Enhanced therapeutic efficacy and amelioration of cisplatin-induced nephrotoxicity by quercetin in 1,2-dimethyl hydrazine-induced colon cancer in rats

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ABSTRACT

Objective: The aim of quercetin treatment in combination of cisplatin (CP)-induced nephrotoxicity as well on 1,2-dimethyl hydrazine (DMH)-induced colon cancer. Materials and Methods: In this study, animals are exposed to DMH hydrochloride to induce colon cancer. In these groups, nephrotoxicity was assessed with the help of blood urea nitrogen, urea, and creatinine. The antitumor activity of quercetin and CP assessed with the help of number of aberrant crypts and foci formation. Tumor size in different treatment group determined with the help of vernier caliper.

Results: CP is one of the most widely used antineoplastic drugs against colon cancer, but it has a major dose-limiting drawbacks of causing nephrotoxicity. Therefore, there is a need for a novel therapeutic regimen which will reduce the nephrotoxicity and enhance the anticancer activity of CP. The protective effect of quercetin on CP-induced nephrotoxicity is well-known. Moreover, quercetin is proven to have antitumor activity in colon cancer. Keeping all the facts in view, this study was conceived to evaluate the role of quercetin on therapeutic efficacy and nephrotoxicity of CP in DMH-induced colon cancer in male Sprague–Dawley rats. Treatment of quercetin with CP (7.5 mg/kg) prevents the CP-induced nephrotoxicity along with enhance the anticancer activity confirmed by the reduction of aberrant crypt foci number. Treatment of CP and quercetin alone leads to significant increase in the anticancer activity as compared to control colon tumor rats.

Conclusion: In DMH-induced colon cancer model, combination of quercetin and CP ameliorates CP-induced nephrotoxicity as well as enhanced antitumor activity.

KEY WORDS: 1,2-dimethyl hydrazine, aberrant crypt foci, cisplatin, colon cancer and quercetin, nephrotoxicity

Introduction

Colon cancer is the second most common cancer in Western societies and is the third most common cause of cancer deaths in the United States.¹ It is estimated that half of the Western population can expect to develop at least one colorectal tumor by the age of 70.² Colorectal cancer (CRC) generally arises from benign adenomas, which progress into malignant adenocarcinomas. CRC develops through complex mechanism which includes genetic mutations and various environmental factor dysplasia of epithelial cells progressing in neoplasia.³

Chemotherapy for colon cancer is restricted to only a few drugs among which cisplatin (CP) is the preferably used.⁴ The antitumor action of CP is by its property of DNA intercalation

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and cell growth prevention, thus arrest proliferation of cancer cells. It is also a drug of choice in the various cancers such as testicular cancer, lung cancer, endometrial cancer, head and neck cancer; and ovarian cancer.

Despite CP being the most potent drug in cancer chemotherapy, its use in clinical practice is often limited because of dose-related nephrotoxicity. The high efficacy of CP is compromised by its ability to cause dose-limiting toxicities such as bone marrow toxicity and nephrotoxicity. The nephrotoxic potential of CP has been ascribed to its accumulation in the renal tubular cells generating ROS and activation of certain inflammatory mediators such as cytokines. These toxicities of CP chemotherapy along with the development of resistance provoke the need of its combination with a drug that can reduce its toxicity, at the same time keeping intact or increasing its efficacy.

Beneficial effects of quercetin have been reported in various drug-induced nephrotoxicity models. The mechanism underlying protection by quercetin against nephrotoxicity include the ROS inhibition, scavenging of radicals. Moreover, quercetin has been reported anticancer activity against various cancers such as colon cancer cells (SW-480), human colon cancer cells (LoVo), and human breast cancer cells (MCF-7). Quercetin supplements improved the efficacy of chemotherapy-induced mitochondrial apoptosis in head and neck cancer reducing side effects of chemotherapy. Quercetin and CP show synergism in chemotherapy by increasing sensitization of cytotoxic drug. Hence, it is reasonable to conclude that quercetin combination with CP provides a novel and more effective chemotherapy against CRC. Hence, this study was designed with an objective to elucidate the role of quercetin on nephrotoxicity and chemotherapeutic activity of CP in 1,2-dimethyl hydrazine (DMH)-induced colon cancer model in Sprague-Dawley (SD) rats.

Materials and Methods

Reagents and Solutions

Cis-diaminoplatinum dichloride (CP) was purchased from QiLu Pharma Co., China. Quercetin was purchased from the National Institute for Drugs and Bioproducts Quality Control (Beijing, China). CP solution was made freshly in saline (0.9% NaCl). DMH solution neutralized before injecting to animals. Quercetin solution was made in ethanol.

Animal Treatment and Experimental Protocol

All the experiments were approved by the Animal Ethics Committee (Protocol No. CJUH 146/13) and complied with the NIH guidelines on handling of experimental animals. Experiments were performed on male SD rats in the weight range of 170–190 g which were procured from the central animal facility of the institute kept in controlled environmental conditions with room temperature 22 ± 2°C and 12 h light/dark cycles. The animals are classified into four groups. Group 1: Disease control DMH-induced colon cancer; Group 2: Disease control DMH-induced colon cancer treated with quercetin; Group 3: Disease control DMH-induced colon cancer treated with CP; and Group 4: Disease control DMH-induced colon cancer treated with quercetin and CP. Colon cancer was induced by five subsequent subcutaneous injections of DMH at subcutaneous injection of 20 mg/kg/week for 10 weeks. After the development of colon cancer, rats were divided at random into different groups, eight animals in each group. A single dose of quercetin (50 mg/kg) daily was administered just before a week the DMH exposure. Alkaline phosphatase is used as marker of tumorigenesis by which at 15th week after DMH exposure observed 25% increase in alkaline phosphatase. In group, three consecutive doses of CP (7.5 mg/kg) were given at 12th, 16th, and 20th week as residual effect of CP is minimized. Drugs were administered by intraperitoneal route. Tumor size evaluated at termination on the 20th week.

Estimation of Plasma Blood Urea Nitrogen, Albumin, and Creatinine

Blood samples were collected from the retro-orbital plexus of rats under light ether anesthesia and plasma was separated. The plasma was used for the estimation of blood urea nitrogen (BUN), creatinine, and albumin at the 20th week of CP treatment.

Identification of Aberrant Crypt Foci

For aberrant crypt foci (ACF) assessment, distal segments from all the colons were examined for the number of ACF. The colon was opened longitudinally, rinsed in ice-cold physiological saline, placed on flat mucosal side up between wet filter papers, and fixed in 10% normal saline for 24 h. The colon was then stained with 0.2% methylene blue. After staining, the entire colonic mucosa was observed using a light microscope. The frequency (number of lesions/cm²), multiplicity (number of crypts/lesion), and size (average of the long and short diameters) were measured on each section.

Measurement of Tumor Size

Tumor size was measured at the termination of the study. Tumors were measured using vernier calipers, and tumor size was calculated as (length + width) × 0.5². Tumor size of disease control expressed in 100%.

Statistical Analysis

Experimental values are expressed as mean ± standard error of mean. Comparison of mean values between various groups was performed by one-way analysis of variance followed by multiple comparisons by Tukey test. P <0.05 is considered to be statistically significant.

Results

Changes in Blood Urea Nitrogen, Creatinine, and Albumin in Plasma After CP Administration Colon Tumor Rats

The CP-treated rats developed colon cancer developed polyuric acute renal failure assessed by measuring the level of biochemical parameters such as BUN, albumin, and creatinine [Table 1]. The BUN and creatinine level in CP-treated rats were increased significantly as compared to control untreated rats. Similarly, the plasma albumin level decreased significantly as compared to control untreated rats. Administration of quercetin with CP further decreases the nephrotoxicity as assessed by a significant decrease in BUN, plasma creatinine and decrease plasma albumin levels. The BUN and plasma creatinine level in quercetin -treated rats without significant changes as compared to control animals.

Aberrant Crypt Foci Analysis

AGF, a colon carcinoma precursor in human and rats, are selected as one of the feasible tools and as a sensitive, reliable, and rapidly appearing biomarker. AGF incidence, total AGF number of AC/AGF (crypt multiplicity), and percentage
inhibition of ACF in experimental groups, reduction in total ACF number of AC, AC/ACF were observed for apparent abnormality. CP-treated rats showed a significant decrease in the number of aberrant crypts as compared to control; similarly, a significant decrease in CP-treated and quercetin-treated rats have been observed [Figure 1]. The decrease in the number of AC was more significant in pretreated quercetin with CP as compared to CP and quercetin alone. There was also a significant decrease in the number of ACF in CP-treated and quercetin-treated as compared to control [Table 2].

**Tumor Size Inhibition**

In the quercetin-treated group decreased tumor size as compared to disease control. CP-treated colon tumors decreased tumor size has been observed. Administration of quercetin before exposing DMH with CP treatment 12th, 16th, and 20th week resulted in remarkable improvement in the antineoplastic effect of colon tumor [Figure 2].

**Discussion**

The most effective chemotherapeutic agent targeted against colon cancer, CP, acts by its propensity to cause DNA intercalation and thus causing cell cycle arrest. However, the chemotherapy with CP has a drawback of causing dose-related toxicities such as nephrotoxicity and bone marrow toxicity limiting its use to a large extent. Quercetin ameliorates CP-induced nephrotoxicity through reducing oxidative stress in adult Wistar rats. Moreover, quercetin has been reported to be an anticancer agent in several animal models. Keeping these facts in mind, we studied the effect of quercetin on CP therapeutic efficacy and nephrotoxicity in colon cancer rat model. The objective of the current study was to reduce the nephrotoxicity and to enhance the efficacy of the CP against colon cancer using the combination of CP with quercetin.

The mechanism for prevention of nephrotoxic damage by quercetin may be due to its antioxidant activity. In this study, treatment of quercetin prevented the nephrotoxic damage of CP in DMH-induced colon cancer model in SD male rats. Flavonoids induce proapoptotic pathway and cell cycle arrest. Quercetin has also been reported as a chemoprotective agent and antitumor promoter against various cancers. Antitumor activity of quercetin has been reported against various cancer cell lines and in vivo cancer models of prostate cancer and skin tumors. Quercetin downregulates pro-inflammatory mediator by which suppresses early preneoplastic lesions with reduce proliferation and increase apoptosis in colon carcinogenesis. Endocannabinoids receptor (CB1-R) expression increased by quercetin treatment resulted in antineoplastic effect of human colon adenocarcinoma cells. Quercetin decreasing polyamine biosynthesis and inducing apoptosis decrease growth of DLD-1 cells. Combinations of quercetin and polyamine inhibitors would enhance their properties, in the treatment of colon cancer. Quercetin changes the resistance of tumor cells, and it could distribute death receptors again at the cell surface, accelerates death signaling through caspase activation. Curcumin potentiates antitumor activity of celecoxib possibly inhibition of cyclooxygenase-2 (COX-2) in different colorectal cancer cells. Quercetin synergistic action on FU-induced apoptosis through p53 modulation in CRC cells. Synergistic effect of quercetin and CP combination in nasopharyngeal carcinoma cells and human head and neck cancer while combinations of platinum analogs with quercetin and thymoquinone showed synergism in human ovarian cancer. Curcumin and 5-flourouracil exhibited synergism at higher doses with changed expression of COX-2 HT-29 cells human colon cancer cell line. Moreover, it also potentiates the antitumor activity of chemotherapeutic agents.

In accordance with these reports, our results also indicate that quercetin enhances the activity of CP against colon cancer. ACF is a colon carcinoma precursor in human and rats, which is sensitive, reliable, and rapidly appearing biomarker and can be used to detect and compare possible effects of a chemoprotective agent in rat colon carcinogenesis. Here, we provide the evidence that quercetin potentiates the anticancer effect of CP against colon cancer.
Table 2:

Effect of quercetin and cisplatin on aberrant crypt in colon tumored rats

| Treatment groups                      | Number of crypt foci | Number of aberrant crypts |
|---------------------------------------|-----------------------|---------------------------|
| Disease control n=6 (0.9% saline)     | 53.17±0.60            | 192.5±2.69                |
| Quercetin (50 mg/kg i.p. daily)       | 44±0.58***            | 170±2.66***               |
| CP-treated (7.5 mg/kg) at 12th, 16th and 20th week | 38.5±0.76***          | 152.83±2.89***            |
| CP (7.5 mg/kg) at 12th, 16th and 20th week + quercetin (50 mg/kg i.p. daily) | 34.17±1.19***         | 125.5±2.36***             |

All values are expressed as 38.5 meansSEM (n=6), ***P<0.001, **P<0.01, *P<0.05, Versus disease control group, Versus CP-treated group, CP=Cisplatin, SEM=Standard error of mean, BUN=Blood urea nitrogen, i.p.=Intraperitoneal

Figure 2: Percentage inhibition of colon tumor by treatment with quercetin, cisplatin and quercetin pretreated and cisplatin

As discussed, quercetin is a TLR4 agonist that can induce autophagy, which may contribute to its antitumor effects. Furthermore, quercetin has been shown to activate AMPK and inhibit mTOR, both of which are important in cancer cell metabolism and survival. These mechanisms may contribute to the synergistic effects observed in the current study.

Conclusion

This study demonstrated the potential of quercetin as a therapeutic agent for colon cancer, both alone and in combination with cisplatin. Further studies are needed to better understand the mechanisms of action and to translate these findings into clinical practice.

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Nil.

Conflicts of Interest

There are no conflicts of interest.

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