Dietary Tryptophan Enhances Platelet Aggregation in Rats

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Summary The effect of diets enriched with tryptophan (4 and 10 g/kg) on ADP induced platelet aggregation and on plasma lipids was studied with growing rats, following feeding periods of up to 3 weeks. Dietary tryptophan was found to enhance markedly ADP induced platelet aggregation. It appears that this effect was not related to plasma lipid levels. In vitro studies aimed to clarify the mechanism by which tryptophan exerts its action showed that the latter, at concentrations up to 100 μg/ml, had no effect on ADP induced platelet aggregation. On the other hand, serotonin, the metabolic product of tryptophan, increased the ADP induced platelet aggregation in a dose dependent pattern. The possible involvement of serotonin in the observed enhancement of platelet aggregation was further substantiated by the observed high levels of 5-hydroxyindole acetic acid, which is a catabolite of serotonin, in the urine of the experimental animals. It is conceivable that tryptophan enhances platelet aggregation in rats via its metabolite, serotonin, and this may in turn contribute to increased atherosclerotic risk.

Key Words dietary tryptophan, platelets aggregation, serotonin, 5-hydroxyindole acetic acid, blood lipids, atherosclerosis risk

Serotonin, a brain neurotransmitter which is involved in appetite regulation (4) and in sleep induction (5) is a very potent vasoconstrictor and also plays a role in platelet activation (6).

Platelets accumulate serotonin and may be activated by several agents including serotonin itself. During activation, serotonin is released and enhances platelet aggregation. Platelet activation initiate several processes such as smooth muscle cell proliferation, endothelial injury, accumulation of cholesterol in macrophages and modification of lipoproteins (7).

Dietary tryptophan was shown to affect lipid metabolism (8). Thus, diets containing 10% tryptophan were found to lower plasma cholesterol by 35% (9). However, administration of high doses of tryptophan were shown to produce fatty liver in rats and other changes in liver morphology (10).

The present study was undertaken to investigate the effect of tryptophan feeding on both platelet activation and plasma lipids concentration.

Materials and methods

Weanling rats of the Charles River CD strain (animal colony, Dept. of Food Engineering & Biotechnology, Technion, Haifa, Israel) were fed up to 3 weeks 10% casein diets (11) with or without the supplementation of 0.4 or 1% tryptophan. Diets and water were provided ad libitum. At the end of the feeding period the rats were sacrificed. Blood was collected into 3.8% concentrate (v:v = 6:1). Platelet-rich plasma (PRP) was prepared by low-speed centrifugation at 200 × g for 10 min at 23°C. ADP induced platelet aggregation in platelet-rich plasma was analyzed according to Viener et al. (12). Plasma lipids were determined as described elsewhere (13).

Statistical analysis was carried out using...
Results and discussion

The effect of dietary tryptophan on body weight of female and male weanling rats following 3 week feeding is shown in Fig. 1. Inclusion of tryptophan had very little influence on the body weight of both sexes. However, a decrease in the liver weight of the animals fed the diet containing 1% tryptophan was observed (Fig. 2). When the effect of dietary tryptophan on ADP induced in vitro platelet aggregation was examined, it was found that tryptophan containing diets substantially increased platelet aggregation, the effect being higher in females than in males (Fig. 3). Thus, it was decided to conduct future experiments employing female rats and diets enriched with only 0.4% tryptophan.

The pattern of platelet aggregation following 1, 3, 7, and 21 day feeding is presented in Fig. 4. A significant effect of tryptophan on platelet aggregation was seen only at the end of 3 week feeding. This may suggest that during short feeding periods of up to 7 days the level of accumulated serotonin was insufficient to induce platelet aggregation.

In vitro studies, aimed to clarify the mechanism by which tryptophan exerts its effect showed that the latter, at concentrations up to 100 µg/ml, had
no effect on ADP induced platelet aggregation (Fig. 5). On the other hand, serotonin, the metabolic product of tryptophan catabolism, increased ADP induced platelet aggregation in a dose dependent pattern (Fig. 6).

Serotonin is metabolized in the body to 5-hydroxyindole acetic acid (5HIAA). The concentration of 5HIAA in the urine of the rats fed the tryptophan enriched diet was significantly higher than the respective level in the urine of the control animals (Fig. 7). This is indicative of the presence of elevated levels of serotonin in the plasma of the experimental animals.

It is conceivable that tryptophan enhances platelet aggregation in rats via its metabolite serotonin, and this may in turn contribute to increased atherosclerotic risk. Indeed, it is well documented that increased platelets activity is observed with patients suffering from atherosclerosis (14).

There was no significant effect of the tryptophan enriched diet on plasma concentration of cholesterol, triglycerides and HDL-cholesterol following 21 day feeding (Fig. 8). This observation may suggest

Fig. 5. Platelet aggregation in the presence of varying concentrations of tryptophan added to the reaction system.

Fig. 6. Platelet aggregation in the presence of varying concentrations of serotonin added to the reaction system.

Fig. 7. Dietary tryptophan supplementation and urine levels of 5-hydroxyindole acetic acid (5HIAA). Values are mean of 6 samples ± SD. * Significant ly different from the control, p < 0.05.

Fig. 8. Blood lipid levels of female rats fed control (◼) and 0.4% tryptophan supplemented diet (■). Values are mean of 4 animals ± SD. * Significantly different from the respective control, p < 0.05.
that the effect of a tryptophan enriched diet on platelet aggregation is not mediated by changes in plasma lipids concentration which are known to be modulators of platelet activity (13). The higher levels of the tested blood lipids observed in the plasma of the rats fed the tryptophan supplemented diets up to 1 week, can not be explained at the moment.

The levels of tryptophan used in the present study are only 4 times higher than the RDA for growing rats (15). Our data show that dietary enrichment with such a small amount of tryptophan causes enhancement of platelet function. These results may be of importance in light of the recent wide-spread self administration of tryptophan for induction of sleep (16). The amount recommended for this purpose is a daily dose of 1–2 g (17). Moreover, high doses of tryptophan (6–9 g) are available in some countries as a prescription of antidepressant drug (16).

In conclusion, this study demonstrates that tryptophan consumption enhances platelet aggregation and thus may contribute to increased atherosclerosis risk.

REFERENCES

1) Lin, F. D., Smith, T. K., and Bayley, H. S. (1987): A role for tryptophan in regulation of protein synthesis in porcine muscle. J. Nutr., 118, 445–449.
2) Horwitt, M. K., Harper, A. E., and Henderson, L. M. (1981): Niacin-tryptophan relationships for evaluating niacin equivalents. Am. J. Clin. Nutr., 34, 423–427.
3) Weber, L. J., and Horita, A. (1965): A study of 5-hydroxytryptamine formation from L-tryptophan in the brain and other tissues. Biochem. Pharmacol., 14, 1141–1149.
4) Fernstone, J. D. (1985): Dietary effects of brain serotonin synthesis: relationship to appetite regulation. Am. J. Clin. Nutr., 42, 1072–1082.
5) Hartman, E., and Spinweber, C. L. (1979): Sleep induced by tryptophan. J. Nerv. Ment. Dis., 167, 497–499.
6) Tiez, N. W. (1982): Serotonin and its metabolite 5-hydroxyindole acetic acid (5-HIAA), in Fundamentals of Clinical Chemistry, W. B. Saunders Company, pp. 812–821.
7) Aviram, M. (1990): The effect of lipoproteins and platelets on macrophage cholesterol metabolism. Blood Cell Biochemistry. Vol. 2, Megakaryocytes, Platelets, Macrophages and Eosinophils, Harris ed. Academic Press, New York, pp. 179–208.
8) Raja, P. K., and Jarowski, C. I. (1975): Lowering of human plasma cholesterol and triglyceride levels by lysine and tryptophan supplementation. J. Pharm. Sci., 64, 691–692.
9) Serouge, C., and Rukaj, A. (1983): Plasma and lipoprotein cholesterol in rats fed L-amino acid supplemented diets. Ann. Nutr. Metab., 27, 386–395.
10) Trulson, M. E., and Sampson, H. W. (1986): Ultrastructural changes of the liver following L-tryptophan injection in rats. J. Nutr., 116, 1109–1115.
11) Association of Official Analytical Chemists (1988): Official Methods of Analysis, 14th ed., AOAC, Arlington, VA, pp. 877–878.
12) Viener, A., Brook, J. G., and Aviram, M. (1984): Abnormal plasma lipoprotein composition in hypercholesterolemic patients induces platelet activation. Eur. J. Clin. Invest., 14, 207–213.
13) Aviram, M. (1989): Modified forms of low density lipoprotein affect platelet aggregation in vitro. Thromb. Res., 53, 561–567.
14) Aviram, M., and Brook, J. G. (1987): Platelet activation by plasma lipoproteins. Prog. Cardiovasc. Dis., 30, 61–72.
15) American Institute of Nutrition (1977): Report on the AIN Ad-Hoc Committee on standards for nutritional studies. J. Nutr., 107, 1340–1348.
16) Pollack, R. L., and Kravitz, E. (1987): Effects of tryptophan injection. J. Nutr., 117, 1315–1316.
17) Hartman, E. (1987): Possible effects of tryptophan injection. J. Nutr., 117, 1314.