Effects of Chlorella Alkali Extract on Blood Pressure in SHR

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We studied dietary effects on blood pressure and the incidence of stroke in SHRSP, and reported that a diet containing white fish meal, casein, euphausia etc, as a protein source could markedly depress the elevation of blood pressure, prevent the incidence of stroke and elongate the life span of SHRSP.

We artificially digested these protein sources to clarify their effective substances (Presentation at this Meeting). We discovered that chlorella was effective in preventing incidence of stroke but that it was difficult to study it in the same way as other protein sources. Therefore, we tried to search for its effective substances by different methods.

Materials and Methods:

1. Removal of pigments in chlorella

Chlorella contains several kinds of pigments (chlorophyll etc). As these pigments easily move into water or alcoholic layers and disturb the experiment, they were removed in advance by repeated treatment with methyl alcohol at room temperature until the extractive solvent was decolorized.

2. Alkali treatment of chlorella

Twenty Gm of decolorized chlorella was suspended in 2 L of distilled water. The pH was adjusted to 9.3. The solution was stirred slowly at 60°C for 15 hours and then centrifuged at 4,000 rpm for 5 min. Supernatants were condensed to about 30 ml by a rotary evaporator, cold ethyl alcohol was added to make the ethyl alcohol content 70%, and the results were kept in cold water for 3 hours. After centrifugation (15,000 rpm for 20 min), the alcohol soluble portion (alkali extract) was condensed to 30 ml.

3. Separation of chlorella alkali extract

The chlorella alkali extract was fractionated by gel filtration on Sephadex G-25 fine (column: 2.5 × 50 cm), eluted with distilled water, collected 5 ml in each tube, and with the absorption spectra at 280 nm, separated into 4 fractions (A, B, C, and D).

4. Effects on blood pressure

The alkali extracts were administered to SHRSP and SHR (blood pressure 180 to 230 mmHg) either intravenously, intraperitoneally, or intragastrically; and injected into normotensive Wistar rats intravenously.

Blood pressure was measured chronologically without anesthesia by the tail-pulse pickup method. Five to 16 animals were used in each group.

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Results :

1. Since fraction A showed a depressive effect, it was fractionated by gel filtration on Sephadex G-75, and separated into 2 fractions (A_1 and A_2). When these fractions were injected into SHRSP and SHR intravenously, the depressive effect of fraction A_1 was stronger than that of fraction A_2 .

The following results for fraction A_1 were obtained.

1) Intravenous injection (3 mg/100 Gm of body weight): Blood pressure in SHRSP and SHR fell after 30 min and showed a fall of 63 ± 21 ($M \pm SD$) mmHg after 60 min. The blood pressure in normotensive rats also showed a fall of 32 ± 0.6 mmHg after 60 min.

2) Intraperitoneal administration (15 mg/rat): Blood pressure in SHRSP and SHR showed a fall of 47 ± 27 mmHg after 2 hours, and of 27 mmHg after 3 hours.

3) Intragastric administration (30 mg/rat): Blood pressure of SHRSP and SHR showed a fall of 20 mmHg after 3 hours, and 16 or 11 mmHg after 4 or 5 hours, respectively.

2. Fraction A_1 showed a minimum of 257 nm and a maximum of 278 nm in UV absorption spectra, and qualitatively was positive in ninhydrin reaction and in the phenol-sulphuric acid method.

Summary :

When chlorella was treated with alkali after decolorization by methyl alcohol and then fractionated by gel filtration, the fraction showed a depressive effect. The blood pressure of SHRSP and SHR showed a fall of an average of 63 mmHg 1 hour after intravenous administration, 47 mmHg 2 hours after intraperitoneal administration. The blood pressure of normotensive Wistar rats also showed a fall of an average of 32 mmHg 1 hour after intravenous administration.

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