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ScienceDirect
Toxicology and Applied Pharmacology 222 (2007) 152–158
Toxicology and Applied Pharmacology
www.elsevier.com/locate/ytaap

The influence of active hexose correlated compound (AHCC) on cisplatin-evoked chemotherapeutic and side effects in tumor-bearing mice

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Received 14 December 2006; revised 11 March 2007; accepted 17 March 2007
Available online 20 April 2007

Abstract

Cisplatin (*cis*-diaminedichloroplatinum (II) or CDDP) (a widely used platinum-containing anticancer drug) is nephrotoxic and has a low percentage of tolerance in patients during chemotherapy. The active hexose correlated compound (AHCC) is an extract of Basidiomycotina marketed as a supplement for cancer patients due to its nutrients and fibre content and its ability to strengthen and optimize the capacity of the immune system. The possibility that AHCC could reduce the side effects of cisplatin was assessed in the tumor-bearing BALB/cA mice on the basis of the ability to ameliorate the cisplatin-induced body weight loss, anorexia, nephrotoxicity and hematopoietic toxicity. Although cisplatin (8 mg/kg body weight) reduced the size and weight of the solid tumors, supplementation with AHCC significantly enhanced cisplatin-induced antitumor effect in both the size ($p<0.05$) and weight ($p<0.05$). Food intake in the cisplatin-treated mice were decreased following commencement of treatment and this remained low compared with the cisplatin-untreated group (control) throughout the experiment period. Supplementation with AHCC increased the food intake in the cisplatin-treated mice. The blood urea nitrogen and serum creatinine concentrations, and the ratio of blood urea nitrogen to serum creatinine were significantly increased in the cisplatin alone treated group compared to the control group. Their increased levels were mitigated by supplementation with AHCC (100 mg/kg body weight) in the cisplatin-treated group. AHCC was also able to modulate the suppression of bone marrow due to cisplatin and the improvement was statistically significant. The histopathological examination of the kidney revealed the presence of cisplatin-induced damage and this was modulated by AHCC treatment. The potential for AHCC to ameliorate the cisplatin-evoked toxicity as well as the chemotherapeutic effect could have beneficial economic implications for patients undergoing chemotherapy with cisplatin.

Keywords: Active hexose correlated compounds (AHCC); Cisplatin; *Cis*-diaminedichloroplatinum (II); Anticancer drugs; Cancer chemotherapy-side effects; Nephrotoxicity; Chemoprevention strategies; Kidney failure; Chronic inflammation; Redox biochemistry; Economics of cancer therapy; Complementary medicine

Introduction

Cisplatin (*cis*-diaminedichloroplatinum (II) or CDDP, Fig. 1A), a platinum-containing anticancer drug, is one of the most commonly used cytotoxic agents in the treatment of a variety of solid malignant tumors, for example in the head and neck, lungs, ovaries, bladder and testicles (Ali and Al Moundhri, 2006; Jordan and Carmo-Fonseca, 2000; van den Berg et al., 2006; Pectasides et al., 2005, 2007; Kollmannsberger et al., 2001; Lebwohl and Canetta, 1998; Chester et al., 2004; Rybak and Whitworth, 2005; Benedetti Panici et al., 1993; Taguchi et al., 2005). Although treatment with this drug is often effective, serious side effects such as nausea, nephrotoxicity, neurotoxicity, ototoxicity, poor Karnofsky performance status and co-morbidities occur often. Focal encephalopathy and neurological deficits of higher function (including cortical blindness and aphasia with or without seizure and confusion) have been documented (Higa et al., 1995). These side effects interfere with the treatment and often force a reduction of the dosage, frequency and duration of the cisplatin therapy necessitating the search for alternative therapy with less toxicity. The cytotoxicity of cisplatin has been primarily ascribed to its interaction with nucleophilic N7-sites of purine bases in DNA to form both DNA–

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doi:10.1016/j.taap.2007.03.031

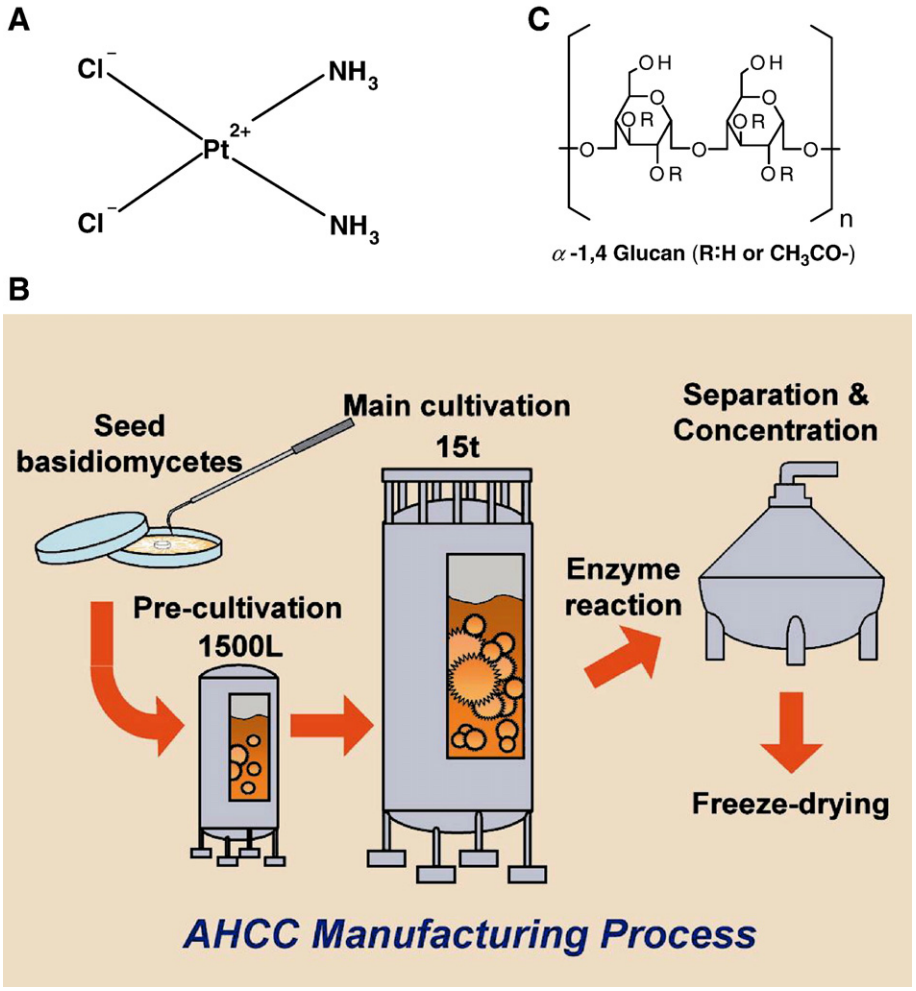

Fig. 1. (A) Structure of cisplatin. The mechanism of action of cisplatin involves entering the cell, where Cl^- dissociates from a reactive complex that reacts with water and then the activated complex interacts with DNA. (B) The manufacturing process of AHCC. The Basidoimysete is pre-cultured in the 1500-L tank following the seed culture on dishes, and then is cultured in the main tank (15 tons) for 45 day. After the fermentation, AHCC is produced in a procedure involving enzymatic reaction, sterilization, concentration and freeze-drying. (C) Structural features of AHCC. The active ingredients of AHCC are partially acetylated α-glucan, and β-glucan. Acetylated α-1,4 glucan, which is a main ingredient, has a low molecular weight of 5000 Da.

protein and DNA–DNA inter-strand and intrastrand cross-links. Binding of the drug causes physical distortions in DNA that interrupt DNA repair and stress response machinery (Wilson et al., 2006; Eastman and Barry, 1987). The intrastrand *cis*-Pt$(NH_3)_2$–d(GpG) and *cis*-Pt$(NH_3)_2$–d (ApG) crosslinks represent approximately 65% and 25%, respectively, of the total lesions present in DNA and the interstrand crosslinks although less common, may play a role in the cytotoxicity of the cisplatin (Wilson et al., 2006; Eastman and Barry, 1987; Jones et al., 1991; Fichtinger-Schepman et al., 1985; Plooy et al., 1985; Siddik, 2003). Cisplatin also damages cell mitochondria, arrests cell cycle in the G2 phase, inhibits ATPase activity, alters the cellular transport system, and eventually causes apoptosis, inflammation, necrosis and death in cells (Ali and Al Moundhri, 2006; Taguchi et al., 2005).

The CDDP-based chemotherapy may work well with younger patients with aggressive and extensive disease. However for the elderly patients with poor Karnofsky Performance Status (a Karnofsky PS of ≤ 70 was associated with a worse prognosis), use of the CDDP analogues such as the carboplatin (CBDCA)-based treatment is advocated (Ali and Al Moundhri, 2006; Eastman and Barry, 1987; Marcuello et al., 1990; Stinchcombe et al., 2006; Paesmans et al., 1995). There has been continued attempts to develop other platinum drugs in an attempt to improve on cisplatin. Oxaliplatin (synthesised by substituting the amine radicals of cisplatin (Chaney et al., 2004)) is a used as a treatment for colon cancer in combination with 5-fluorouracil. Oxaliplatin itself is a better tolerated chemotherapeutic than cisplatin but while both are known to cause neurotoxicity, the toxicity of oxaliplatin is more rapidly reversible. Oxaliplatin has been widely regarded as potentially useful for the treatment of cisplatin-resistant cancer (Culy et al., 2000; Grothey, 2005). Interestingly, patients with inoperable or recurrent loco-regional disease without distant metastases tend to have favourable prognosis compared to patients with visceral metastases. These patients respond favourably to CDDP-

based chemotherapy with 5-year survival rates ranging from 10% to 30%. However, most of these patients relapse and die from transitional cell carcinoma (a chemosensitive tumor that accounts for more than 90% of the bladder cancers) (Ali and Al Moundhri, 2006; Pectasides et al., 2006; Chester et al., 2004; Rybak and Whitworth, 2005; Stinchcombe et al., 2006). The context of cisplatin's synergistic cytotoxic action with radiation and other chemotherapeutic agents is widely reported but the major limitation in the clinical applications of cisplatin has been the development of cisplatin resistance by tumors (Ali and Al Moundhri, 2006; Boulikas and Vougiouka, 2003; Ozben, 2006; Ohno et al., 2006). Given that patients who respond completely to the CDDP-based chemotherapy are usually long-term survivors, maintaining the quality of life of these patients is a unique bridge that complementary medicine based on food supplements aims to provide.

The active hexose correlated compound (AHCC) is a mixture of polysaccharides, amino acids, lipids and minerals derived from cultured mycelia of a Basidiomycete mushroom. The LD_{50} was 8490 mg/kg in male rats and 9849 mg/kg in female rats. The minimal lethal dose of intraperitoneally administered AHCC was lower in the male rats than in the female rats, at 7430 mg/kg and 8340 mg/kg, respectively. AHCC has been implicated to modulate immune functions and plays a protective role against infection. AHCC treatment has been shown to significantly delay tumor development after inoculation of either melanoma cell line B16F0 or lymphoma cell line EL4 to C57BL/6 mice. AHCC enhanced both antigen (Ag)-specific activation and proliferation of CD4(+) and CD8(+) T cells and increased the number of tumor Ag-specific CD8(+) T cells, and more importantly, increased the frequency of tumor Ag-specific IFN-gamma producing CD8(+) T cells (Gao et al., 2005). AHCC treatment tended to increase the cell number of NK and gammadelta T cells, indicating the role of AHCC in activating these innate-like lymphocytes (Gao et al., 2005). The potential that AHCC can act as a biological response modifier (hence the concept of immunoceuticals) has been reported by Cowawintaweewat et al. (2006). In this study, the possibility that AHCC could reduce the side effects of CDDP was assessed by using tumor-bearing mice to investigate the effect on CDDP-induced body weight loss, anorexia, nephrotoxicity and hematopoietic toxicity.

Materials and methods

Source of AHCC. AHCC is extracted from a myceloid of a Basidiomycete mushroom, which is cultured in a large tank in a process comparable to the GMP standards of manufacturing in quality control for medical products, ISO 9001 and HACCP certification. The Basidiomycete forms colonies during their pre-cultivation phase, and then is cultured further in the main tank (15 tons at largest) for 45 days. AHCC is obtained after undergoing cultivation, enzymatic reaction, sterilization, concentration, and freeze-drying (a patented process) (Fig. 1B). AHCC's active ingredients are partially acetylated α-glucan, and β-glucan. The acetylated α-glucan (Fig. 1C) is an oligosaccharide obtained during the basidiomycete's cultivation process and has a low molecular weight of about 5000 Da, making it easily absorbable in the gut.

Animals and treatment. Female BALB/cA SPF mice were purchased from CLEA Japan Inc, and used at 6 weeks of age. Mice were randomly divided into three groups (control, CDDP, and CDDP+AHCC), and each group consisted of 17 mice. Colon-26 tumor cells (kindly provided from Cell Resource Center for Biomedical Research, Tohoku University, Japan) were inoculated into subcutaneous dorsal right region of mice with 5×10^5 cells/mouse 3 days before an initial injection of cisplatin. Cisplatin was a commercially available drug as Randa 10 mg (Lot. 654820 and 754830, 0.5 mg/ml) (NIPPON KAYAKU CO., LTD, Japan) and was kept at room temperature until usage. Cisplatin was intraperitoneally administered into mice at 8 mg/kg body weight (BW) on day 0, 6, 13 and 20 (total 4 injections). AHCC (Lot. 44-0722) was prepared at 10 mg/ml in distilled water each time, and the solution was immediately given to mice at 0.01 ml/g BW (100 mg/kg BW) by gavage everyday from day 0 to day 28. The scheme of the administration procedure is summarised in Fig. 2. Matsushita et al. (1998) have shown that AHCC at 100 mg/kg BW (*p.o.*) significantly reduces the metastasis of rat mammary adenocarcinoma by combination therapy with UFT (tegafur and uracil in a 4:1 molar concentration) in tumor-bearing rats. Hence the working dose of AHCC at 100 mg/kg BW was chosen.

Evaluation of biochemical parameters. The following parameters were assessed: tumor size and weight, body weight, food intake, kidney function (blood urea nitrogen and creatine), and bone marrow suppression. Blood urea nitrogen (BUN) and serum creatinine were measured using Urea Nitrogen B-test WAKO and Creatinine-test WAKO assay kits (Wako Pure Chemical Industries Limited, Japan), respectively. Bone marrow cells collected from mice with or without cisplatin injection were suspended in 0.83% NH_4Cl solution to haemolyze red blood cells, and incubated at 37°C for 10 min. After centrifuge, the bone marrow cells were prepared at a concentration of 1×10^7 cells/ml in DMEM supplemented with 10% FBS. Then, a 100-μl aliquot of the suspension was cultured in a 96-well plate for 3 days. Bone marrow suppression was estimated from cell viability (% of control group) in the 3-day culture using the MTT assay. On day 21, five mice from each group were dissected to measure bone marrow suppression. Then, at the end of the study, the remaining 12 mice of each group were euthanized to assess other parameters.

Statistical analysis. Data presented mean±S.D. and were analyzed by one-way analysis of variance (ANOVA). Fisher's Protected Least Significant

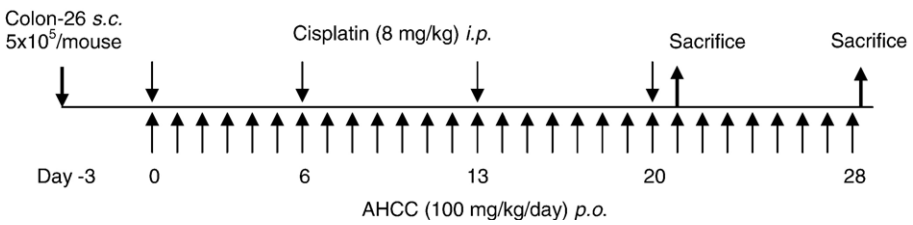

Fig. 2. The experimental schedule used for assessing the efficacy of AHCC following treatment with cisplatin. Colon-26 tumor cells (5×10^5 cells/mouse) were inoculated into subcutaneous dorsal right region of female BALB/cA mice before an initial injection of cisplatin. Cisplatin was intraperitoneally administered into mice at 8 mg/kg body weight on day 0, 6, 13 and 20, and AHCC was daily given by gavage at 100 mg/kg body weight from day 0 to day 28. On day 21 and at the end of the experiment (day 28), 5 mice of each group and all residual mice were euthanized, respectively, to evaluate various parameters such as tumor size and weight.

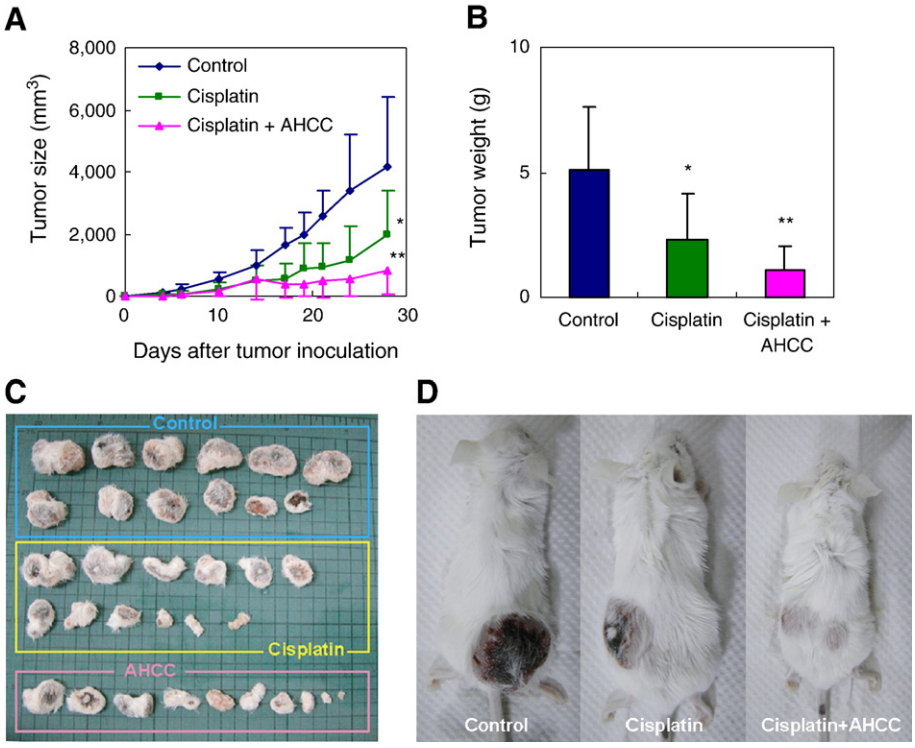

Fig. 3. Anti-tumor effect of cisplatin alone and co-treatment of cisplatin plus AHCC. Colon-26 tumor bearing BALB/cA mice were treated with vehicle (control), cisplatin (8 mg/kg) and cisplatin (8 mg/kg) plus AHCC (100 mg/kg), respectively, as described in Materials and methods. Growth curves of colon-26 tumor cells in BALB/cA mice (A). *$p<0.01$ vs control, **$p<0.01$ vs control and $p<0.05$ vs cisplatin. Anti-tumor effect of AHCC in combination with cisplatin on tumor weight (day 28) (B). The actual weights (g) of control, cisplatin, and cisplatin plus AHCC groups were 5.09±2.51, 2.32±1.81, and 1.06±0.97, respectively. *$p<0.01$ vs control, **$p<0.01$ vs control and $p<0.05$ vs cisplatin. Photographs of colon-26 solid tumor taken from all mice of each group (day 28) (C). Representative external appearance of tumors (day 28) (D).

Difference (PLSD) was used as post hoc test and values of p less than 0.05 were assessed statistically significant.

Results

The effect of AHCC and cisplatin on the growth curves of colon-26 cells in BALB/cA mice and the integrity of solid tumors due to cisplatin treatment are shown in Fig. 3. Although cisplatin reduced the size and weight of the solid tumors, supplementation with AHCC significantly resulted in the further reduction in both the size (Fig. 3A, $p<0.05$) and weight (Fig. 3B, $p<0.05$). Photographs of the same are shown in Figs. 3C and D, respectively.

Food intake in the cisplatin-treated mice were decreased by 55% 4 days after commencement of treatment and this remained low compared with the control group throughout the experiment period (Fig. 4). AHCC supplementation increased the food intake in the cisplatin-treated mice, but this was not statistically significant (Fig. 4). There was however an indication that AHCC improved anorexia as well as body weight loss in normal mice treated with cisplatin (data not shown). The ability of AHCC to ameliorate weight reduction was not assessed in this study, because body weight would be depended on tumor growth.

Kidney function parameters were assessed following the treatment of mice with cisplatin and supplementation with

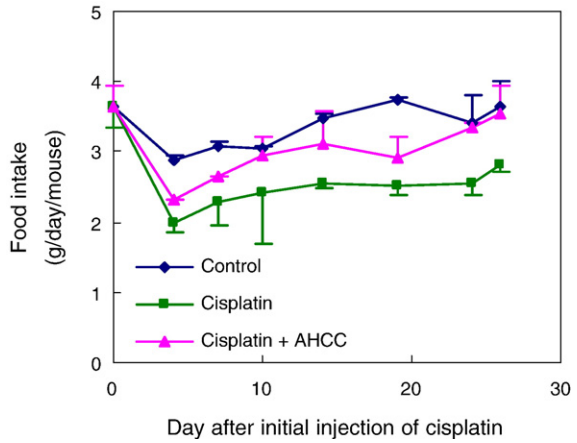

Fig. 4. Effect of AHCC on food intake. All mice were given CE-2 solid diet (CLEA Japan, Inc.). Food intake of each breeding cage was regularly estimated, and calculated as the intake amount per day of a mouse (g/day/mouse).

Table 1
Levels of BUN and serum creatinine, and the ratio of BUN to creatinine on day 28

Group	BUN (mg/dl)	Creatinine (mg/dl)	BUN/creatinine
Control	18.8±3.1	0.86±0.04	21.9±3.9
Cisplatin	26.3±5.1*	0.97±0.05*	27.0±5.1
Cisplatin+AHCC	22.0±2.5	0.87±0.07**	25.4±1.4

Values represent the mean±S.D. *$p<0.01$ vs control, **$p<0.01$ vs cisplatin, ***$p<0.05$ vs control.

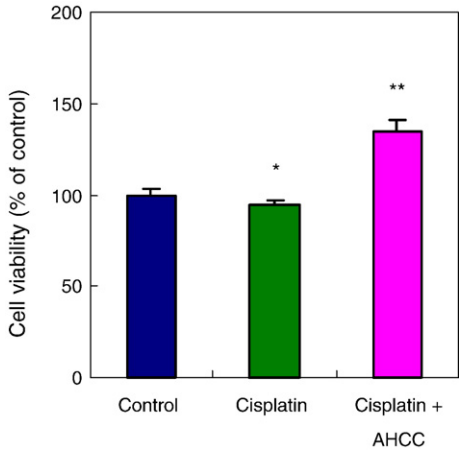

Fig. 5. Ameliorative effect of AHCC against cisplatin-suppressed bone marrow cells. Bone marrow cells were collected from mice with or without cisplatin injection on day 21, and cultured in a 96-well plate for 3 days. Bone marrow suppression was assessed as cell viability using MTT assay. The values (% of control) of bone marrow cells viability in control, cisplatin, and cisplatin plus AHCC groups were 100±2.5, 94.4±6.0, and 135.2±6.0, respectively. *$p<0.01$ vs control, **$p<0.01$ vs control and cisplatin.

AHCC (as scheduled in Fig. 2). The concentrations of BUN and serum creatinine as well as the ratio of BUN to serum creatinine on day 28 were significantly increased in the cisplatin alone treated group compared to the control group (Table 1). AHCC administration reduced the levels of BUN and serum creatinine, and the ratio. In particular, the creatinine level of cisplatin group was significantly alleviated by combination with AHCC. Thus it is suggested that AHCC may to ameliorate the nephrotoxicity of cisplatin.

The effect of AHCC on the cisplatin-induced bone marrow suppression was assessed on day 21. As shown in Fig. 5, AHCC was able to improve the bone marrow repression caused by cisplatin with a significant difference ($p<0.01$). Moreover, since the cell viability of AHCC group was significantly much higher than that of the control group ($p<0.01$), it was suggested that AHCC might recover immune depression induced by tumor cells themselves as well as cisplatin. In normal mice, cisplatin (8 mg/kg) greatly and significantly suppressed the viability (% of control) of bone marrow cells by 40.3±2.5% ($p<0.01$) as compared to no-treatment group (control, 100±7.5%). However, the cisplatin-induced suppression was recovered up to 85.2±8.3% ($p<0.01$) by co-treatment of AHCC (100 mg/kg) with a statistically significant difference (data not shown).

The nephrotoxicity was assessed using right kidneys removed from 12 mice of each group on day 28, and the representative result of each group is shown in Fig. 6. The histopathological examination of the kidney revealed that significant change of renal tissue was observed in cisplatin alone group (Fig. 6B) compared to control group (Fig. 6A) and cisplatin+AHCC group (Fig. 6C). No obvious histopathological change was observed in control and cisplatin+AHCC groups. Cisplatin-induced damage as seen by tubular cell destruction and the failure was improved by co-treatment with AHCC. However, given that the damages caused by cisplatin were not observed in cisplatin plus AHCC group as well as control group, AHCC can be suggested to have the propensity to alleviate the cisplatin-induced nephrotoxicity in mice.

Discussions

It is becoming clear that the nephrotoxicity of cisplatin is due to a complex metabolic pathway that activates the drug to a potent kidney toxin, and the various metabolic responses, cell cycle events and the inflammatory cascade are argued to be important determinants of the degree of renal failure induced by cisplatin (Ali and Al Moundhri, 2006; van den Berg et al., 2006; Taguchi et al., 2005, Higa et al., 1995; Basnakian et al., 2005; Arany et al., 2004). There has been active interest in devising strategies to reduce the side effects of cisplatin therapy, including the use of less intensive treatment, replacement of the nephro- and neurotoxic cisplatin by its less toxic analogue carboplatin (which has different pharmacokinetic and toxicological characteristics compared with cisplatin (Ali and Al Moundhri, 2006; Taguchi et al., 2005) and dietary supplements (Taguchi et al., 2005; Ajith et al., 2007; Soobrattee et al., 2006; Weijl et al., 2004; Kadikoylu et al., 2004; Kondo et al., 2004; Pardini, 2006). Ajith et al. (2007) reported the results of their comparative study on the nephroprotective effects of antioxidant vitamins (250 and 500 mg/kg, p.o.), vitamin C (ascorbic acid) and vitamin E (α-tocopherol), which were evaluated using cisplatin (10 mg/kg BW, i.p.) induced oxidative renal damage in mice, and concluded that the high doses of vitamins are effective to protect oxidative renal damage (Ajith et al., 2007). Consumption of fruits, vegetables and beverages like teas continues to be

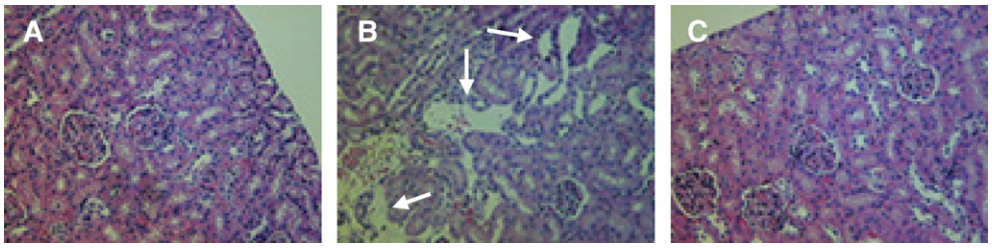

Fig. 6. Histopathological examination of kidney. Right kidney from 12 mice of each group on day 28 was removed and fixed overnight in 10% buffered formalin. The paraffin-embedded sections (4-μm thick) were stained with hematoxylin and eosin (H&E) for histolopathological examination and observed under light microscope at ×160 magnifications. The result shown here is representative cortex from each group. (A) Control, cortex (×160), (B) Cisplatin, cortex (×160), renal tubule cell destruction (marked by arrows, →), (C) Cisplatin+AHCC, cortex (×160).

suggested to have the capacity to reduce the incidence of cancer. The bioactive compounds including phenolics may be responsible for the chemopreventive effects. While the free radical scavenging and antioxidant properties of phenolics are well established, emerging literature reports suggest that their chemopreventive effects may also be ascribed to their ability to modulate components of cell signaling pathways (Soobrattee et al., 2006). Ramesh and Reeves (2005) have suggested that enhanced tumor necrosis factor-α (TNF-α) production may mediate cisplatin nephrotoxicity and that this could involve the activation of p38 mitogen-activated protein kinase. Litterst and Schweitzer (1988) have argued that the renal accumulation of platinum and covalent binding of platinum to renal protein may also play a role in the nephrotoxicity.

Lee et al. (2001) have indicated that treatment of M-1 cells (derived from the outer cortical collecting duct cells of SV40 transgenic mice) with high concentrations of cisplatin (0.5 and 1 mM) for 2 h led to necrotic cell death, whereas a 24-hr treatment with 5–20 μM cisplatin led to apoptosis. The authors further argued that antioxidants protected against cisplatin-induced necrosis but not apoptosis, indicating that reactive oxygen species play a role in mediating necrosis but not apoptosis induced by cisplatin and that the mechanism of cell death induced by cisplatin is concentration-dependent. Experimental studies in animals have shown that a minimum dose of cisplatin (5 mg/kg BW, i.p.) was sufficient to induce nephrotoxicity in rats, which is corresponding to use in clinical practice. In this study, administration of cisplatin at 8 mg/kg BW significantly increased in serum creatinine and BUN concentrations compared to control, which clearly indicates the intrinsic renal acute renal failure. Although cisplatin (8 mg/kg BW) reduced the size and weight of the solid tumors, supplementation with AHCC significantly strengthened cisplatin-induced anticancer effect in both the size ($p<0.05$) and weight ($p<0.05$). It is worth pointing out, although not in direct parallel with this study, that previous studies by Burikhanova et al. (2000) have shown that AHCC can suppress thymic apoptosis induced by dexamethasone. Food intake in the cisplatin treated mice was decreased following the commencement of treatment and this remained low compared with the control group throughout the experiment period. Supplementation with AHCC increased the food intake in the cisplatin-treated mice although this was not statistically significant. However, preliminary but ongoing studies appear to indicate that AHCC improves anorexia as well as body weight loss in normal mice treated with cisplatin. The serum creatinine and BUN concentrations, and the ratio of BUN to creatinine were significantly increased in the cisplatin alone treated group compared to the control group. Their increased levels were mitigated by supplementation with AHCC (100 mg/kg BW) in the cisplatin-treated group and this effect by AHCC on the creatinine level was statistically significant. AHCC was also able to improve the suppression of bone marrow induced by cisplatin. The histopathological examination of the kidney revealed the presence of cisplatin-induced damage and this was modulated by AHCC treatment. Thus AHCC was able to ameliorate the toxicity associated with cisplatin treatment as well as augmenting its antitumor effect. This could have beneficial implication for patients undergoing chemotherapy with this drug. For novel dietary supplements, the real proof of efficacy must come from a demonstration of clinical efficacy on defined therapeutic categories. The economic impact of cancer in health care systems remains one that much attention in the context of complementary medicine needs to be directed. This line of investigation makes a significant contribution to this endeavor.

Acknowledgments

We are deeply grateful to Cell Resource Center for Biomedical Research, Tohoku University (Japan), which kindly provided colon-26 tumor cells. Professor Aruoma is also Adjunct Research Professor at the University of Mauritius.

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