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Tumor-promoting activity of *p*-hydroxybenzenediazonium is accelerated in Mg-deficient rats

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Abstract

Tumor-promoting activity caused by the short-term administration of *p*-hydroxybenzenediazonium (PDQ) has been assayed in rats fed on a Mg-deficient diet as a reference model versus rats fed on a standard diet as controls. For 5 weeks groups of 20 rats, fed either on the Mg-deficient or standard diet, were treated simultaneously with PDQ. A group of 10 Mg-deficient rats remained untreated. Topical application of PDQ was followed by the appearance of macroscopic alterations in the skin, which were more evident in the Mg-deficient rats. No deaths occurred during the treatment. After 5 weeks' PDQ treatment the rats were killed and histological analyses were made. Tissues from the skin, liver, heart, kidney, lung and thymus were screened by conventional staining methods. Both the PDQ-treated Mg-deficient and PDQ-treated control rats presented tissue lesions, although to a different extent. The untreated Mg-deficient rats showed no such lesions. Mg-deficient rats treated with PDQ developed significant incipient fibrosarcomas ($p < 0.05$) and extended hyperplasia ($p < 0.001$), particularly in the skin, accompanied by a significant increase in the thickness of collagen (mean value: 445.4 ± 47.2 μm, $p < 0.05$) compared to the control PDQ-treated group (mean values: 258.7 ± 36.4 μm). The overall results provide objective proof of tumor-promoting activity after 5 weeks' treatment with PDQ. Such a fast response is interpreted as being linked to the increased vulnerability of the membrane caused by Mg deficiency, which would more readily facilitate the toxic activity of *p*-hydroxybenzenediazonium ions.

Keywords: Tumor-promoting activity; Arenediazonium ions; *p*-Hydroxybenzenediazonium ion; Mg-deficient rats; Dediazoniation; Histological analysis

1. Introduction

Arenediazonium ions (ADIs) are known to have mutagenic and carcinogenic capacity [1] but the mechanisms involved in their genotoxicity are still not fully understood. In early studies ADIs themselves were judged to exert direct genotoxic activity [2]. Genotoxicity deriving from the formation of triazene adducts has also been blamed, either directly [3,4] or as an intermediate step in the arylation of adenine residues in DNA [5]. Thus the observed damage caused to DNA has been put down to the simultaneous action of arenediazonium

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ions and aryl radicals deriving from them [6]. Nevertheless, in the light of the ease with which aryl radicals cause arylation in vitro to nucleobases and nucleosides [7] and their ability to produce unspecific DNA strand breakage [8], the most commonly given explanation for the genotoxicity of ADIs is one of a direct attack on DNA by aryl radicals. More recently reported has been the capacity of carbon-centered radicals deriving from the *p*-methylbenzenediazonium ion to activate the AP-1 system and thus stimulate the phosphorylation of ERK1, ERK2 and p38 proteins, in a similar way to that observed for superoxide anion and hydroxyl radicals [9].

p-Hydroxybenzenediazonium (PDQ) is an arenediazonium ion which cleaves DNA [10], is responsible for the formation of the C8-guanine adduct [11] and in the long term causes tumors when repeatedly administered as a sulphate salt by subcutaneous injection [12]. In previous studies into PDQ dediazoniation [13–15] our results have shown that in a neutral aqueous medium in the dark PDQ dediazoniation is a homolytic process. This homolytic degradation is triggered by the hydroxyl anion but the primary products then produce a self-catalyzed reaction in which aryl, hydroxyphenylperoxyl and semiquinone radicals appear to be involved.

Magnesium ions are one of the most important elements involved in cell metabolism. The intracellular concentration of Mg^{2+} is very tightly controlled because of its involvement in crucial cell processes such as the hydrolysis of ATP and ADP, protein biosynthesis and apoptosis. There is evidence from epidemiological studies that in 15–20% of the population of industrialized countries Mg intake is approximately 30% below the recommended daily allowance and that Mg deficiency, together with inadequate dietary habits, can lead to many pathological conditions [16]. It is known to be linked to cancer [17], cardiovascular alterations [18] and many renal, gastrointestinal, neurological and muscular disorders [19]. Other effects associated with Mg deficiency are changes in the composition and fluidity of the fatty acids in the erythrocyte membrane [20] and complex electrolytic alterations secondary to the mineral deficit [21–24].

We report here on the results of experiments with Mg-deficient rats to evaluate the tumor-promoting capacity of PDQ, taking into account alterations to the cell membrane that accompany Mg deficiency and any possible synergic effect caused as PDQ is administered simultaneously. To this end we applied PDQ to the skin of the rats. In the case of Mg-deficient animals, this non-aggressive method was deemed to be more appropriate than a subcutaneous injection. The effects deriving from the application of PDQ were monitored over the short term by the appearance of histological alterations considered to be biomarkers of tumor promotion in mouse skin [25]

2. Materials and methods

2.1. Chemicals

Sodium tetrafluoroborate, perchloric acid, *p*-aminophenol, sodium nitrite, absolute ethanol and diethyl ether were bought from Merck and Sigma (Madrid, Spain). PDQ was synthesized in our laboratory according to the procedure indicated elsewhere [13] and stored at −18 °C in darkness to avoid degradation by exposure to light. Dimethylsulfoxide (DMSO) was from Merck (Madrid, Spain) and was used as received without further purification. Doubly distilled water from a Millipore system was always used. HNO_3 and $HClO_4$ (Merck, Spain) were used for atomic absorption measurements. Bovine liver (Certified Reference Material CRM 185, Community Bureau of Reference, Brussels, Belgium) was used for magnesium quality-control assays.

2.2. Analytical techniques

Spectrophotometric analyses of PDQ in DMSO and DMSO:HCl aqueous solutions were made in a Cintra 10 spectrophotometer (GBC Scientific Equipment, Hucoa Erlöss, Madrid, Spain) equipped with a Frigomix external thermostatic water bath (B. Braun-Biotech S.A., Barcelona, Spain). The Mg^{2+} concentrations in both plasma and diet were determined by atomic absorption spectrometry (AAS) using an Aanalyst'300 spectrometer (Perkin-Elmer, Madrid, Spain).

2.3. Animals and diets

Thirty recently weaned, male, Wistar rats (provided by the animal laboratory of the Scientific Instrument Centre, University of Granada) were fed on a standard commercial diet (Panlab, Barcelona) until they reached a body weight of 192 ± 17 g. Only male rats were used since no significant difference has been found in the past between genders in the appearance of tumors induced by arenediazonium ions [26]. Thereafter they were allowed access ad libitum to doubly distilled water and a semi-synthetic, Mg-deficient diet for 6 weeks. The diet [27] contained (g/kg) casein (Musal & Chemical, Granada, Spain) 140; cysteine (Roche SA, Madrid) 1.8; sucrose (Musal & Chemical) 100; wheat starch (Musal & Chemical) 622; fiber (cellulose) (Musal & Chemical) 50; olive oil 40; AIN-93 mineral mix (without magnesium