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Carcinogen-induced early molecular events and its implication in the initiation of chemical hepatocarcinogenesis in rats: Chemopreventive role of vanadium on this process

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Abstract

Carcinogen-induced formation of DNA adducts and other types of DNA lesions are the critical molecular events in the initiation of chemical carcinogenesis and modulation of such events by chemopreventive agents could be an important step in limiting neoplastic transformation in vivo. Vanadium, a dietary micronutrient has been found to be effective in several types of cancers both in vivo and in vitro and also possesses profound anticarcinogenicity against rat models of mammary, colon and hepatocarcinogenesis. Presently, we report the chemopreventive potential of vanadium on diethylnitrosamine (DEN)-induced early DNA damages in rat liver. Hepatocarcinogenesis was induced in male Sprague–Dawley rats with a single, necrogenic, intraperitoneal (i.p.) injection of DEN (200 mg/kg body weight) at week 4. There was a significant induction of tissue-specific ethylguanines, steady elevation of modified DNA bases 8-hydroxy-2′-deoxyguanosines (8-OHdGs) ($P<0.0001$; 89.93%) along with substantial increment of the extent of single-strand breaks (SSBs) ($P<0.0001$) following DEN exposure. Supplementation of 0.5 ppm of vanadium throughout the experiment abated the formations of O^6-ethylguanines and 7-ethylguanines ($P<0.0001$; 48.71% and 67.54% respectively), 8-OHdGs ($P<0.0001$; 81.37%), length:width (L:W) of DNA mass ($P<0.01$; 62.12%) and the mean frequency of tailed DNA ($P<0.001$; 53.58%), and hepatic nodulogenesis in preneoplastic rat liver. The study indicates that 0.5 ppm vanadium is potentially and optimally effective, as derived from dose–response studies, in limiting early molecular events and preneoplastic lesions, thereby modulating the initiation stage of hepatocarcinogenesis. Vanadium is chemopreventive against DEN-induced genotoxicity and resulting hepatocellular transformation in rats.

Keywords: Vanadium; Hepatocarcinogenesis; DNA adduct; Ethylguanines; 8-OHdGs; DNA strand-break

1. Introduction

Vanadium, a group VB, first transition series, ultra-trace element (molecular weight 50.942) with various oxidation states ranging from −1 to +5, is an endogenous constituent of plants, animals and most mammalian tissues [1,2]. This dietary micronutrient is believed to have a regulatory role in biological systems and is very probably an essential element, just like other 40 essential micronutrients, requiring small amount for normal cell metabolism as well as for proper growth and development of mammals [1–3]. It influences the behaviour of enzymes, regulates the activities of second messengers, signal transduction cascades and carbohydrate metabolism, mimics insulin and growth factor activities, stimulates protein tyrosine kinase and inhibits phosphotyrosine phosphatases and modulates gene expression [4]. This nutritional element has further been considered as a potential agent owing to its ability to prevent regular wear and tear of the genome and accordingly, it is involved in various DNA maintenance reactions and thereby may prevent genomic instability leading to cancers [2,5]. Vanadium compounds have been found to be potentially effective against murine leukaemia, fluid and solid Ehrlich ascites tumour [6], murine mammary adenocarcinoma and HEp-2 human epidermoid carcinomal cells [7] and human carcinomas of lung, breast, and gastrointestinal tract [Köpf-Maier, 1994] [8]. Furthermore, in vivo and in vitro antitumour activities of different vanadium compounds have been

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documented by several workers [9–11]. Sakurai et al. [12] 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. 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 [13]. 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 leukemia cells, multiple myeloma cells and a number of solid tumours derived from cancer patients [14]. Again, bisperoxovanadium (bpV) compounds as irreversible protein tyrosine phosphatase inhibitors with broad-spectrum antineoplastic activities are also under investigation [15].

A series of studies from our laboratory has shown that supplementation of 0.5 ppm vanadium in drinking water was quite effective in suppressing chemical hepatocarcinogenesis in rats without any toxic manifestations. Reports indicate that the anti-tumour effect of 0.5 ppm vanadium may be mediated through selective induction and stabilization of hepatic xenobiotic biotransforming enzymes [16], inhibitions of γ-glutamyl transpeptidase (GGT)- [17] and placental glutathione S-transferase (PGST)-positive foci [18], suppression of 2-AAF-induced DNA–protein crosslinks formation [19] and reduction of proliferating cell nuclear antigen immunoreactivity [20] in preneoplastic rat liver. The 0.5 ppm (4.27 μmol/L) concentration of vanadium was chosen exclusively by dose–response studies made in our laboratory much earlier in untreated normal rats with respect to inductions of hepatic glutathione (GSH) and Glutathione S-transferase (GST) activity [21]. This particular dose of 0.5 ppm has been found to be well tolerated and suitable with adequate growth responsive effect. Vanadium at this concentration was also found to be devoid of toxicity, since no histological abnormalities or histopathological changes were noticed neither in the liver and kidney nor in the stomach of the rats studied [21]. In this paper, we have further detailed the dose–response studies of vanadium, which we have made in recent times with respect to carcinogen-induced DNA damage and development of preneoplastic lesions (nodulogenesis) in Sprague–Dawley rat liver chemical carcinogenesis model.

We preferred rat model over mouse, because: first, many mouse strains seem to yield high rates of spontaneous liver tumours. Spontaneous liver tumours in rats are more rare [22]. High background incidence of tumours in mice liver may also confound the interpretation of dose response in chemical carcinogenicity studies in murine liver [23]; second, rat liver is a frequent target for the development of chemically induced cancer in rodents, and it is the most commonly used experimental model for investigating multistage carcinogenesis in vivo [24]. Furthermore, in the rat liver carcinogenesis models, a variety of enzyme-altered condition has been studied for their relevance to preneoplastic and neoplastic developments. For example, an immunohistochemically demonstrable enzyme marker, PGST has been utilized for the identification of liver preneoplastic focal lesions. In contrast, no equivalent markers for preneoplastic foci are available for mice [25]. In addition, rats have got several other advantages [26], like (a) the many similarities between the rat and human metabolic pathways; (b) the many similar anatomical and physiological characteristics that allow for comparisons in pharmacokinetics; (c) the short life span, especially for carcinogenesis study, which allows observation of DNA transformation from its initial stage to full-blown malignancy; (d) high levels of natural killer (NK) cell immunity; and (e) the easy availability, ease of breeding, and the existence of a large database to enable comparison of present to reported literature findings.

Presently we extend our study further to have insights into the early molecular events associated with the 'initiation' of carcinogenesis in order to have an understanding of the underlying basis of chemopreventive potential of vanadium in modulating the initiation event and thereby limiting DNA damage in vivo. Experimental hepatocarcinogenesis can be induced by various chemical carcinogens, such as, diethylnitrosamine (DEN), 2-acetylaminofluorene (2-AAF), aflatoxin B1. DEN is a potent hepatocarcinogen in rats influencing the initiation stage of carcinogenesis during a period of enhanced cell proliferation induced by hepatocellular necrosis and forming DNA–carcinogen adducts, inducing DNA-strand breaks and in turn hepatocellular carcinomas (HCCs) without cirrhosis through the development of putative preneoplastic focal lesions [27]. Formation of DNA–carcinogen adduct is therefore a prerequisite for chemical carcinogenesis. Besides, carcinogen-induced alterations of the DNA helix include helical distortion, oxidative base modifications, single-strand and double-strand breaks, DNA–DNA inter-strand as well as DNA–protein crosslinks and chromosomal aberrations, and as such carcinogen-induced DNA damage has been implicated as one of the early steps in chemical carcinogenesis [28]. Among the most abundant and mutagenic oxidative base modifications, 8-hydroxy-2′-deoxyguanosine (8-OHdG), produced by the oxidation of deoxyguanosine is considered as the most sensitive and potential marker of oxidative DNA damage [29]. 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 [30,31]. Thus, studying the pattern of changes in the levels of tissue-specific DEN-induced alkylated DNA adducts as well as 8-OHdGs following carcinogen assaults could be quite relevant in understanding the 'initiation' event of carcinogenesis. ^{32}P-post-labeling and high performance liquid chromatography (HPLC) provide sensitive techniques to quantify respectively ethylated DNA bases and oxidative bases in tissue DNA samples. Besides 8-OHdGs, the magnitude of DNA single-strand breaks (SSBs) is a measure of genotoxicity following carcinogen exposure. The inability of cells to repair such damage adequately is a putative causal event in chemical carcinogenesis [31,32]. Single Cell Gel Electrophoresis (SCGE) or the Comet assay, in particular the alkaline version of the assay, has become a popular method for the sensitive analysis, detection and quantitation of genotoxic DNA damage caused by various chemical and physical agents.