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Polyol profile as an early diagnostic and prognostic marker in natural product chemoprevention of hepatocellular carcinoma in diabetic rats

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ABSTRACT

Aim: Diabetes mellitus (DM) is a risk factor for hepatocellular carcinoma (HCC). It directs glucose to sorbitol and fructose in polyol pathway (PP). To pursue contribution of PP in hepatocarcinogenesis.

Methods: We utilized ascorbic acid (AA) and diallyl sulfide (DAS) in experimental DM and HCC against control. HCC was induced by diethyl nitrosamine (DENA, one intraperitoneal (IP) dose 125 mg/kg), DM, by streptozotocin (STZ, IP dose 65 mg/kg). AA was given as 7.4 g/kg/d, I.P., DAS 200 mg/kg/d, orally. All animals were killed after 10 weeks.

Results: DENA elevated serum AFP, erythrocyte sorbitol (ES), neoplastic changes in liver, lowered blood glucose, increased hepatocyte aldose reductase (AR) and sorbitol dehydrogenase (SDH), significantly alleviated by DAS/AA combination. DM elevated ES activating AR, inhibiting SDH, improved by DAS and AA.

Conclusion: Co-induction of DM and HCC increased liver tissue lesion, serum AFP, ES, liver AR and SDH. Co-administration of DAS/AA reduced ES, AR without changing SDH. DAS/AA co-therapy lowered ES by depressing AR without affecting SDH, meaning that AR is activated by cancer and DM in different ways. PP is early marker for HCC detection and response to chemoprevention. DAS/AA combination is promising cost effective chemopreventive and anti-diabetic combination.

1. Introduction

Hepatocellular carcinoma (HCC) is the third deadliest and fifth most common malignancy worldwide [1,2]. It is a highly malignant tumor with a very high morbidity, mortality and poor prognosis due to its rapid infiltrating power and complicating liver cirrhosis [3]. Its incidence is increasing worldwide ranging between 3% and 9% annually [4]. The possibility that diabetes mellitus (DM) may increase the risk of HCC has been raised in a number of cohort and case control studies [5]. DM is now considered as an independent risk factor for HCC, despite the presence of hepatitis C virus (HCV), hepatitis B virus (HBV), alcoholic liver disease, or nonspecific cirrhosis [6,7]. Since most cases with DM are non-insulin dependent and characterized by hyperinsulinemia, insulin may interact with hepatocytes to stimulate mitogenesis or carcinogenesis [7]. Activation of the polyol pathway (PP) is a key metabolic change caused by

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hyperglycemia [8]. Aldose reductase (AR) was first identified as the rate-limiting enzyme in PP, through which glucose is reduced to sorbitol [9]. Excessive accumulation of intracellular sorbitol in various tissues contributes to the pathogenesis of diabetic complications [10]. Excessive accumulation of intracellular sorbitol in various tissues contributes to the pathogenesis of diabetic complications [9]. Many studies indicated that in addition to sugars, AR reduces lipid peroxidation-derived aldehydes as well as their glutathione conjugates [11–14]. Sorbitol dehydrogenase (SDH), the second key enzyme in PP, catalyzes the interconversion of polyols, such as sorbitol and xylitol to their respective ketones. SDH deficiency leads to subsequent accumulation of sorbitol within the cell, leading to diabetic complications as cataracts and microvascular abnormalities [15]. During hyperglycemia, AR activity diminishes the cellular antioxidant capacity [16,17]. As PP converts glucose to fructose and its metabolites fructose-3-phosphate and 3-deoxyglucosone being more potent non-enzymatic glycation agents than glucose, the flux of glucose through the PP would increase advanced glycation end products (AGEs) formation which are known to cause oxidative stress [18–20]. Chemoprevention serves as an attractive alternative to control malignancy by a pharmacological intervention aiming to arrest the process of carcinogenesis [21]. The major focus of research in cancer chemoprevention includes the identification, characterization and development of new and safe cancer chemopreventive agents [22].

Some dietary components were utilized as potent tactic for enhancing the therapeutic efficacy of chemotherapy, radiotherapy, or other standard therapeutics for the treatment of resistant cancers [23,24]. Some AR inhibitors (ARIs) were tried over the past few decades to prevent long-lasting diabetic complications including carcinogenic transformation. However, the clinical potential of ARIs in diabetic patients still controversial due to the lack of conclusive evidence and the safety of this category of drugs is also uncertain. Of these drugs, Alrestatin, Tolrestat, Ponalrestat, Epalrestat, Zopolrestat, Zenarestat, Fidarestat, were used [25]. Epidemiologic and observational studies provided an evidence for strong protective role of ascorbic acid (AA) against cancer [26,27]. Additionally, the therapeutic use of garlic and its application to cancer have been widely studied [28,29]. Diallyl sulfide (DAS) is derived from garlic and is sequentially converted to diallyl sulfoxide (DASO) and diallyl sulfone ($DASO_2$) by cytochrome P450 2E1 (CYP2E1). This component has been described to exhibit positive effects towards decreasing the incidence of experimental malignancies [30], inhibiting tumor cell growth [31] and suppressing the formation of DNA-adducts caused by N-nitroso compounds in vitro [32].

The present study aims mainly to uncover the possible link between DM and HCC using PP profile (erythrocyte sorbitol (ES) content, AR and SDH liver tissue expression), along with blood glucose, alpha fetoprotein (AFP) and histological examination of liver sections in experimental HCC in albino rats. This profile will be utilized also in following the possible chemopreventive utility of a suggested ARI (AA) and a naturally occurring anticancer (DAS) combination in diethyl nitrosamine (DENA)-induced HCC among both euglycemic (EG) and DM rat models.

2. Materials and methods

2.1. Materials

2.1.1. Animals and experimental design

One hundred and twelve male Wistar rats (3 months old), weighing 145–180 g were used in this study, kept for 2 weeks to accommodate with laboratory conditions under constant environmental and nutritional conditions. Animals were given a standard food of 26% casein and water ad liitum. Individual body weights were recorded weekly. Zero time is considered as dosing after 2 weeks accommodation. They were divided into equal 14 experimental groups (eight rats per group). During the period of experiment; animals were kept at 12 h light/12 h dark cycle. Both AA and DAS were used as possible cancer protectors among both EG and DM rats. The total period of the experiment was 10 weeks.

2.2. Animal Approval Committee

An approval was taken from the University committee resident in College of Medicine/Minia University.

The groups were classified and dosed as follows (Table 1).

2.3. Methods

By the end of the study period, all rats were fasted overnight then killed by exposure to increasing concentration of CO_2. Blood samples were obtained by cardiac puncture and divided into 2 portions, heparinized, for determination of ES as the ratio of sorbitol concentration to hemoglobin concentration after measuring blood hemoglobin [37] and erythrocytes sorbitol contents [38,39]. The other was centrifuged to separate serum samples and kept at −80 °C for glucose determination [40], using Randox diagnostic kits (UK) and AFP determination [41], using rat AFP kit from USCN life company/China, following the instructions of the manufacturer. Liver tissues were quickly removed, washed thoroughly in ice-cold phosphate buffer solution (PBS). Individual liver weights were recorded, then divided into 2 portions, one kept in formol saline for histological examination after Hematoxylin and Eosin staining [42], the second portion was immediately frozen in liquid nitrogen, kept at −80 °C for determination of both AR and SDH expression in hepatic cytosol by western blotting.

Liver tissue portions (approx. 100 mg/sample) were pulverized separately under liquid nitrogen, re-suspended in 1 ml of ice-cold 50 mM HEPES buffer (pH 7.5) containing 150 mM NaCl and 1 mM dithiothreitol. This buffer was fortified with complete EDTA-free protease inhibitor cocktail tablets (blend of protease inhibitors formulated as a ready to use water soluble tablets) at a dose of one tablet/10 ml buffer (Roche Diagnostics Ltd., UK). The pulverized hepatic material was placed on ice and homogenized mechanically using an Omni homogenizer. The extracts were subjected to 2 centrifugation steps at 4 °C (15,000 × *g* for 45 min, followed by 100,000 × *g* for 90 min). The final supernatants (cytosols) were collected for enzyme analyses [43]. Individual protein concentrations in cytosols were determined using Bradford assay [44] and