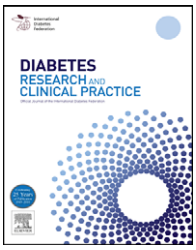
Contents lists available at ScienceDirect

Diabetes Research and Clinical Practice

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

International Diabetes Federation



Salicylate prevents hepatic oxidative stress activation caused by short-term elevation of free fatty acids in vivo

Bing He[a], *Sheng Zhao*[a], *Wei Zhang*[b], *Yan Li*[a], *Yan Lu*[a], *Ping Han*[a,*]

[a] *Department of Endocrinology, Shengjing Hospital of China Medical University, Shenyang 110004, China*
[b] *Department of Internal Medicine, Affiliated Fourth Hospital, China Medical University, Shenyang, China*

ARTICLE INFO

Article history:
Received 12 February 2010
Received in revised form
26 March 2010
Accepted 12 April 2010
Published on line 20 May 2010

Keywords:
Sodium salicylate
Insulin resistance
Oxidative stress
Free fatty acids

ABSTRACT

It has been reported that high-dose salicylates, an IKKß inhibitor, may prevent FFAs-induced insulin resistance. In previous study, we found that in FFAs-induced insulin resistant rats, administration of salicylate was associated with a reduction of plasma malondialdehyde (MDA). In the present study, we investigated the effects of sodium salicylate on FFAs-induced insulin resistance and on oxidative stress in liver.

Overnight-fasted Wistar rats were subject to 7 h i.v. infusion of either saline or Intralipid plus 20 U/ml heparin (IH) with or without salicylate. Hyperinsulinemic–euglycemic clamp with tracer infusion was performed to assess insulin-induced suppression of endogenous glucose production (EGP). Oxidative antioxidant markers, immunohistochemical inducible nitric oxide synthase (iNOS) stain, nitric oxide (NO), MDA, Superoxide dismutase (SOD) activity in liver was measured.

Infusion of IH markedly decreased insulin-induced suppression of EGP, which were completely prevented by salicylate co-infusion. Furthermore, salicylate reversed IH-induced (1) increase in iNOS and NO expression in the liver; (2) increase in MDA/SOD in the liver.

This study provides preliminary assessments of efficacy of sodium salicylate as a new treatment for FFAs-induced insulin resistances. The effect of increasing insulin sensitivity by salicylate in part may be secondary to reduce the oxidative stress in liver.

1. Introduction

Numerous studies have established a close relationship between obesity, insulin resistance, and type 2 diabetes. This link is attributed to greater release of various adipocyte products, such as cytokines, resistin, and free fatty acids (FFAs), from the expanded adipose tissue in obese individuals. In particular, elevated circulating levels of FFA cause insulin resistance in both animals and humans [1,2]. However, precise mechanisms by which FFAs impair insulin action in the liver and peripheral tissues are incompletely understood. Recent studies have implicated inflammatory pathway, particularly IκB kinase β (IKKß)/nuclear factor κB (NFκ B) system, is a causal mediator of insulin resistance induced by FFA [3,4]. Inactivation of IKKß by salicylates might improve insulin sensitivity, and aspirin administered at high doses results in a marked reduction of fasting glycemia in patients with T2DM [5].

In previous study, we found that in FFAs-induced insulin resistant rats, administration of anti-inflammatory drug sodium salicylate was associated with a reduction of plasma malondialdehyde (MDA), a maker of lipid peroxidation. One of the characteristic features of oxidative stress is enhancement of lipid peroxidation. Oxidative stress is associated with a wide variety of pathologies, including diabetes, cardiovascular,

* *Corresponding author*. Tel.: +86 24 96615 23121; fax: +86 24 23845739.
E-mail address: hb3h@hotmail.com (P. Han).
0168-8227/$ – see front matter
doi:10.1016/j.diabres.2010.04.004

cancer. Diabetes mellitus is strongly associated with it in particular [6]. The effect of salicylate on oxidative stress has not yet been investigated. It is unclear whether salicylate prevents insulin resistance induced by FFA via reducing oxidative stress pathway.

It is well known that the liver plays a central role in the regulation of whole-body glucose and is the major target for insulin resistance. Thus, in the present study, we investigated the effects of sodium salicylate on FFAs-induced insulin resistance and on oxidative stress in liver.

2. Materials and methods

2.1. Animal models

Male Wistar rats weighing 230–260 g were obtained from China Medical University Laboratories (Shenyang, Liaoning, China) and studied at least 5 days after arrival. Rats were housed under controlled temperature (23 °C) and lighting (12 h of light, 0600–1800 h; 12 h of dark, 1800–0600 h) with *ad libitum* access to water and standard rat chow. All procedures were approved by the China Medical University Animal Care and Use Committee.

2.2. Surgery

The rats were anesthetized with a ketamine (100 mg/ml), xylazine(0.1 mg/ml) and acepromazine (0.5 mg/ml) cocktail(1 μl/g body weight), and indwelling catheters were inserted into the right internal jugular vein for infusion and the left carotid artery for sampling, as previously described [1]. Polyethylene catheters (PE-50; Cay Adams, Boston, MA, USA), each extended with a segment of silastic tubing (length 3 cm, internal diameter 0.02 in.; Care Express Products, Inc., NY, USA), were used. The venous catheter was extended to the level of the right atrium, and the arterial catheter was advanced to the level of the aortic arch. Both catheters were tunneled subcutaneously, exteriorized. The catheters and filled with a mixture of 60% polyvinylpyrrolidone and heparin (1000 U/ml) to maintain patency and were closed with metal pins. The rats were allowed 3 days to recover before experiments.

2.3. Experimental design

After overnight fasting 12 h, the rats ($n = 12$/group) were subject to a 7 h i.v. infusion of either saline (SAL), Intralipid plus heparin (IH; 20% Intralipid plus 20 U/ml heparin at 5.5 μl/min), IH plus sodium salicylate (IHSS; 7 mg/kg bolus plus 0.117 mg/kg/min), or sodium salicylate (SS) alone. The dose of sodium salicylate (Sigma–Aldrich) used in the study was derived from a previous in vivo study by Kim et al. [7] in which SS treatment was shown to prevent FFA-induced insulin resistance in skeletal muscle. [6-^{3}H] glucose (20 μCi, blous + 0.4 μCi/min infusion) was given during the last 2 h of the experiment to assess endogenous glucose production (EGP) and peripheral glucose utilization. Further, each group rats were divided into 2 groups: a basal infusion group and a hyperinsulinemic–euglycemic clamping group, 6 in each group. The clamp protocols were similar to the basal period, but the clamp had the addition of an intravenous infusion of insulin (5 mU kg^{-1} min^{-1}), resulting in plasma insulin levels in the postprandial range. To maintain euglycemia during insulin infusion, a variable infusion of 20% glucose was given intravenously through the jugular catheter and adjusted according to frequent glycemic determinations (every 5 min). The glucose infusate was radiolabeled with 15 Ci/g [6-^{3}H] glucose to avoid variability in plasma glucose-specific activity due to changes in the rate of the cold glucose infusate. Blood samples for glucose, insulin, C-peptide, FFAs, and [6-^{3}H] glucose-specific activity were taken during the last 30 min (every 10 min) of each experiment. The total blood volume withdrawn was 3.0–3.3 ml during the basal experiments and 3.5–3.8 ml during the clamp experiments. After plasma separation, red blood cells diluted 1:1 in heparinized saline (4 U/ml) were re-infused into the rats. At the end of the experiments, the rats were anesthetized with i.v. administration of ketamine:xylazine: acepromazine cocktail (87:1.7:0.4 mg/ml), immediately after clamping of liver samples, while infusions were maintained through the jugular vein.

Part of liver tissue was fixed with 4% paraformaldehyde solution. The rest of liver tissue used for measurement of oxidative stress markers was kept in deep freezer at −70 °C

2.4. Laboratory methods

Plasma glucose was measured with glucose oxygenase method (BIOSEN5030, Germany). Plasma radioactivity from [6-^{3}H] glucose was determined after deproteinization with $Ba(OH)_2$ and $ZnSO_4$, passage through ion exchange columns, and subsequent evaporation. Aliquots of the [6-^{3}H] glucose and of the tritiated glucose infusate were assayed together with the plasma samples. The intra-assay coefficient of variation was 2.5%, and the inter-assay coefficient of variation was 6.5%. Insulin and C-peptide levels in plasma were determined by radioimmunoassay (Beijing Furui Biological Engineering Co., China). The coefficient of variation were <8% and 10.5%, respectively. Plasma FFAs levels were measured using a colorimetric kit (Nanjing Jiancheng Institute of Bio-engineering Institute, China). Hepatic nitric oxide (NO) content was measured by a rapid and sensitive spectrophotometric method [8]. MDA [9] and Superoxide dismutase (SOD) activity [10] in liver were performed using colorimetric kits (Nanjing Jiancheng Institute of Bio-engineering Institute, China).

2.5. Immunohistochemistry for express of iNOS in liver

Livers were fixed in 4% paraformaldehyde and embedded in paraffin. Each slide prepared with 6-μm thick-sections and placed on microscope slides. After removal of livers, they were then dehydrated in xylene and graded alcohols, and were immersed in distilled water. After being washed by water, 0.05% of H_2O_2–methanol was applied for 15 min. Hepatic sections were transferred to 10 mm/L of pH 6.0 citrated buffer solution for antigen retrieval and microwaved for 10 min. The primary antibody used in this experiment was diluted by 1:1000 of monoclonal anti-iNOS antibody