Effect of IL-21 on CCl_4 Induced Hepatotoxicity

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ABSTRACT

IL-21, a member of a large family of cytokines has receptors who share a common receptor γ chain (γ_c). IL-21 drives B cell differentiation into plasma cells, regulates immunoglobulin production, controls the proliferation and/or effector function of both CD4+ and $CD8^+$ T cells, limits the differentiation of regulatory T cells (Tregs) and can stimulate epithelial cells and fibroblasts to produce inflammatory mediators. Like other cytokines in this family, IL-21 has potent anti-tumor effects due to its ability to expand the pool of cytotoxic CD8+ T cells, NK cells and NKT cells. In line with this notion; IL-21 has been associated with clinical antineoplastic activity. The main aim of our study is to investigate the hepatoprotective activity of IL-21 in mammalian cells against a known carcninogen. Carbon tetrachloride (CCl_4) is widely used for experimental induction of acute liver damage leading to carcinogenesis. The principle causes of hepatotoxicity are induced hepatic damage in lipid peroxidation, decreased activities of antioxidant enzymes and generation of free radicals. Swiss Albino mice (Mus musculus) were exposed to sub-lethal doses of CCl_4 and hepatotoxic effects were observed against control groups by standard anti-oxidant biochemical and cytological parameters. The highest toxic dose was supplemented with IL-21 (i.p.) to check for any anti-mutagenic activities. The hepatoprotective effects demonstrate efficacy of IL-21 and its future use against liver carcinogenesis.

Keywords: Carbon tetrachloride, IL-21, Swiss Albino mice, hepatotoxicity

1. INTRODUCTION

Carbon tetrachloride (CCl_4) has been used extensively to study hepatotoxicity in animal models by initiating lipid peroxidation, thereby causing injuries to kidney, heart, testis and brain [1-3], in addition to liver pathogenesis [4]. Liver is particularly susceptible to oxidative stress due to the direct release of CCl_4 metabolites and cytokines, which propagate inflammatory response [5-7]. CCl_4 exposure induces an increase in lipoperoxide and free peroxide radical concentrations that are highly reactive and cause injury or necrosis [8, 9].

Animals administered carbon tetrachloride (CCl_4) develops a reversible acute centrilobular liver necrosis. The hepatotoxicity is thought to involve two phases. First, CCl_4 metabolization by cytochrome P450 in the hepatocytes produces the highly reactive trichloromethyl radical, which leads to lipid peroxidation and membrane damage.

The second step is an inflammatory response in which Kupffer cells play an important role [10]. Kupffer cells are activated by free radicals and secrete cytokines that attract and activate neutrophils [11-13]. Neutrophils themselves release reactive oxygen species, thereby enhancing the liver injury. Several cytokines were described to be expressed in human liver diseases and experimental liver injury. Among them, tumor necrosis factor-alpha (TNF-α) has emerged as a major endogenous mediator of hepatotoxicity in several experimental models of liver injury [14-16], through direct cytotoxicity [17] and the triggering of an inflammatory cascade.

Interleukin (IL)-21, the most recently described member of the common γ-chain cytokine family, is found to be a potent immune-regulatory cytokine [18]. Recent studies suggest that IL-21 can be used as an immune-modulatory compound [19,20] and its use in the clinical setting is under way[21].IL-21 is produced by activated CD4+ T cells and shares significant sequence homology to IL-2, IL-4, and IL-15[22]. IL-21 has potent effects on all classes of lymphocytes (B, T, and NK cells) [23].

IL-21 costimulates lymphocyte proliferation, modulates gene expression, and displays attributes of both Th1 and Th2 cytokines in vitro. Analysis of IL-21R-deficient mice indicates a role for this cytokine in regulating Ab production and humoral immunity, and a variety of tumor challenge models using gene transfer methodologies indicate that IL-21 can influence both innate and cell-mediated immunity [24].

IL-21 has potent anti-tumor effects due to its ability to expand the pool of cytotoxic CD8+ T cells, NK cells and NKT cells. In view of its antitumor activity documented in pre-clinical studies, IL-21-based therapy has been proposed in the management of malignant neoplasias. In Phase I and Phase IIa clinical trials, IL-21 was well tolerated and showed anti-tumor activity in patients with renal cell carcinoma and metastatic melanoma [25, 26].

However, before generally considering IL-21 as an anti-tumor cytokine, it should be taken into consideration that the majority of preclinical studies investigating the role of IL-21 in tumor development have been conducted on implanted tumor models. It remains unclear whether the anti-

tumor activity of IL-21 can be generalized to spontaneously arising tumors, including those boosted by chronic inflammatory processes [27].

The present study investigated the role of IL-21 in modulating the production of ROS, and the hepatotoxicity and the biochemical and cytological response of hepatocytes after inducing acute CCl_4 liver injury in mice.

2. MATERIALS AND METHODS

CCl_4 and IL-21 treatment groups. Healthy male Swiss Albino mice (*Mus musculus* Linn.), about 3 to 4 months old, and weighing between 20 and 25 gm were procured from M/s Scientific Concern, Kolkata, India, and reared in animal cages. Four mice were used for each treatment group. One group was administered CCl_4 intraperitoneally with two doses @300µl/kg body wt and @600µl/kg body wt.

Two separate groups of mice with same arsenic treatment were co-treated with IL-21 (i.p.) @300µl/kg body wt. All the mice were sacrificed after 14 days of exposure.

Control groups. This group of mice was injected with distilled water @ 1ml/100gm body weight. This group served as the negative control as arsenic was dissolved in distilled water.

Another group of mice were injected only with IL-21 @300µl/kg body wt. to check for any toxicity caused by IL-21 itself.

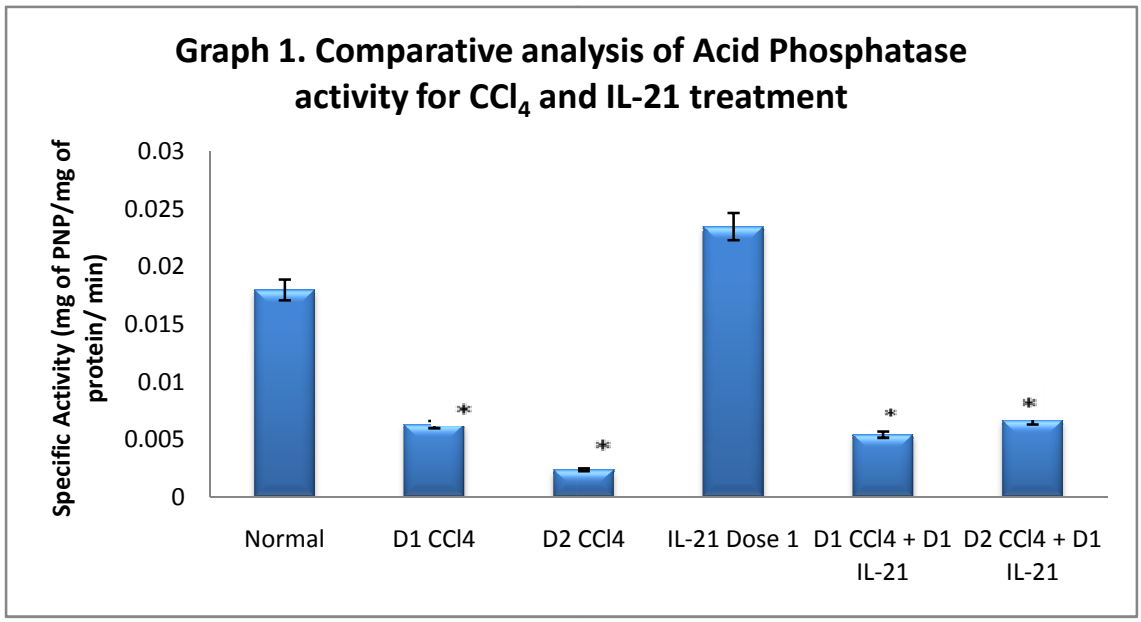
Parameters studied. Specific enzyme activity of Acid phosphatase, Alkaline phosphatase and Glutathione were studied.

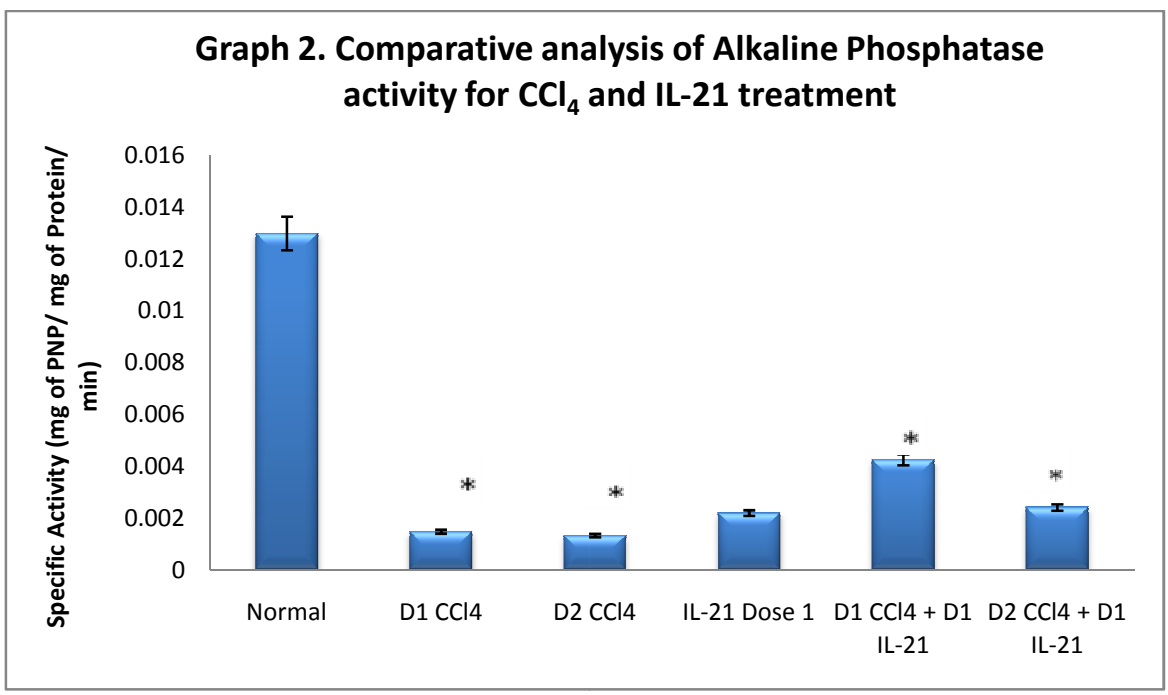
Assay protocols. For estimation of specific activity of phosphatase enzymes, the method of Bergmeyer and Brent [28] was followed with some minor modifications. The specific activity was expressed as mg of PNP produced per mg of protein per minute.

The reduced Glutathione level (GSH) was determined by the method Ellman *et al* [29]. Enzyme activity was expressed as microgram per unit tissue.

Statistical analysis. All the Mean ± S.E. treatment values were checked for statistical significance by Student's t-test compared with the control values. P value of 0.05 was considered as statistically significant.

3. RESULT





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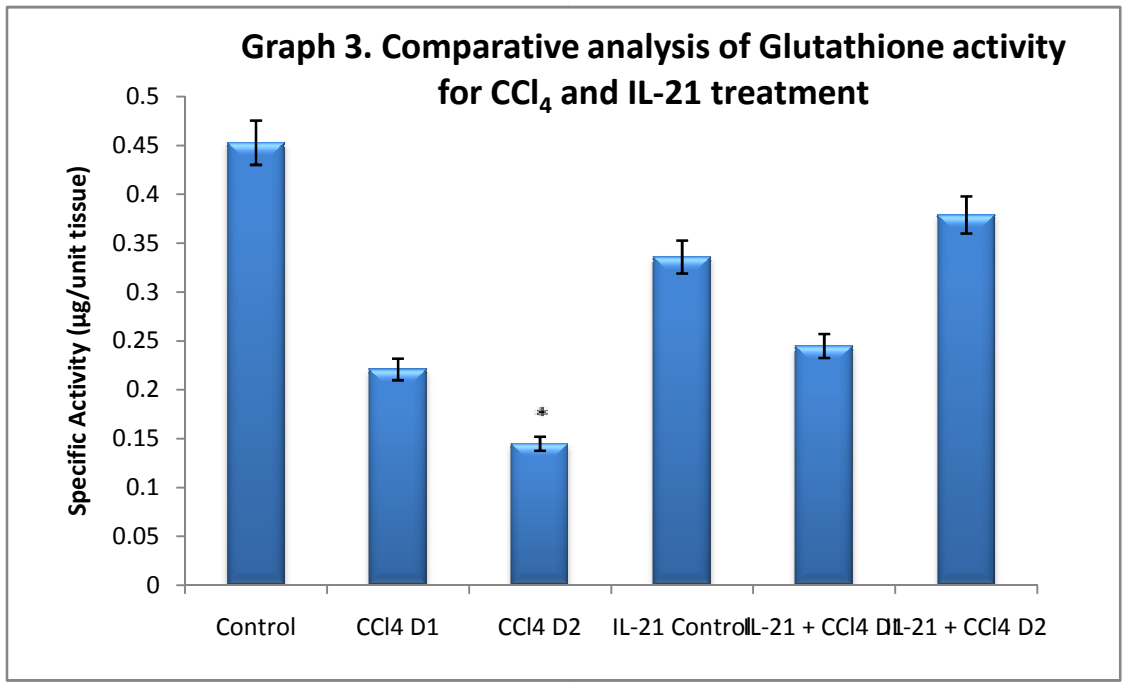

The biochemical activities in the liver were disrupted very much by CCl_4 treatment, which is evident from lowering of activities of acid and alkaline phosphatase as well as depletion of glutathione, which were significantly different from the control values. The enzyme activities became lower with increase in CCl_4 dose. Co-treatment with IL-21 resulted in regaining of activity to a considerable extent. The enzyme activity remained constant even after increase in dose of CCl_4, with the exception of alkaline phosphatase. Acid phosphatase and alkaline phospahatse activity did not show any significant recovery but glutathione activity was restored to a significant level by IL-21 treatment. The vehicle control and beta-carotene control groups of mice showed no significant toxicity.

4. DISCUSSION

The fields of immunotherapy and chemoprevention show considerable effective approaches against oxidative stress and are the focus of research these days [30]. Various studies have shown that several mutagens and carcinogens cause generation of oxygen-free radicals, which play a major role in the emergence of cancer and other health disturbances [31, 32]. The present study revealed that CCl_4-induction in rats remarkably decreased the level of Acid and alkaline phosphatase, GSH, SOD and Mitotic index. CCl_4 causes acute hepatocyte injuries, altered membrane integrity and as a result enzymes in hepatocytes leak out [33]. However, after treatment with IL-21, the pathological

decreases in enzyme activity and mitotic index were restored. These results indicate that IL-21 has the ability to protect against CCl_4-induced hepatocyte injury, which is in agreement with a previous study [34] that reported the protective consequence of another cytokine, IL-10 against CCl_4-induced liver injury. These conditions very often lead to liver carcinogenesis.

Glutathione provides a first line of defense and scavenges free radical oxygen species (ROS). The decreased concentration of GSH in liver may be due to NADPH reduction or GSH utilization in the exclusion of peroxides [35]. GSH-dependent enzymes offer a second line of protection as they primarily detoxify noxious byproducts generated by ROS and help to avert dissemination of free radicals [36].

These results may be explained on the basis that CCl_4 acts as a tumor promoter through increasing the intracellular concentration of ROS necrosis/regeneration and cell proliferation and/or may be due to mutation of p53. Considering its potential for immune activation, it is not surprising that IL-21 has anti-tumor effects. These have been observed in animal models including melanoma, sarcoma, and bladder and renal cell carcinoma. IL-21 can induce antitumor responses not only by activating T and NK cells but also by facilitating tumor-specific antibody production and enhancing antibody-dependent cellular cytotoxicity. In this study, the anti-inflammatory and anti-oxidant properties were responsible for hepatoprotective effects against CCl_4-induced liver stress.

5. CONCLUSION

These results demonstrate that administration of IL-21 may be useful in the treatment and prevention of hepatic stress.

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