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Are Allopurinol and Metabolites Found in HPRT Deficient Erythrocytes Responsible for Increased NAD Synthesis?

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ABSTRACT

Aim of this study was to ascertain whether allopurinol, usually administered to hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficient patients, or metabolites abnormally increased in HPRT deficient erythrocytes (NAD, PPribP) could be directly responsible for the reported increased activities of nicotinic acid phosphoribosyltransferase (NAPRT) and NADsynthetase (NADs) in these patients. No direct effect of the mentioned metabolites was demonstrated.

Key Words: Erythrocytes; NAD synthesis; HPRT deficiency; Allopurinol.

INTRODUCTION

Inborn complete deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT) causes hyperuricemia, gout and severe neurological disturbances (Lesch-Nyhan Syndrome).^[1] HPRT-deficient erythrocytes present additional biochemical alterations: GTP depletion; ZTP, phosphoribosylpyrophosphate (PPribP), NAD and UDP sugars accumulation;^[2] increased activity of adenine phosphoribosyltransferase (APRT),

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IMPdehydrogenase, UMPsynthetase,^[3] 5'nucleotidase,^[4] nicotinate phosphoribosyl-transferase (NAPRT) and NAD synthetase (NADs).^[5,6] Aim of this study was to ascertain whether NAPRT and NADs activity in HPRT deficient erythrocytes are increased by drugs or abnormal metabolites. Experimental conditions mimicking the metabolic situation of HPRT deficient erythrocytes were used to investigate the effect of allopurinol (usually administered to such patients and metabolized to oxypurinol) of NAD and PPribP in normal erythrocytes. Orotate phosphoribosyltransferase (OPRT), OMPdecarboxylase (ODC), known to be raised by allopurinol^[3] and APRT activity, increased in patients, were also checked as control.

MATERIALS AND METHODS

The activities of NAPRT, NADs, APRT, HPRT, OPRT and ODC were measured in erythrocyte lysates by HPLC-linked methods.^[5,6] Experimental conditions: addition of allopurinol, oxypurinol, NAD, PPribP to assay mixtures; lysate heating (60°C) to check enzyme stability; incubation of erythrocytes before lysis with PPribP generating medium either alone or plus nicotinate (increasing NAD production), plus allopurinol or oxypurinol. The endogenous nucleotide pattern was also measured in pre-incubated erythrocytes.

RESULTS AND DISCUSSION

No remarkable increase of NAPRT and NADs activities in the above conditions mimicking HPRT deficient erythrocytes was found. Direct effect by the mentioned metabolites was ruled out (only a mild protection of PPribP on NAPRT activity). Nucleotide pattern did not show any alteration following incubations (except for NAD increase with NA, and for the respective nucleoside monophosphates appearing with allopurinol or oxypurinol), nor were enzyme activities increased, except OPRT and ODC as expected after allopurinol and oxypurinol incubation. Data were confirmed in non-HPRT deficient patients treated with allopurinol.

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