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Formation of 8-nitroguanine in blood of patients with inflammatory gouty arthritis

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Abstract

Background: NOx causes DNA damage due to an inflammatory effect of gouty arthritis. We investigated the concentration of 8-nitroguanine (8-NO_2-G) in the blood of patients with arthritis.
Methods: Subjects were divided into 3 groups: (1) high inflammatory (HI) group ($n=21$) with hyperuricemia (mean, 8.9 mg/dl) and leukocytosis, (2) low inflammatory (LI) group ($n=14$) with mild hyperuricemia (mean, 7.6 mg/dl) but normal leukocyte count, (3) non-inflammatory (NI) healthy control ($n=19$) with mean serum uric acid concentration 5.3 mg/dl and normal leukocyte count. Serum C-reactive protein (CRP) concentrations were measured by a visual agglutination method. The blood concentrations of 8-NO_2-G were determined by high performance liquid chromatography-electrochemical detection and were compared between groups.
Results: There was significant difference in percentage of positive CRP (NI: 55.6%, LI: 64.3%, HI: 100%, $p=0.003$) between the 3 groups. The leukocyte count (mean ± S.E., NI: 7400 ± 528, LI: 7686 ± 433, HI: 10952 ± 691/mm^3, $p<0.001$), uric acid (NI: 5.3 ± 0.24, LI: 7.6 ± 0.4, HI: 8.9 ± 0.36 mg/dl, $p<0.001$), NO_2 (NI: 6.5 ± 1.2, LI: 11.1 ± 2.9, HI: 35.6 ± 5.1 μg/ml, $p<0.001$) and the 8-NO_2-G (NI: 0.08 ± 0.03; LI: 0.34 ± 0.13; HI: 0.59 ± 0.09 ng/μg DNA, $p=0.002$) were significantly increased by inflammation.
Conclusion: Gouty inflammation induces DNA damage by increasing 8-NO_2-G through endogenous NO and ROS formation.

Keywords: Gout; 8-nitroguanine

Abbreviations: 8-NO_2-G, 8-nitroguanine; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; CRP, C-reactive protein; HI, high inflammatory; HPLC-ECD, high performance liquid chromatography-electrochemical detection; LI, low inflammatory; NI, non-inflammatory; ROS, reactive oxygen species.

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1. Introduction

Gout can be viewed as a chronic inflammatory disease in which the presence of cells and monosodium urate crystals in the joint and their constant interaction form the base. During long asymptomatic periods, there is some minimal inflammation in these joints, which becomes intense and symptomatic during attacks [1]. Considerable evidence suggests that oxidant stress and reactive oxygen species (ROS) play significant roles in several aspects of acute and chronic inflammation. Increased ROS causes oxidative damage to cellular constituents, including membrane lipid, protein and DNA. Among many base modifications induced by ROS, 8-hydroxy-2′-deoxyguanosine (8-OH-dG) is one of the most abundant products for assessing oxidative damage to DNA, resulting in mutations through formation of GC to TA transversion. 8-OH-dG is formed from deoxyguanosine in DNA by hydroxy free radicals. Because of its stability, 8-OH-dG is known as one of the reliable markers of oxidative DNA damage. In addition, peroxynitrite has been reported to induce DNA strand breaks and 8-nitroguanine (8-NO_2-G) is one of the products of peroxynitrite reaction with DNA bases [2]. We have recently reported that 8-NO_2-G could act as a specific marker for DNA damage induced by gaseous nitrogen oxides [3]. We also found that cigarette smoke could induce the formation of 8-NO_2-G in the lung of exposed rats [4]. In this study, we investigated the concentration of 8-NO_2-G, to evaluate whether there is oxidative damage to DNA in the blood of patients with active gouty arthritis.

2. Materials and methods

2.1. Study subjects

A total of 49 male and 5 female (aged from 18 to 90, mean 47 y) subjects were recruited and were categorized into 3 groups including: (1) high-inflammatory (HI) group ($n=21$) with active gout arthritis and leukocytosis defined by white blood cell count >11 000/mm^3, (2) low-inflammatory (LI) group ($n=14$) but normal leukocyte count (4000–11 000/mm^3), and (3) non-inflammatory (NI) healthy controls ($n=19$) who were generally healthy without medical (such as inflammatory or cardiovascular diseases) or surgical problems. The age was comparable between groups.

2.2. Chemicals

Flavine adenine dinucleotide (FAD), β-Nicotinamide adenine dinucleotideleotide phosphate reduced form (β-NADPH), nitrate reductase, pyruvate, L-lactic dehydrogenase (LDH), Tris–HCI, isopropyl alcohol, DNase I, Nuclease P1, alkaline phosphatase, citric acid, EDTA (ethylenediaminetetraacetic acid), zinc sulfate ($ZnSO_4$), sulfanilamide were from Sigma Chemical Co. (St. Louis, MO). Sodium nitrate ($NaNO_3$), acetone, sodium acetate anhydrous, glacial acetic acid, methyl alcohol, phosphoric acid, hydrogen chloride (HCl), sodium hydroxide (NaOH) were from Merck (Darmstadt, Germany), *N*-1-naphthylethylenediamine was from Serva (Heidelberg, Germany).

2.3. Measurement of leukocyte count, serum uric acid and C-reactive protein

Blood samples were drawn and leukocyte counts were measured on the Sysmex K-1000 analyzer. Serum uric acid was measured on the Hitachi-7050 analyzer using uricase–peroxidase method. Serum C-reactive protein (CRP) concentrations were measured by a semiquantitative analysis obtained with a visual agglutination method.

2.4. Measurement of nitrite

The concentrations of NO and NO_2 in serum were measured by the formation of nitrite. NO was quantified by nitrite accumulation in the serum, using a procedure involving the Greiss reaction with sodium nitrite as the standard [5].

2.5. DNA digestion

DNA was extracted by the protocol of Dahlhaus and Appel [6] with minor modifications as described previously [7]. Briefly, one volume of nuclear fraction obtained from cell homogenate by centrifugation was mixed with 8 volumes of extraction buffer (1 mol/l NaCl, 10 mmol/l Tris–HCl, 1 mmol/l EDTA, 0.5% SDS, pH 7.4) and 1 volume of chloroform : isoamy-