The Effect of Addition of Tryptophan Metabolites on the ADP-induced Platelet Aggregation in vitro

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SUMMARY

As a preliminary experiment to be clear whether Trp. metabolites give some influences to the platelet aggregation or not in a morbid state, for example vitamin B_6 deficiency, this study was performed *in vitro*. In Amang each metabolites added solely in incuvation medium including platelet rich plasma, 5-HT did not enhance the platelet aggregation but suppressed slightly at the concentration of 37.5 μg/ml. 3-hydroxykynurenine showed the striking suppression to the ADP-induced platelet aggregation at the concentration of 83.3 μg/ml. 5-HT enhanced the platelet aggregation to some extent at the concentration of 85.8 μg/ml.

The addition of equal concentration mixture of kynurenic acid and xanthurenic acid (42.9, 41.6 μg/ml respectively) remarkably suppressed the platelet aggregation. On the contrary to our expectation, the addition of xanthurenic acid and 3-hydroxykynurenine to the medium at the concentration 42.9, 41.6 μg/ml respectively did not show any suppressive effect on the platelet aggregation as in the case of their single addition, but rather enhanced the platelet aggregation. The addition of mixture of kynurenic acid and anthranilic acid (42.6, 37.1 μg/ml) did not suppress the platelet aggregation but enhanced it.

INTRODUCTION

The oyster extract is a rich source of taurine and metals such as zinc[1].

Taurine is an amino acid containing sulfonic acid group in its molecule, and is biosynthesized from methionine via cysteine. Since taurine does not have carboxyl group, it can be a constituent comprising protein molecule. It is mostly distributed as free form or conjugates with cholic acid. The biological function of taurine has well been studied, and its biological activity in preventing cardiovascular dysfunction such as hypertension, stroke, and myocardial infarction[2].

Authors previously reported that the oyster extract suppressed platelet aggregation caused by ADP and epinephrine *in vivo* and *in vitro*[3,4].

Shibata et al. reported that longterm oral taurine administration increased 5-hydroxytryptamine (5-HT; or serotonin) level in liver[5]. Similar result obtained in our study with rat brain. In our experiment monoamine oxidase activity was enhanced also by continuous longterm taurine administration[6].

The platelet aggregation has been thought to be somewhat enhanced by 5-HT[7], one of Trp. metabolites (Fig. 1). It seems likely that various factors affect Trp metabolism especially under

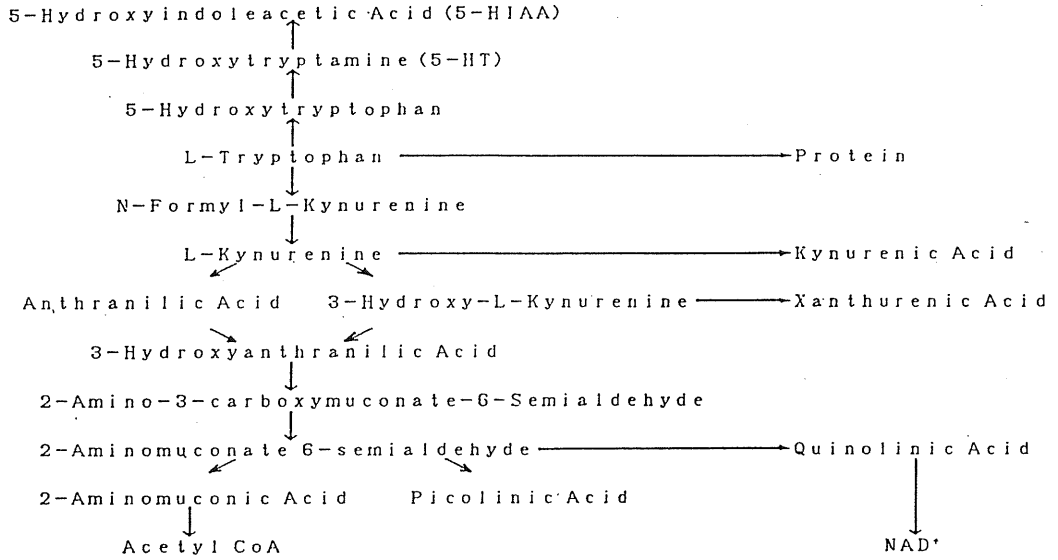

Fig. 1. Tryptophan Pathway. *This figure was quoted from the paper Shibata *et al.* reported.

morbid condition[8]. For instance, under vitamin B_6 deficient state urinary xanthurenic acid level increases. However, the interrelation between Trp. metabolites, taurine, oyster extract and platelet aggregation has remained unknown. The authors speculated that one or more constituents of the oyster extract affect Trp. metabolism to influence the ADP-induced platelet aggregation. In other words, Trp. metabolites are supposed to participate in regulating the ADP-induced platelet aggregation. This is a preliminary work to examine the relation between ADP-induced platelet aggregation and Trp. metabolites.

MATERIALS AND METHODS

Chemicals used for platelet aggregation

Tryptophan metabolites examined for platelet aggregation test were kynurenine sulfate, 3-hydroxykynurenine, anthranilic acid, 3-hydroxyanthranilic acid, 5-hydroxyindole acetic acid (5-HIAA), which were purchased from Sigma Chemical Co. St. Louis, Mo, and 5-hydroxytryptamine (5-HT) purchased from Aldrich Chemical Co., Milwaukee, Wis. L-Tryptophan was purchased from Ajinomoto Co., Tokyo. All the chemicals used in the present work were guaranteed reagent grade.

Animals

Experimental animals used for platelet aggregation were male Wistar rats of ca. 150 gram. They were fed a diet for 21 days and kept in the cage. Two hundred mg of the oyster extract/kg/day, 80 μ

M taurine as aqueous solution/kg/day, 80 μM each of serine and methionine mixture/kg/day were orally adiministered for 21 days.

To examine platelet aggregation of human blood, fresh blood were collected from two healthy male volunteers of 29 and 36 years of age without any medication.

Platelet aggregation test

To examine the rats' platelet aggregation, blood was collected from hearts by punctuation of six rats without starvation. Fresh blood thus collected was gently mixed with 3.8% sodium citrate, then centrifuged at 1000rpm for 10 min. to obtain platelet rich plasma (PRP). Platelet poor plasma (PPP) prepared by centrifugation of the remaining precipitates of the blood was used as the reference of platelet aggregation test. The platelet aggregation test was evaluated by measuring turbidity of the incubation mixture using Agricorder (Kyoto Daiichi Kagaku co. Model PA-3210) according to the standard manual using ADP.

To examine the effect of addition of Trp. metabolites on the ADP-induced platelet aggregation, Trp metabolites were added in the incubation medium at the final concentration as shown in Table 2.

In some experiments metabolites were added as a mixture of two compounds with equal concentrations (Table 3).

Determination of urinary tryptophan metabolites

To survey the influence of tryptophan and its metabolites on platelet aggregation, L-tryptophan was injected to rats intraperitoneally at the dose of 10mg/100g body weight. In some experiments chelating compounds injected together with L-Trp[9]. The amount of excreted tryptophan metabolites into urine was measured by Brown *et al's* method[10].

RESULTS

Level of Urinary Tryptophan Metabolites of Rats under Different Conditions

Quantitative analysis of Trp metabolites secreted into urine was done for Wistar rats kept under different nutritional conditions. Data are showed in Table 1. Rats to which taurine was administered orally for 21 days showed no significant difference in xanthurenic acid level between control diet fed rats. Meanwhile xanthurenic acid secretion rose about 1.4 times in the urine of rats administered methionine and serine simultaneously. Rats fed oyster extract, on the other hand, showed significantly lower level of urinary xanthurenic acid than control diet fed rats.

Influence of in vitro Addition of Tryptophan Metabolites on ADP-induced Platelet Aggregation.

Different kinds of Trp metabolites were added solely or as mixture of two metabolites in the incubation medium to measure ADP-induced platelet aggregation. Data are shown in Tables 2 and 3.

Externally added 5-HT at the concentration of 37.5 μg/ml of incubation medium did not enhance the platelet aggregation but rather suppressed slightly. This result suggests that an increase in the internal 5-HT accompanied with platelet aggregation and the externally added 5-HT should not be dealt equally in its role on the platelet aggregation.

Table 1. Excretion amount of xanthurernic acid in urine after oral Trp. administration

	μg/day in urin
Taurine fed group	2689.1
Ser + Met fed group	3667.4
Oyster extract fed group	1851.1
Control group	2681.4

*Each group except control group was given the feed described in the table added to normal diet. Control group was fed only normal diet.

Table 2. Influence of Trp metabolites on ADP-induced platet aggregation *in vitro*

Trp. metabolites	Final concentration (μg/ml)	Platelet aggregation
5-HIAA	83.3	↓ ↑
5-HT	37.5	↓
Anthranilic acid	74.2	↓ ↑
3-Hydroxanthranilic acid	83.3	↓ ↑
Kynurenic acid	83.3	↓
Xanthurenic acid	85.8	↓
3-Hydroxy-L-Kynurenine	83.3	↓ ↓
5-Hydroxytryptophan	85.8	↑

↑ ↑ : Strongly accelerate
↑ : Weakly accelerate
↓ ↑ : no effect
↓ ↓ : Strongly inhibit
↓ : Weakly inhibit

Table 3. Influence of Trp metabolites mixture on ADP-induced platelet aggregation *in vitro*

Metabolites mixture	Final concentration (μg/ml)	Platelet aggregation
Kynurenic acid : Anthranic acid	41.6 : 37.1	(↑)
Kynurenic acid : Xanthurenic acid	41.6 : 42.9	(↓ ↓)
Xanthurenic acid : 3-hydroxyanthrenilic acid	42.9 : 41.6	(↓ ↑)
Xanthurenic acid : 3-hydroxy-L-Kynurenine	42.9 : 41.6	(↑)
L-Kynurenine : Kynurenic acid	20.8 : 41.6	(↓)
L-Kynurenine : Anthranilic acid	20.8 : 37.1	(↓ ↑)

*Meaning of symbol ( ) is the same as former table.

3-Hydroxykynurenine strikingly suppressed the ADP-induced platelet aggregation. In the meantime 5-hydroxytryptophan enhanced the platelet aggregation to some extent.

In the experiments to examine the effect of combined addition of mixture of equal amount of two different Trp. metabolites, we examined the addition of Trp. metabolites in several combinations. The addition of mixture of kynurenic acid and xanthurenic acid remarkably suppressed the platelet aggregation. On the contrary to our expectation, the addition of xanthurenic acid and 3-hydroxykynurenine to the medium did not show any suppressive effect on the platelet aggregation as in the case of their single addition, but rather enhanced the platelet aggregation. The addition of mixture of kynurenic acid and anthranilic acid did not suppress the platelet aggregation, but enhanced it.

DISCUSSION

In the present study the authors aimed to know if the imbalance of Trp. metabolism causes changes of the susceptibility to the ADP-induced platelet aggregation. As described in the previous paper the oyster extract suppresses the ADP-induced platelet aggregation, however, it is not necessarily due to the direct effect of taurine contained in the extract. Then we speculated the suppressive effect of the longterm oral administration of oyster extract on the platelet aggregation could be explained by ruling Trp. metabolism.

In the rat injected Trp. together with chelating agent, the urinary xanthurenic acid level rose than control rats, and on the contrary, anthranilic acid level in the urine decreased. Standing on the previous result that the addition of chelating agent such as EDTA along with high level of Trp. stimulated the secretion of xathurenic acid into urine, and kynureninase that catalyzes the conversion of kynurenine to anthranilic acid requires zinc as cofactor, it seems highly likely that the oyster extract has something to do with Trp. metabolism. Furthermore, the facts that platelet aggregation has been reported to be suppressed under the vitamin B_6 supplemented condition[11] and the vitamin B_6 is required for the enzymatic conversion of kynurenine to anthranilic acid by kynureninase[12] suggest the close relation between the serum Trp. metabolites level and the platelet aggregation.

However, it still is too early to give any conclusive comments. We need to examine if Trp. metabolites in serum really participate in the platelet aggregation *in vivo,* and if so to what extent does it contribute to the suppression of platelet aggregation *in vivo.*

The blood levels of Trp. metabolites especially xanthurenic acid, kynurenic acid and anthranilic acid under various physiological conditions are now under the investigation.

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REFERENCES

1. Arakawa, K. and Yamasaki, Y. "KAKI(Oyster)" Shibata Shoten.
2. Huxtable, R. and Barbeau, A. (1976): ed. Taurine, Raven Press, New York.
3. Ohta, T., Ohkubo, M., Okumura, S. *et al.* (1985): Effect of extract oyster on human platelets aggregation. Proceedings the 2nd symposium on trace nutrients research Tokyo, pp. 169–180.
4. Ohta, T., Ohkubo, M. and Shibata, Y. (1986): Effect of oyster extract on rat platelets aggregation. In Proceedings of the 3rd symposium on trace nutrients researh, Nagona, pp. 61–68.
5. Shibata, Y., Sotokawa, Y., Iwata, S., Takeuchi, F., Tsubouchi, R., Okubo, T., Hattori, M., Kotake, Y., Shiraishi, S., Sugino, N. and Okumura, S. (1986): On the regulation of serotonin (one of neurotransmitter) metabolism through the compoment of oyster. In Proceedings of the 3rd Symposium on trance nutrients research, Nagoya, pp. 75–87.
6. Shibata, Y., Hattori, M., Takeuchi, F. *et al.* (1986): On the serotonin metabolism. In Proceedings of International study group for tryptophan research 5th international meeting.
7. a) Clerck, F. De., Xhonneux, B. and Wiele, R. Van De (1985): Agents and Actions, Vol. 17.2:220–228.
   b) Bevan, J. and Heptinstall, S. (1986): Nannyn-Schmiedeberg's Arch Pharmacol 334:341–345.
8. Igari, T., Tsuchizawa, M., Tohoku J. (1987): exp Med. 153, 79–86.
9. Shibata, T. *et al.* (1985): Trptophan metabolism and Mineral Proceedings The 2nd Symposium on Trace Natrients Research.
10. a) Lepkovsky, S., Roboz, E. and Haggen-Smit, A. J. (1943): Xanthurenic acid and its role in tryptophan metabolism of pyridoxine deficient rats. J. Biol. Chem., 149, 195–201.
    b) Brown, R. R. and Price, J. M. (1956): Quantitative studies on metabolites of Try in the urine of the dog, cat, rat and man. J. Biol. Chem., 291, 985–997.
11. Schoene, N. W., Chanmugam, P. and Reynolds, R. D. (1986): Effect of oral Vitamin B_6 supplementation on *in vitro* platelet aggregation. Am. J. Clin. Nutr., 43, 825–830.
12. Ogasawara, N., Hagino, Y. and Kotake. Y. (1962): Kynurenine-transaminase and the increase of xanthurenic acid excretion. J. Biochem., 52, 162–166.