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Molecular basis of anticlastogenic potential of vanadium *in vivo* during the early stages of diethylnitrosamine-induced hepatocarcinogenesis in rats

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Abstract

Carcinogen-induced DNA base modification and subsequent DNA lesions are the critical events for the expression of premalignant phenotype of the cell. We have therefore investigated the chemopreventive efficacy of a vanadium salt against diethylnitrosamine (DEN)-induced early DNA and chromosomal damages in rat liver. Hepatocarcinogenesis was induced in male Sprague–Dawley rats with a single, necrogenic, intraperitoneal injection of DEN (200 mg/kg body weight). 8-Hydroxy-2′-deoxyguanosines (8-OHdGs), strand-breaks and DNA–protein crosslinks (DPCs) were measured by HPLC, comet assay and spectrofluorimetry, respectively. There was a significant and steady elevation of modified bases 8-OHdGs along with substantial increments of the extent of single-strand-breaks (SSBs), DPCs and chromosomal aberrations (CAs) following DEN exposure. Supplementation of vanadium as ammonium metavanadate (NH_4VO_3, +V oxidation state) at a dose of 0.5 ppm in terms of the salt weight throughout the experiment abated the formations of 8-OHdGs ($P < 0.0001$; 79.54%), tailed DNA ($P < 0.05$; 31.55%) and length:width of DNA mass ($P < 0.02$; 61.25%) in preneoplastic rat liver. Vanadium treatment also inhibited DPCs ($P < 0.0001$; 58.47%) and CAs ($P < 0.001$; 45.17%) studied at various time points. The results indicate that the anticlastogenic potential of vanadium *in vivo* might be due to the observed reductions in liver-specific 8-OHdGs, SSBs and/or DPCs by this trace metal. We conclude that, vanadium plays a significant role in limiting DEN-induced genotoxicity and clastogenicity during the early stages of hepatocarcinogenesis in rats.

Keywords: 8-OHdG; DNA strand-breaks; DNA–protein crosslinks; Chromosomal aberrations; Vanadium; Hepatocarcinogenesis

1. Introduction

Liver is a frequent site for the development of chemically induced cancer in rodents [1]. Experimental hepatocarcinogenesis can be induced by various chemical carcinogens, such as: diethylnitrosamine (DEN), 2-acetylaminofluorene (2-AAF), and aflatoxin B1. DEN is a potent hepatocarcinogen in rats influencing the initiation stage of carcinogenesis and induces DNA base modifications, DNA strand-breaks and in turn hepatocellular carcinomas (HCCs) without cirrhosis through the development of putative preneoplastic focal lesions [2]. The DEN model of experimental hepatocarcinogenesis provides a unique tool for studying molecular

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and cellular changes resulting from the administration of the carcinogen to the development of premalignant phenotype of the cell, mechanisms of cell growth, differentiation and cell death [1].

In recent years, vanadium, a dietary micronutrient present in mammalian tissues has received considerable attention as a limiting agent because of its ability to prevent regular wear and tear of the genome [3,4]. Vanadium influences the behaviour of enzymes, mimics insulin and growth factor activities and regulates gene expression [5,6]. Vanadium compounds have been found to be potentially effective against murine leukaemia, fluid and solid Ehrlich ascites tumour [7] and mammary adenocarcinoma [8]. Sakurai et al. have found strong antitumour chemopreventive activities of vanadyl complexes of 1,10-phenanthroline [$VO(Phen)^{2+}$] and related derivatives against human nasopharyngeal carcinoma and the observed effects were found to be superior than the chemotherapeutic drug, *cis*-diamminedichloroplatinum [9]. Recently, organometallic vanadocene compounds have been found to be potent anti-proliferative agents disrupting bipolar mitotic spindle formation and inducing cell cycle growth arrest in cancer cell lines [10]. Bis(4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium(IV) or Metvan is equally the most promising multitargeted antitumour vanadium complex with apoptosis-inducing property against human leukaemia cells, multiple myeloma cells and a number of solid tumours derived from cancer patients [11]. Again, bisperoxovanadium (bpV) compounds as irreversible protein tyrosine phosphatase inhibitors with broad-spectrum antineoplastic activities [12] are also under investigations.

A series of studies from our laboratory has shown that, supplementation of 0.5 ppm vanadium in drinking water was quite effective in suppressing DEN-induced hepatocarcinogenesis in rats without any toxic manifestations [13–17]. The 0.5 ppm (4.27 μmol/l) concentration of vanadium was chosen by dose–response studies made in our laboratory much earlier [18]. This particular dose of 0.5 ppm has been found to be well tolerated with adequate growth responsive effect. Vanadium at this concentration was also found to be nontoxic since no histological abnormalities or histopathological changes were noticed neither in the liver and kidney nor in the stomach of the rats studied [18]. Moreover, the dose used in our study is 600 times less than the toxic dose as reported by several workers in rat models [19–21].

Previous reports indicate that, the antitumour effect of vanadium may be mediated through selective induction and stabilization of hepatic xenobiotic biotransforming enzymes [15], inhibition of γ-glutamyltranspeptidase (GGT)- and placental glutathione *S*-transferase (PGST)-positive foci in preneoplastic rat liver [16,17]. This study is an attempt to have insights into the molecular events associated with the 'initiation' of premalignant phenotype of the cell in order to have an understanding of the underlying basis of anticlastogenic and thereby anticarcinogenic potentials of 0.5 ppm vanadium *in vivo* in the inhibition of rat liver preneoplasia. Carcinogen-induced alterations of the DNA helix include helical distortion, base modifications, single-strand and double-strand-breaks, DNA–DNA inter-strand as well as DNA–protein crosslinks (DPCs) and chromosomal aberrations (CAs) [22]. Among the most abundant and mutagenic oxidative base modifications, 8-hydroxy-2′-deoxyguanosine (8-OHdG), produced by the oxidation of deoxyguanosine (dG) is considered as the most sensitive and potential marker of oxidative DNA damage [23]. It has been shown that 8-OHdG is closely associated with certain diseases, including cancer, and is produced in various experimental models of chemical carcinogenesis [24]. Thus, studying the pattern of changes in the levels of tissue-specific 8-OHdGs following carcinogen assaults could be quite relevant in understanding the 'initiation' event of carcinogenesis. Besides 8-OHdGs, the magnitudes of DNA single-strand-breaks (SSBs) and DPCs are the measures of genotoxicity following carcinogen exposure. The inability of cells to repair such damage adequately is a putative causal event in chemical carcinogenesis. Single cell gel electrophoresis (SCGE) or comet assay, the alkaline version in particular has become a very popular and sensitive method for analysis, detection and quantitation of genotoxic DNA damages caused by various chemical and physical agents, including DNA strand-breaks, alkali-labile damage and excision-repair sites in an individual cell in interphase [25]. Furthermore, CAs, which occur with greatest frequency in cells and involved in the origin, progression and diversification of cancers are considered to be good somatic markers as well [26].

The present study thus aims to define the potentials of vanadium in limiting the actions of DEN by studying these molecular markers, such as tissue-specific 8-OHdGs, SSBs, DPCs and CAs in liver during the early preneoplastic stages of hepatocarcinogenesis in rats. The principal aim of the study therefore definitely is to elucidate mechanisms underlying anticlastogenic and thereby anticarcinogenic effects of vanadium in a defined rodent model of chemical hepatocarcinogenesis. The study further involves interactions of the dietary micronutrient vanadium with the critical molecule like DNA and inhibition of early DNA and chromosomal damages by this trace metal.