A promising chemosesitization role of inulin in management of experimentally induced hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is the most known primary liver cancer. It represents the third cause of cancer deaths all over the world, and is the principal cause of death in patients with liver cirrhosis. The known risk factors of HCC possess hepatitis C and B, alcoholic steatohepatitis, aflatoxins, cirrhosis and some metabolic diseases. Although, among 25% of HCC cases still unrecognized, chronic active hepatitis is the common risk for HCC, associated with hepaticocyte inflammation, cytokine deregulation and cirrhosis. Liver cirrhosis usually develops within a period of 20–40 years after persistent hepatic disease. TAA is a well-established inducer of liver cirrhosis. The active metabolites of TAA, as TAA-sulfoxide and TAA-sulfodioxide, are hepatotoxic intermediates. Administration of TAA for long times causes hyperplastic hepatic nodules, hepatocyte adenomas and HCC. HCC risk exponentially increases during the cirrhosis stage, then, carcinogenic transformation takes place. Cisplatin (CP) is a key drug in standard regimens in treatment of many cancers, but its clinical use is narrowed due to its side effects as nephrotoxicity, neurotoxicity, ototoxicity and hepatotoxicity. Furthermore, increasing the dose of chemotherapy to overcome resistant cancer stem cells can result in severe side effects for the patient including bone loss, hair loss and mucositis. Presentation of new chemotherapeutics or enhancing the effectiveness of current chemotherapeutics could prove vital cancer care. Many phytochemical antioxidants showed favourable effects on human diseases. Inulin (IN) is obtained from chicory roots (Cichorium intybus L.), as a mixture of linear polymers and oligomers composed of fructose joined through β(2–1) glycosidic linkage mostly by glucose ending. It shows antioxidant activity, preventing liver cell damage, through capturing oxidants, maintaining the native mechanisms that protect the cell. Thus, it was thought worthwhile to investigate the ability of supplementa-

Keywords

Chicory seed extract, hepatocellular carcinoma, inulin, oxidative stress and inflammation

Materials and Methods

Experimental animals

Two months old adult male Wistar rats (120–150 g), have been purchased from the Institute of Ophthalmic Disease Research in Cairo (Egypt). They were kept in stainless cages in a clean room with adjusted temperature (22–25°C) and standard chow and water ad libitum. Light cycle as equal light and darkness periods was maintained.

Chemicals and drugs

TAA and IN were purchased from Sigma Chemical Co., St. Louis, MO, USA. CP was purchased from local Egyptian market (Mansoura, Egypt). TAA was used to induce chronic liver damage and hepatocarcinoma.

Experimental design

Rats were classified into five groups (six rats per each): group I, rats served as control received intra peritoneal (IP) saline daily, group II rats, injected by fresh TTA solution twice weekly at a dose of 200 mg/kg b.wt (IP) 18, for 4 months, group III rats given CP (7.5 mg/kg/bw) as a single IP dose in addition to TAA, as
mentioned in group II. Group IV rats were orally administered IN (10 mg/kg/bw) in addition to TAA with for 4 months and group V rats were given CP (7.5 mg/kg bw) and IN in addition to TAA (200 mg/kg bw), as before, for 4 months.

**Collection of samples**

By the last day of work, all animals were kept fasting to the morning, venous blood samples were withdrawn, centrifuged at 860 × g for 20 min at 4°C and kept at −20°C right analysis. Rats were dissected and the liver tissue was divided into 2 parts, one was kept in formal saline for microscopic investigation and the other was weighed, minced in saline with Potter-Elvehjem type homogenizer on an ice container. Homogenate was centrifuged at 860 × g for 20 min at 40°C, and supernatants were frozen at −20°C right analysis.

**Biochemical analysis**

These investigations comprised two parts, one in the serum as: Alpha fetoprotein (AFP), TAC, tumor necrosis factor (TNF)-α, P53 and B-cell lymphoma 2 (Bcl), the other in liver tissue as: MDA, GHS, PC, AO, XO and CAT.

Quantitative measurement of AFP level in serum was executed by ELISA according to the method of Acosta (1983), kits were purchased from Diagnostic, Pacific Concourse, Drive, Los Angeles, USA. Malondialdehyde (MDA) and protein carbonyl (PC) contents were assessed according to the methods of Ohkawa et al. and Smith et al., respectively. Xanthine oxidase (XO) and aldehyde oxidase (AO) activity were determined as described previously.

Reduced glutathione (GSH) content was assayed by the method of Prins and Losse. Total antioxidant capacity (TAC), superoxide dismutase (SOD) and catalase (CAT) activities were estimated as described previously.

Serum TNF-α level was determined by ELISA (Diagnostic Products Corp, Los Angeles, CA, USA) as described by Aggarawal.

Bcl, and P53 levels were computed.

**Statistical analysis**

Results were recorded as mean ± SE. Statistical significance was computed exploiting one-way analysis of variance (ANOVA). We used SPSS 12.00 software. Variations of t-value less than P ≤ 0.05, were considered significant.

**Results**

Histological examination showed normal tissue architecture of control rats, while HCC group showed fibrosis as blue collagen fibers around CV with few nuclear/cytoplasmic ratio increments. Liver sections of group 2 (HCC + Cis) showed little strands of blue collagen in hepatocytes. HCC + IN liver sections showed similar features to control rats group. HCC + Cis + IN showed approximately normal hepatocytes (Fig. 1).

As shown in Table 1, a significant increase in oxidative stress biomarkers (MDA and PC) as well as free radical enzymes activity (AO and XO accompanied with significant decrease in enzymatic and non-enzymatic antioxidants (GSH, TAC, SOD and CAT), in HCC rats comparing to control. In contrast, administration of IN or CP to HCC rats showed amelioration in the tested parameters. Moreover, the results in Table 2, showed a significant increase in the level of serum AFP, TNF-α and P53 but decrease in BCl level in HCC rats than control. However, administration of IN or CP to HCC rats succeeded to attenuate these changes. Furthermore, the IN is more effective than CP and could sensitize cellular response to CP.

**Discussion**

HCC seems to be the most lethal of all cancers. A lot of dietary, endogenous and environmental, stimulimmediate hepatocarcinogenesis. Carcinogenesis may result from biological or chemical effects on normal cells through multistage and complicated steps, involving changes of the initiation step, promotion, progression that ends by malignant transformation.

Chemical carcinogens initiate oxidative stress on different cellular compartments, ultimately complicate to malignancy.

TAA was adopted as a well trusted a chemical that can induce liver cirrhosis in rats, with a very close human cirrhotic analogy. Intra peritoneal TAA generates fibrosis, cirrhosis and HCC in rats. It is a sulfur(thio( containing necrogenic potentials. Experimentally, it is acceptable inducer of fulminant hepatic failure and liver cirrhosis in animals. Biotransformation of TAA into oxidative products is responsible for hepatic damage, so that, flavin-containing monooxygenase (FMO) and cytochrome P450 reduces dioxyn into superoxide anion, that is metabolized into H₂O₂.

HCC disturbs liver functions through oxidative cellular derangements induced by reactive oxygen species (ROS) causing lipid peroxidation, a risk for damage to the macromolecules in most of bio membranes. In our study, administration of a single dose of TAA could induce premalignant
In our work, the significant downregulation of SOD and CAT activities to elevated ROS, which interferes with the enzyme through denaturation and inactivation. The current study indicated that HCC causes increase in inflammatory chemokines as TNF-α and apoptotic cell marker (p53) accompanied with decrease anti-apoptotic proteins (Bcl-2). These findings were reported in cirrhosis, fibrosis and cancer. HCC was reported to be associated with failure in the anti-apoptotic protein Bcl, maintenance and its translocation into the mitochondria, this hinders apoptosis. Further studies published that the ROS induced by TAA metabolites induce necrosis. In our work, the significant downregulation of blood level of Bcl-2 protein interprets the liability of cells to apoptosis by TAA actions. A dilemma of mechanisms are sought in CP resistance, as depressed intracellular accumulation, drug deactivation by elevated contents of cellular thiols, changes in drug target, metabolism or drug-induced damage by elevated nucleotide excision-repair activity and decreased mismatch-repair activity and evasion of apoptosis.

On the other hand, it has been established that chicyco-supplemented diet improved antioxidant activities against ROS responsible for cellular damage and elevated liver functions. Previous records reported that chicyco significantly reduces the oxidative stress, restoring GSH and CAT levels, thereby up-regulates the native antioxidant defense networking. In this study, administration of IN sensitized the used chemotherapy through a dilemma of mechanism of action.

It was frequently observed that oxidative stress contributed in carcinogenesis. XO and AO are molybdo-flavoenzyme containing hydroxylases, known to contribute in various forms of injury. Elevated activity of these enzymes produce ROS and therefore lead to oxidative stress. These enzymes contribute to the catabolism of purines in humans. In the present project, superoxide dismutase (SOD) activity was reduced, TAC and CAT activities, and reduced GSH content. The downregulated SOD activity assumes deactivation of this enzyme, presumably due to elevated superoxide release or depression by H₂O₂ due to decreased CAT activity, that preferentially breaks H₂O₂. The depressed GSH, SOD and CAT in HCC rats can be attributed to elevation of ROS. SOD is deemed to be the confront of defense against harmful effects of intracellular ROS through catalysis by superoxide radical (O₂⁻) to H₂O₂ and O₂. CAT detoxifies H₂O₂ which in turn is potent inhibitor of SOD. Kregel and Zhang attributed the downregulation in SOD and CAT activities to elevated ROS, which interferes with the enzyme through denaturation and inactivation. The current study indicated that HCC causes increase in inflammatory chemokines as TNF-α and apoptotic cell marker (p53) accompanied with decrease anti-apoptotic proteins (Bcl-2). These findings were reported in cirrhosis, fibrosis and cancer. HCC was reported to be associated with failure in the anti-apoptotic protein Bcl, maintenance and its translocation into the mitochondria, this hinders apoptosis. Further studies published that the ROS induced by TAA metabolites induce necrosis. In our work, the significant downregulation of blood level of Bcl-2 protein interprets the liability of cells to apoptosis by TAA actions. A dilemma of mechanisms are sought in CP resistance, as depressed intracellular accumulation, drug deactivation by elevated contents of cellular thiols, changes in drug target, metabolism or drug-induced damage by elevated nucleotide excision-repair activity and decreased mismatch-repair activity and evasion of apoptosis.

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antioxidant defense with decreased apoptotic cell marker (p53) and inflammatory cytokines (TNF-α) accompanied with increased anti apoptotic cell marker (BCL-2). IN is scavenger to ROS possibly due to the NH₄ that grazed in its molecule. NH₄ can be converted to NH₃ by absorbing hydrogen ion from media, then react with −OH. IN is extracted from chicory, bacterial fermentation of chicory fructans yields short-chain fatty acids in colon, as butyric acid. It activates the immune system, prevents inflammation and bacterial infections.

Conclusion

Our data indicated the efficacy of IN as a chemo-sensitizer for CP treatment in experimentally induced HCC. This hypothesis is supported by up-regulation of the native antioxidant system and capturing ROS responsible for oxidative stress and cell damage. It also elevates the apoptotic potential of hepatocytes, through modulation of TNF-α, p53 and Bcl-, which is manifested by decreased serum AFP and normalized liver tissue.

References


Author Contributions

Nabil contributed in conception, design and critical review, Hanaa Hassan and Hanaa Serag, in conception, analysis and drafting. Mahmoud Amr contributed to analysis and drafting of the manuscript.

Declaration of Conflicting Interests

The authors declare no conflict of interest with respect to the manuscript, authorship, and/or publication of the manuscript.

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Ethical Approval

All procedures performed in this study comply with ethical guidelines of the University Research Committee.


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