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Short Communication

D-galactose induced inflammation lipid peroxidation and platelet activation in rats

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ABSTRACT

Background: To investigate events possibly related to the development of D-galactose induced senescence, we examined whether 8-iso $PGF_{2\alpha}$ formation, a marker of *in vivo* lipid peroxidation is altered and whether its biosynthesis is associated with 11-dehydro-TXB_2 excretion rate, as a marker of *in vivo* platelet activation. In this setting, we also investigated the relationship between proinflammatory mediators (IL-6 and TNF-α from one, and lipid peroxidation and platelet activation, from another aspect.
Methods and results: Forty animals were divided, depending on treatment with D-galactose into: placebo and D-galactose treated rats. 8-iso-$PGF_{2\alpha}$, IL-6 and TNF-α were measured in plasma, while 11-dehydro-TXB_2 was determined in the urine after a six week treatment with D-galactose. Compared to placebo, D-galactose treated animals showed significantly higher levels of all measured parameters.
Conclusions: D-galactose induced changes in the rate of F_2-isoprostane formation are associated with the changes in the excretion rate of 11-dehydro-TXB_2.

1. Introduction

The D-galactose (D-gal), is a reducing sugar and can be metabolized at normal concentration. However, at high levels, it induces the production of reactive oxygen species (ROS) and advanced glycation end products (AGEs) [1]. Recent findings show that ROS and AGEs induced by continuous injection of D-gal in rodent lead to pathological processes of age-related disease such as diabetes, atherosclerosis, nephropathy, infection, and Alzheimer's disease [2]. Especially, the latest reports suggest that AGEs binding to its receptor for advanced glycation end products (RAGE) in many cell types induces pathophysiological cascades linked to the downstream activation of NF-κB and other signaling pathways that lead to ROS generation and certain proinflammatory responses measured trough interleukin-1β IL-1β, and tumor necrosis factor-α TNF-α) [3,4]. ROS generated in such a way, may cause oxidation of arachidonic acid (AA) followed by production of 8-iso-$PGF_{2\alpha}$ as a marker of lipid peroxidation that can be reliably measured in plasma and urine [4], and have been shown to be increased in association with advanced age [4]. However, the relative contribution of the proinflammatory markers IL-1β and TNF-α in enhanced 8-iso-$PGF_{2\alpha}$ biosynthesis as a complementary onset during D-gal induced senescence is not examined yet.

It is also interesting that *in vivo* degradation of AA can be carried through non-enzymatic pathway ending with biosynthesis of 8-iso-$PGF_{2\alpha}$ [5], and through enzymatic (cyclooxygenases) pathway ending with the production of thromboxane [6]. Such one very important thromboxane product of AA enzymatic degradation is 11-dehydro-thromboxane B_2 (11-dehydro-TXB_2) that is proposed as a marker for platelet activation during different age-related metabolic settings [6,7]. Considering mentioned reports together with the reported ability of D-gal to induce metabolic setting similar with aging, we decided to examine D-gal-related urinary 11-dehydro-TXB_2 excretion rate, as one of the determining variables of platelet events during aging.

All data mentioned above, in combination with the data published from Davì with coworkers [6], that inflammation could contribute in enhancing of the formation of F_2-isoprostane and 11-dehydro-TXB_2 during different metabolic settings [6–8], lay down the basis for hypothesis that: enhanced formation of F_2-isoprostane in the plasma, can be taken as a reliable marker for enhanced 11-dehydro-TXB_2 excretion during the D-gal induced setting.

2. Materials and methods

2.1. Animals and experimental design

All experimental procedures were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Ani-

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mals approved by the Macedonian Center for Bioethics. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the Institutional Animal Care and Use Committee, April 1997, Oakland University, MI, USA. Anesthetics were applied according to the standards given by the Guide of the Oakland University. Male Wistar rats ($n = 40$) were used for all protocols and were maintained on a 12:12 light: dark cycle and fed with standard rat chow and water, *ad libitum*. All animals were divided depending on the treatment with D-gal into: placebo (P) and D-gal treated (D-gal-T) rats.

Rats in (D-gal-T) group were treated intra-gastrally with a water solution of D-gal (100 mg kg^{-1} b.wt) for 42 consecutive days. Previously, it was shown that the used dose of D-gal induces senescence in the rats [9]. The rats in the placebo group were treated intra-gastrally with physiological salt solution. During the period of treatment, all animals were housed at 20 ± 2 °C.

2.2. Blood collection

Rat venous blood samples (from an abdominal vein) were taken at 11:00 O'clock on the day of sacrifice and maintained at room temperature until processed. Blood samples were withdrawn into tubes containing 3.8% sodium citrate (0.9 ml of blood to 0.1 ml of sodium citrate). Plasma was prepared by centrifugation (2000*g* for 20 min), separated into aliquots, and stored at −80 °C, until analyses were performed.

2.3. Urine collection

Before sacrifice, urine samples were obtained from each animal housed in a metabolic cage, where fecal contamination was avoided. They were collected in bottles within 24 h cycles. The volume of each urine sample was centrifuged at 3000*g* for 5 min, separated into aliquots, and stored at −80 °C until analyses were performed.

2.4. IL-1β and TNF-α immunoassay

Plasma levels of IL-1β and TNF-α were determined using commercially available enzyme-linked immunosorbent assay kits (Rat IL-1β and TNF-α Platinum ELISA, Bender Med-Systems, Vienna, Austria). Results are expressed as pg mL^{-1}. All assays were performed as outlined in the protocols enclosed in each kit. Intra-assay and inter-assay coefficients of variation were <10%.

2.5. 8-iso-$PGF_{2\alpha}$ and 11-dehydro-TXB2 immunoassays

8-iso-$PGF_{2\alpha}$ in the plasma and 11-dehydro-TXB_2 in the urine were analyzed by a newly developed ELISA for quantitative analysis of 8-iso-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 levels in biological fluids from Cayman Chemical Company. The total amounts of 8-iso-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 were analyzed after hydrolysis and extraction. The limits of detection of the assays were about 2.7 pg mL^{-1} for 8-iso-$PGF_{2\alpha}$ and about 15.6 pg mL^{-1} for 11-dehydro-TXB_2. Intra-assay and inter-assay coefficients of variation were <10%.

2.6. Creatinine assay

The levels of creatinine in the urine were estimated spectrophotometrically using the Jaffe's reaction [10].

2.7. Statistical analysis

Data were analyzed by one-way ANOVA, followed by the Newman–Keulls multiple comparison test between all groups. The correlation between different parameters was assessed by Spearman's test. Multiple linear regression analysis was performed to assess independent predictors of 8-iso-$PGF_{2\alpha}$ biosynthesis and 11-dehydro-TXB_2 excretion rate. Only 2-tailed probabilities were used for testing statistical significance. Probability values <0.05

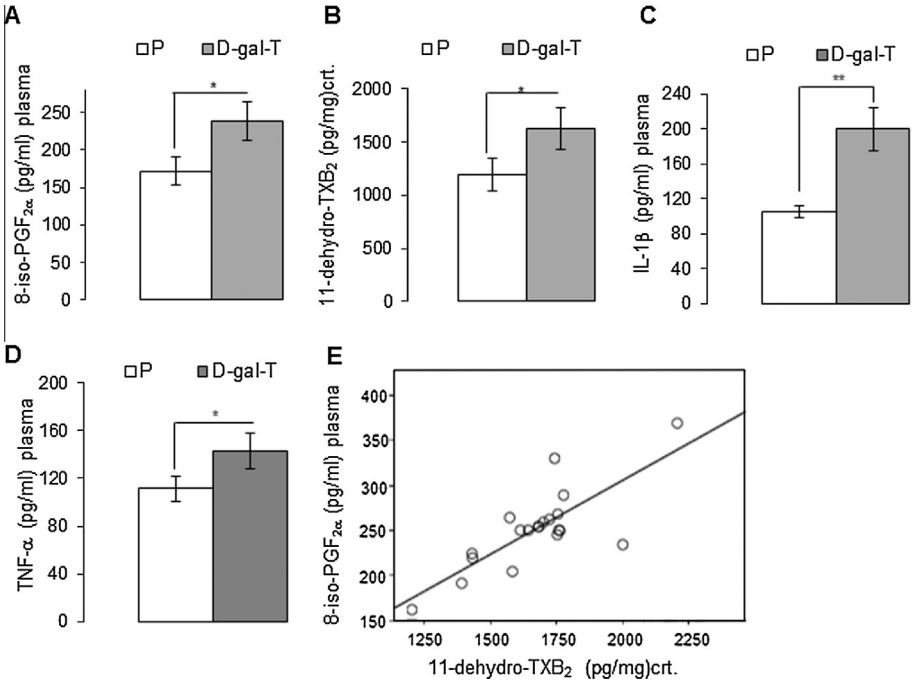

Fig. 1. (A) 8-iso-prostaglandin $F_{2\alpha}$ level in plasma (8-iso-$PGF_{2\alpha}$, mean ± SD). (B) 11-dehydro-TXB_2 level in urine (11-dH-TXB_2, mean ± SD). (C) Interleukin-1β in plasma (IL-1β, mean ± SD). (D) Tumor necrosis factor-α in plasma (TNF-α, mean ± SE). Placebo (P) and D-galactose treated (D-gal-T): *effect of D-gal, $^{*}p < 0.05$; $^{**}p < 0.001$. (E) Correlation between 8-iso-prostaglandin $F_{2\alpha}$ formation and 11-dehydro-TXB_2 excretion rate in D-gal treated rats.

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