Preventive Effects of AHCC on Carbon Tetrachloride Induced Liver Injury in Mice

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Introduction

In the natural world, it is said that approximately 5,900 genera, 6,400 kinds of mycelia inhabit. Lingzhi {Ganoderma lucidum, reishi), zhuling (Polyporus umbellatus, chorei) are popular Chinese traditional medicine derived from fungi of basidiomycetes family. Those mycelia are identified to contain various physiologically active substances, such as polysaccharide with b-1, 3-glucan structure. It has been reported its activities; anti-tumor activity, accommodation activity on immune system, hypoglycemic activity, etc. Recent advance of culturing techniques has enabled artificial culture of basidiomycetes. Active Hexose Correlated Compounds (AHCC; from Amino Up Chemical Co., Ltd.) is a mixture containing polysaccharide obtained by culturing in a liquid culture tank followed by enzyme reactions and hot water extraction.

AHCC has been observed and reported its bioactivity as Biological Response Modifiers (BRM) or nonspecific immunoreactive activator. Especially in clinical studies, the effects of AHCC have been reported; improvement of adult diseases such as diabetes or hepatic disease, cancer cell atrophy and inhibition of metastases in tumor patients, the survival time prolongation, reducing side effects caused by chemotherapy, etc.
Although there are many clinical studies reported, actual pharmacological mechanism is known only a little. To investigate its pharmacological mechanism as the first step, we prepared an acute carbon tetrachloride hepatitis model in mice which symptom is said to be similar to drug liver injury in human. This is the report of the investigation of the effect of AHCC administered per os (p.o.) on liver function change and drug metabolizing enzymes in liver.

Materials and Methods

Animals. Male ICR mice of 7 weeks old, body weight 34-38 gm. were purchased from Nippon Clea Co., Ltd. The animals were kept free access to food (CE-2; Nippon Clear) and water for one week as pre-breeding period.

Materials. All the material drugs were purchased from Wakopure Industry Co., Ltd. and Sigma Chemical Co. AHCC freeze-dried powder was supplied by AMINO UP CHEMICAL CO., LTD.
Methods

Preparation of food and its administration- AHCC was dissolved in water at 50% concentration. Mice were treated with the solution by oral at the dose of 1g/kg/day for three days. On the forth day, 20% solution of CCl4 diluted with olive oil (Japan Pharmacopoeia) was administered i.p. at the dose of 2ml/kg/day for five days. Those mice were divided into four groups with five mice each: control group with normal mice, injured group treated with CCl4, AHCC treated group, AHCC and CC14 co-administrated group (CCl4 was administered after AHCC treatment).

Blood and organ preparation- On the final day of CCl4 administration, mice were given free access to water not giving any food. 12 hours later, blood sample was collected via carotid. The samples were centrifuged (3,000 r.p.m,, 5 min) and serum was obtained. The liver removed was rinsed with in ice-cold saline solution (0.9%) and weighted. Livers were immediately stored at -80°C.
Serum analysis - Glutamate-pyruvate transaminase (GPT) activity in serum was measured by Rate method

(GPT-UV Test Wako), Albumin was measured by BCG method (Albumin-B Test Wako), Total protein (TP) was measured by Lowry method, Globulin (G) was calculated by TP-A, Triglyceride (TG) was measured by Acetylacetone method (Triglyceride Test Wako)

Hepatic enzyme preparation- Frozen stored livers were homogenated at ice by adding 4 times of 0.154MKCI-0.05M Tris (pH 7.4)-lmM EDTA. This liver homogenize was first centrifuged 1,000r.p.m., 5°C for 5 min, then lO.OOOr.p.m. for 5°C for l5min., finally the supernatant fraction was obtained. Then the supernatant fraction obtained by lO.OOOr.p.m. was centrifuged 38,000r.p.m., 5°C for 60min and supernatant (cytosol) fraction was obtained for GST measurement. Microsome fraction was obtained by adding twice as much as the liver weight of cold 0.154MKCI-0.05M Tris (pH 7.4)-lmM EDTA-20% glycerol solution in the precipitate gained from 38,000r.p.m. The microsome fraction was applied for the measurements of Protein, P450, LPO, UDP-GT, ERDM and AHH.

Lipid peroxidation (LPO) measurement- The measurement was based on Yagi method. 0.05ml of liver microsome fraction was centrifuged 3,000r.p.m., lOmin with 0.5ml of 1/12N H2S04 4.0ml, 10% phosphotungstic acid aqueous solution, and TBA reagent was added in the precipitate for 60min heating in boiling water. Obtained fluosubstance was extracted by n-butanol and measured its fluorescence intensity with spectrophotofluorometer.

The measurement of P450 content- The measurement was based on Omura and Sato method. Two cells of enzyme solution 3ml which is equivalent to liver weight (250mg) were prepared with hydrosulfite sodium OlM in each cell. One of them were treated with CO for 30 seconds, and another was used as the control. The content of P450 was calculated from the highest peak of 450nm.

The measurement of each enzyme activity- Glutathione S-transferase (GST) measurement was measured by Habig method. Uridine phosphate glucuronyl transferase (UDP-GT) by Mulder method, Arylhydrocarbon Hydroxylase (AHH) by Dehnen method, Benzyloxyresorufin 0-dealkylation (BROD) by Lubet method, Eryhromycin N-demethylase (ERMD) by Wrighton. Histopatbological examination- The liver was fixed in formalin and the prepared slide was stained with haematoxylin-eosin (HE).
Statistical analysis- All the data were expressed as means ±SD. Statistical analysis was performed by ANOVA method and significant difference was judged by Kruskall-wallis test. A P value less than 0.05 was considered as significant difference.

Results

1. General condition in mice
In CCI4 administration group, some toxicity was observed: the decrease of active movement, hair xanthosis, and inhibition of body weight gain. On the other hand, in the AHCC and CCI4 combined treatment group, those symptoms were reduced. Comparing to the CCI4 group, which showed liver weight gain by more than 70%, a significant inhibition in liver weight gain was observed in AHCC co-administration group.

2. Biochemical parameter change in serum and microsome fraction
Comparing to the serum GPT activity increased to 369.5 (IU) in CCI4 group, it was 164.7 (IU) in AHCC co-administration group. Not any significant change in triglyceride, albumin or globulin was observed. LPO in liver microsome fraction in CCI4 group was 287 nmol/ml which was more than twice as much as in control group. On the other hand, LPO in AHCC co-administration group was 152 nmol/ml, the lipid hyperoxidation was significantly inhibited. AHCC administration group also showed lower lipid hyperoxidation comparing to control group).

3. Phase I drug metabolizing enzyme activity
Cytochrome P450 content ia liver microsome franction was decreased in CCI4 group, however, AHCC co-administration inhibit P450 reduction. P450 content in AHCC group was increased compared with control group. By the measurement of drug metabolizing enzyme activities, BROD activity was not observed. Also, ERDM activity was remarkably inhibited in CCI4 group and induced in AHCC group, however, there was no significant difference between AHCC co-administration group and control group.
AHH activity was tending to be induced in CCI4 group and AHCC group, the significance is not clear as in co-administration group (Fig. 2).

4. Phase u drug metabolizing enzyme activity

The measurement of drug metabolizing enzymes showed that CCl4 suppressed GST activity and UDP-GT activity. This effect was not observed when AHCC was administrated together. Additionally, GST activity was induced in AHCC group .

5. Histopathological changes

In CCl4 group, widespread centrilobular necrosis and inflammatory cell infiltration were observed. On the other hand, in CCl4 and AHCC co-administration group, centrilobular necrosis was rarely observed and small part of inflammatory cell infiltration was observed.

Discussion

As a liver poisonous substance, which induces experimental liver injury, CCl4 is generally used and known to induce acute liver injury by short-term administration.

Acute liver injury model was used in this experiment to find a protecting effect of AHCC on liver. AHCC is known to have immune stimulate effect or anti-tumor effect as bioactivity, and it is expected to have another various activities in plant polysaccharide like AHCC. It is considered that this experiment made one of its activities clear.

It was observed the degradation of appetite, weight loss, auxesis of liver, sGPT level increase, etc. in CCl4 induced liver injury mice prepared for this experiment.

Regarding organ weight, AHCC significantly inhibit liver weight gain. Liver of mouse administered CCl4 is known to become fatty liver because of disorder of lipid metabolism, and olive oil used as a solubilizer is also a cause of adiposity in liver. As a result of histopathological examination, adipose degeneration in liver was significantly inhibited in AHCC co-administrated group more than in CCl4 group. This result made us think AHCC had some effect on fatty liver.

For serum parameter, sGPT level, a general index of hepatitis, which elevate when liver cell is injured, was suppressed. This result suggests that radical derived from CCl4 has a preventive effect for attacking liver cell.

Liver cytochrome P450 measured in this research was decreased in CCl4 group. For the cause of decreasing P450, since P460 content usually depend on the content of protein, the possibilities are whether heme protein change of composition or metabolism damaged or the change of P450 active part, e.g. CO bind inhibition, has happened. On the other hand, P450 tend to be induced in AHCC group. In AHCC and in CCl4 group, it was considered that P450 content went to the normal level by the counteraction of these two substances.

Furthermore, AHH as P450 metabolic enzyme belongs to CYP1A isozyme. It is a general compound, metabolic enzyme especially for aromatic compound, and has strong relation to metabolic activation of cancergenesis. ERDM belongs to CYP3A molecule and has intimate relation to metabolism of endocrine substances such as antibiotic or hormone. Liver drug metabolizing enzyme is known as the most important enzymes when endogenic substances like hormone or xenobiotics are absorbed and go thorough liver, and also they are known as the enzyme easy to change its amount or activity when liver was injured. It is also known that these enzymes are suppressed and cause endocrine disorder when hepatic failure (liver abortive) is happened, various general symptoms, feebleness or febricula, arise by adrenal hormone disorder or sex hormone metabolism disorder. On the other hand, it is considered that AHCC induce these enzymes and maintain the balance of biofunctions; detoxication of external substances, metabolism, metabolism of endogenic substances (hormone). Also, it was suggested that the dose of AHCC need to have an adjusted when it is administered with P460 drug metabolize enzyme.

Another type of drug metabolizing enzyme is phase II drug metabolizing enzyme: GST and UDP-GT. GST strongly relates to detoxification as glutathione conjugation reaction. That is GST detoxification hydrophobic compound by protein-bounding and operate bioprotective reaction against detriments absorbed or taken in the body or formed by metabolism under normal conditions. UDP-GT is also known to detoxification various kinds of external exogenous materials or endogenic substances such as O-, N-, S- and C-glucuronic acid conjugate as a reaction of glucuronic acid conjugation. AHCC showed inhibition effect on declining phase II drug metabolizing enzyme activity caused by CCl4, also GST activity induced effect was observed in AHCC administered group. That is one of the protective mechanism for liver injury caused by CCl4 is to accelerate detoxification of CCl4. CCl4 becomes trichloromethylradical. This radical is supposed to form lipid peroxidation followed by hyperoxidation reaction to damage cytoplasmic or cell

membrane which cause liver injury. LPO level in AHC and CCl4 co-administrated group suppressed compared more than in CCl4 group, and it was reduced more in AHCC group than in control group. According to these results, it is considered that AHCC had another effect to erase radical and prevent liver injury caused by the radical.

In conclusion, AHCC showed protective effect for acute liver injury in mice regarding general condition, serum parameter, liver drug metabolize enzymes. The most significant effects of AHCC are the inhibition of liver auxesis, reducing general poisoning symptom, and inducement of detoxic enzymes. It is necessary to explicate the pharmacological functions of these protective effects.

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Abbreviations

A: albumin, AHCC: active hexose correlatedd compounds, AHH: arylhydrocarbon hydroxylase, BRM: biological response modifiers, BROD: benzyl oxyresorufm 0-dealkylation, CCl4: carbon tetrachloride, ERMD: erythromycin N -demethylase, G: Globulin, GPT: glutamate pyruvate transaminase, GST: glutathione S-transferase, TG; tryglyceride, TP: total protein, UDP-GT: uridine phosphate glucuronyl transferase.