東北大学機関リポジトリ
TOUR
Tohoku University Repository

Studies on the Depressive Factors in Heterochordaria abietina Affecting the Blood Cholesterol Level in Rats II. Hypocholesterolemic Activity of Chlorophyll, Its Derivatives and Magnesium

著者	ITO Koichi, TSUCHIYA Yasuhiko
journal or publication title	Tohoku journal of agricultural research
volume	27
number	1
page range	46-52
year	1976-09-01
URL	http://hdl.handle.net/10097/29711

Tohoku Journal of Agricultural Research
Vol. 27, No. 1, August 1976
Printed in Japan

Studies on the Depressive Factors in *Heterochordaria abietina* Affecting the Blood Cholesterol Level in Rats

II. Hypocholesterolemic Activity of Chlorophyll, Its Derivatives and Magnesium

Koichi Ito and Yasuhiko Tsuchiya*

Department of Fisheries, Faculty of Agriculture, Tohoku University, Sendai, Japan

(Received, March 26, 1976)

Summary

To evaluate the effect of chlorophyll and its derivatives on the blood cholesterol level in rats, the chlorophyll content of marine algae was determined. It occurred more richly in a small brown alga *Heterochordaria abietina* than in other brown seaweeds tested and was at the same level as that of a green alga, *Enteromorpha sp.*.

Chlorophyll, Na-Cu-chlorophyllin, phaeophytin and phaeophorbide included in basal diet supplementing with 1 percent cholesterol a casein-sucrose were tested on rats for 6 weeks at 12.5 and 125 mg per day per animal and the hypocholesterolemic activity was compared. The phaeophytin and phaeophorbide were effective in lowering blood cholesterol level in rats at a dose of 25 mg per animal per day, but chlorophyll and Na-Cu-chlorophyllin were ineffective. Magnesium demonstrated a significant hypocholesterolemic activity.

Introduction

It was reported by the authors in 1964 that a supplemental diet of brown alga called Matsumo, *Heterochordaria abietina*, impeded blood cholesterol elevations in cholesterol-fed rats (1). To identify the substances responsible for the effect of this seaweed, we have attempted to investigate the comparative hypocholesterolemic activities of its fractionated components for rats (2). It was found that alginic acid present in brown marine algae was effective in lowering the plasma cholesterol levels in rat (3). Both saturated and unsaturated fatty acid fractions were also physiologically active. A number of investigators had reported that highly unsaturated fatty acids has a potency of lowering the serum cholesterol level in rats (4–6). Therefore, the hypocholesterolemic activity of the unsaturated acid

* Present address: Kitasato University, School of Fisheries Sciences, Sanriku-cho, Iwate Prefecture.

fraction is due to the presence of the polyunsaturated fatty acids in this seaweed. It is surprising for us to note that the saturated acid fraction, at which palmitic acid was present in large amounts, had depressing effect on the cholesterol level. We suggested that an efficacious substance in this fraction might be plant pigment such as chorophyll derivatives extracted, together with the fatty acids. Therefore, the experiment reported here was designed to clarify the effect of chlorophyll, phaeophytin, phaeophorbide, Na-Cu-chlorophyllin and magnesium, which is in the porphyrin ring of chlorophyll, on the blood cholesterol level in rats.

Experimental Methods and Results

Feeding of Animal

Male rats of the Wistar strain were used throughout the five feeding experiments and kept on the basal diet for 7 days prior to the beginning of the tests. They were housed in individual cages which were automatically maintained at 22°C and 60 percent relative humidity with an abundant supply of water. Body weights were recorded weekly. The basal diet was the same as that previously reported by Ito and Tsuchiya (2). The quantity of sucrose was modified by the addition of each supplemental substance, so the caloric value of the diet supplied to the rats was adjusted to approximately the same on all.

Determination of Cholesterol

At the termination of the experiments, the rats were starved for 12 hours prior to the collection of blood in order to avoid any short term change in plasma lipid due to recent ingestion of food, The blood was obtained using heparin as an anticoagulant from their hearts by cardiac pancture, while they were under light ether anesthesia. Blood samples from rats were pooled in three lots and the plasma was collected in the usual manner. The total cholesterol concentration of the blood and plasma was determined by the method of Abell *et al.* (7).

Preparation of Chlorophyll and Phaeophytin

The chlorophyll was prepared by a slight modification of the procedures described by Mackinney (8) and Jacobs *et al.* (9). It was extracted from 8 Kg of fresh homogenized spinach with 20 l acetone and the pigments extracted were transferred to 2 l water. The petroleum ether layer freed from acetone was washed successively with 90 percent aqueous methanol to remove yellow xanthophylls. The solution was then washed with water until chlorophyll precipitated. The pigment was collected on talc, washed with petroleum ether to remove all traces of carotene, and redissolved in an acetone. After transfer to ethyl ether, the acetone was removed by washing with distilled water, and the solution was dried under vacuum. The yield of chlorophylls was about 11 g from 8 Kg of fresh spinach.

The preparation of phaeophytin was performed by the following method. The chlorophylls were dissolved in ether and the ether solution was acidified with dilute hydrochloric acid at about pH 3.0 at ordinary temperature. The ether solution was washed out the excess acid with water, and concentrated and chromatographed on sugar column according to the usual method for chlorophyll separation.

Determination on Chlorophylls in Several Marine Algae

The materials used were as follows: marine algae, Matsumo (*Heterochordaria abietina*), Aonori (*Enteromopha sp.*), Wakame (*Undaria pinnatifida*), Hiziki (*Hizikia fusiforme*), Tsunomata (*Chondrus ocellatus*), and Shōjōkenori (*Polysiphonia urceolata*), which were collected in Onagawa Bay. The total concentration of chlorophylls was determined by measuring the optical density in ether solution at 600 and 642.5 nm respectively and applying to the equations given by Comar and Zscheile (10).

The results obtained are given in Table 1. It shows that the chlorophyll concentration of *Heterochordaria abietina* was 0.63 percent which was superior to that of other brown and red seaweeds tested, and was at the same level as that of a green alga, *Enteromorpha sp.*.

TABLE 1. *Chlorophyll Contents of Algae*

algae	Chlorophyll (dry matter %)
Aonori, *Enteromorpha sp.*	0.67
Matsumo, *Heterochordaria abietina*	0.63
Wakame, *Undaria pinnatifida*	0.26
Hiziki, *Hizikia fusiforme*	0.15
Tsunomata, *Chondrus ocellatus*	0.17
Shōjōkenori, *Polysiphonia urceolata*	0.21

Feeding Experiments

Trial 1. The purpose of this trial was to compare the effect of 25 mg chlorophyll or phaeophytin per day per rat and the control diet on the blood cholesterol level of animal.

The results are summarized in Table 2. It should be noted that both chlorophyll and phaeophytin supplementation showed slightly lower rates of growth than the control diet. The phaeophytin had the ability to reduce the blood cholesterol level in rat. On the contrary, the chlorophyll was a substance insignificant for the hypocholesterolemic activity.

Trial 2. The purpose of this trial was to test the efficacy of dietary phaeophorbide and Na-Cu-chlorophyllin.

Table 3 shows that weight gain was insignificantly affected by the type and

TABLE 2. *Effect of Chlorophyll and Phaeophytin on the Blood Cholesterol Level in Rats** (Trail 1)

Dietary treatment	No. in group	Weight gain (g/rat)	Whole blood*** cholesterol (mg/dl)
Basic diet +1% cholesterol	6	120	178.8± 5.5
Basic +1% Cholesterol +25 mg chlorophyll**/day/rat	6	111	205.6±10.3
Basic +1% cholesterol +25 mg phaeophytin**/day/rat	7	110	163.0± 7.5

* The average initial body weight was 198 g (rang; 188 to 216 g) and the experimental period was 6 weeks.

** Chlorophyll and phaeophytin was extracted from spinach.

*** Mean ±stand. dev. $\sigma = \sqrt{\frac{\Sigma d^2}{n}}$

TABLE 3. *Effect of Na-Cu-chlorophyllin and Various Concentration of Phaeophorbide on the Blood Cholesterol Level in Rats** (Trial 2)

Dietary treatment	No. in group	Weight gain (g/rat)	Whole blood*** cholesterol (mg/dl)
Experiment 1			
Basic diet+1% cholesterol	7	162	182.4± 5.7
Basic +1% cholesterol +12.5 mg phaeophorbide**/day/rat	7	153	176.4± 3.4
Basic +1% cholesterol +25 mg phaeophorbide**/day/rat	7	168	162.4± 6.5
Experiment 2			
Basic diet +1% cholesterol	7	114	186.8± 7.8
Basic +1% cholesterol +25 mg Na-Cu-chlorophyllin/day/rat	7	120	219.9±19.8
Basic +1% cholesterol +25 mg phaeophorbide**/day/rat	6	113	159.1± 8.2
Basic +1% cholesterol +125 mg phaeophorbide**/day/rat	6	113	164.2± 8.8

* The average initial body weight in experiment 1 was 130 g (range: 113 to 146 g), and the body weight in experiment 2 was 204 g (range: 187 to 242 g). Both experimental period was 6 weeks.

** Preparation of the phaeophorbide; dissolve Na-Cu-chlorophyllin (Wako pure chemical industries, LTD.) in water. Boil the mixture 5 minutes with ethanolic 30% hydrochloric acid. Cool to 0°C and extract the phaeophorbide with ether.

*** Mean±stand. dev. $\sigma = \sqrt{\frac{\Sigma d^2}{n}}$

concentrations of the substance used. The blood cholesterol level of rat was much depressed by phaeophorbide at a dose of 25 mg per day per animal. However, the hypocholesterolemic activity did not augment with increasing the dose. On the contrary, the Na-Cu-chlorophyllin raised the blood cholesterol level in rat.

Trial 3. The purpose of this trial was to investigate the effect of magnesium,

TABLE 4. *Effect of Magnesium on the Blood Cholesterol Level in Rats** (Trial 3)

Dietary treatment	No. in group	Weight gain (g/rat)	Whole blood**** cholesterol (mg/dl)
Basic diet+1% cholesterol	6	120	178.8± 5.5
Basic +1% cholesterol −Magnesium**	6	109	186.9±10.2
Basic +1% cholesterol +Magensium***	6	111	159.2± 8.6

* The average initial body weight was 197 g (range: 176 to 216 g) and the experimental period was 6 weeks.

** Removed magnesium from salt mixture of basal diets.

*** Doubled magnesium contents of salt mixture

**** Mean±stand. dev. $\sigma = \sqrt{\frac{\Sigma d^2}{n}}$

which is in the porphyrin ring of chlorophyll, on the blood cholesterol level in rat.

The results are summarized in Table 4. It shows that the weight gain was little affected by removal of magnesium from the salt mixture in the basal diet. Likewise the further addition of magnesium to the basal diet containing salt mixture showed little lower rate of growth. It is noteworthy that magnesium significantly depressed the blood cholesterol level in rats. On the contrary, the magnesium free diet was ineffective.

Discussion

Phaeophytin can easily be obtained from chlorophyll, removing the magnesium of porphyrin ring with the dilute acid, and then readily converted to phaeophorbide by removing the phytol with the alkali.

The effect of chlorophyll and its derivatives of the response of depressing the blood cholesterol level in rats was investegated (Table 2 and 3). Among them, both phaeophytin and phaeophorbide demonstrated a significant hypocholesterolemic activity at a level of 25 or 125 mg per day per animal. On the contrary, chlorophyll and Na-Cu-chlorophyllin raised the concentration of blood cholesterol. It would appear plausible that there was no definite relation between the hypocholesterolemic efficacy and the growth rate of rat.

Although the phaeophytin and phaeophorbide of which magnesium is removed from the porphyrin ring, had a significant hypocholesterolemic activity, the chlorophyll and Na-Cu-chlorophyllin, which constitute the metal in the ring, were almost inactive. Therefore, it could be conclusively said that the substances freed from the metal of the porphyrin ring had the ability to decrease the blood cholesterol level in rat. However the mechanism of the anticholesterolic activity is not clear. According to Hill (11), chlorophyll is first converted into phaeophytin by the loss of magnesium and then phaeophytin is mainly converted to phaeophorbide in the digestive tract of animals. These products are not absorbed

from the gut and excreted in the faeces. Therefor, we assumed that both phaeophytin and phaeophorbide interfered with the absorption of cholesterol through the gut in rats. To re-examine the efficacy of chlorophyll and phaeophytin extracted from Wakame, the brown alga, *Undaria pinnatifida*, we performed the feeding experiment (12). The results obtained were similar to those of the preceeding experiments. However, we are unable to explain the ineffectiveness of chlorophyll or chlorophyllin in lowering blood cholesterol level in rats.

Next the effect of the magnesium in the porphyrin ring of chlorophyll was investigated. It showed that the blood cholesterol level of animal was remarkably higher in the group of magnesium-free diet than the control (Table 4). Curran (13–16) had investigated the influence of the synthesis of the liver cholesterol in rats by the various concentrations of metalic ion and assumed that the synthesis of cholesterol was interfered with Fe^{++}, V^{++++}, Co^{++} and Mn^{++} ion. We proposed first that magnesium depressed the blood cholesterol level in rats, but the mechanism whereby these results were obtained remains undetermined. It would appear that there was no definite association between some chlorophyll derivatives and mangesium in the hypocholesterolemic activity.

TABLE 5. *Effect of Matsumo and Phaeophytin on Plasma Cholesterol Level in Rats**

Dietary Treatment	No. in group	Weight gain (g/rat)	Plasma*** cholesterol (mg/dl)
Basic diet	7	130	130.8±11.5
Basic +1% cholesterol	7	139	346.2±13.7
Basic +1% cholesterol +5% Matsumo	7	125	235.9±11.8
Basic +1% cholesterol +25 mg phaeophytin**/day/rat	7	118	245.5±10.7

* The average initial boly weight was 107 g (range: 96 to 124 g) and experimental period was 4 weeks.

** Phaeophytin was extracted from spinach.

*** Mean Stand. dev. $\sigma = \sqrt{\frac{\Sigma d^2}{n}}$

Table 1 indicates that there occurred a fair amount of chlorophyll in *Heterochordaria abietina* that was superior to other brown and red seaweeds and as much as green alga, *Enteromorpha sp.*. Matsumo supplemented to the basal diet at 5 percent level that is equal to about 8 mg of phaeophorbide was effective in depressing the plasma cholesterol concentration in rat as seen in Table 5. From these experiments it is said that besides alginic acid, chlorophyll derivatives and polyunsaturated acids present in *Heterochordaria abietina* are a part of the substances responsible for depressing the blood cholesterol levels in rat.

References

1) Tsuchiya, Y., Takahashi, T., and Ito, K, Annual meeting of the *Jap. Soc. Sci. Fish. at Tokyo*, April 4, (1964)
2) Ito, K., and Tsuchiya. Y., *Tohoku J. Agr. Res.*, **23**, 160, (1972)
3) Ito, K., and Tsuchiya, Y., Proc. Seventh Int. Seaweed Symp., 558 (1972)
4) Peifer, J.J., Janssen, F., Ahn, P., Cox, W., and Lundberg, W.O., *Arch. Biochem. and Biophys.*, **86**, 302 (1960)
5) March. B.E., Jacob Biely, Tothill. J., and Haqq. S.A., *J. Nutrition*, **69**. 105 (1959)
6) Kaneda, T., Arai, K. and Tokuda, S., *Bull. Jap. Soc. Sci. Fish.*, **30**, 419 (1964)
7) Abell, L.L., Levy, B.B., Brodie, B.B., and Kendall, F.E., *J. Biol. Chem.*, **195**, 357 (1952)
8) Mackinney, G., *J. Biol. Chem.*, **132**, 91 (1940)
9) Jacobs, E.E., Vatter, A.E., and Holt, A.S., *Arch. Biochem. Biophys.*, **53**, 228 (1954)
10) Comar, C.L., and Zscheile, F.P., *Plant Physiol.*, **17**, 198 (1942)
11) Hill, R., "*Comparative Bichemistry*" ed. by M. Florkin and E.H. Stotz, Elsevier Publ. Co., 3, 60 (1963)
12) Ito, K., Tsuchiya, Y., and Onodera, I., Annual meeting of the *Jap. Soc. Sci. Fish.*, at Tokyo, April 4, (1970)
13) Curran, G.L., *J. Biol. Chem.*, **200**, 17 (1953)
14) Curran, G.L., and Clute, O.L., J. Biol. Chem., 204, 215 (1953)
15) Curran, G.L., *J. Biol. Chem.*, **210**, 765 (1954)
16) Curran, G.L., *Proc. Soc. Exp. Biol. and Med.*, **88**, 101 (1955)