.16,34 Results of this and previous studies confirm the contradiction in the use of RA in renal patients.

Cellular proteins are believed to be the target of free radical-induced oxidation resulting in increased carbonyl content in the tissue. Protein carbonyls are an irreversible form of protein and can be used as a benchmark for protein damage measurement in cells. The results of the present study showed that atorvastatin can prevent the carbonyl proteins production in the renal tissue and as a result, damage from oxidative stress in HFD. This finding confirms the results of previous studies.35,36 However, in the present study, RA was not effective in reducing the amount of PCO. This sheds doubt on the use of RA in the protection against kidney damages.

Histological studies showed clear changes in renal glomeruli including increased cell mass in glomeruli and apparent decrease in glomerular Bowman’s space in HFD. This is consistent with previous histological studies about the effects of obesity and HFD on renal glomeruli.1,28 Atorvastatin attenuated these histological changes which can be due to modifying and preventing changes in the fat profile as well as strengthening the antioxidant system. With this assumption, the less protective effects of RA can be attributed to the inability of this agent to enhance the antioxidant system in kidneys.

In conclusion, HFD changes the renal function and causes tissue damage in kidney. Treatment with atorvastatin significantly prevents these changes probably due to its potent antioxidant and lipid-lowering effects. The effect of retinoic acid on renal protection in HFD is far less than that of atorvastatin. The protective effect of the combination of these two agents in HFD on the kidneys seems to be due to the effect of atorvastatin.

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Conflict of interest

The authors declare that they have no competing interests regarding this paper.

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