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Effect of taurine concentration on platelet aggregation in gestosis patients with edema, proteinuria and hypertension.

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Abstract

To elucidate the relationship between the high concentration of taurine in platelets and platelet aggregation in patients with EPH gestosis (gestosis with edema, proteinuria and hypertension), platelet aggregation and the platelet release response (release of ATP and beta-thromboglobulin) were studied in the washed platelet suspension (PS) obtained from normal pregnant or non-pregnant women and EPH gestosis patients. Platelet aggregation and platelet release response were significantly lower in EPH gestosis patients than in normal pregnant and non-pregnant women. Platelet aggregation, platelet release response induced by ADP and collagen and the aggregation induced by A23187 were inhibited in taurine-loaded PS from non-pregnant women. These results suggest that the decrease of platelet aggregation in EPH gestosis patients was caused by high concentrations of taurine in platelets, which may inhibit the intracellular Ca^{2+} movement and platelet release response. Therefore, taurine appears to have a protective effect against the hyper-coagulative state in EPH gestosis.

KEYWORDS: platelet, taurine, platelet aggregation, platelet release response, EPH gestosis

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Effect of Taurine Concentration on Platelet Aggregation in Gestosis Patients with Edema, Proteinuria and Hypertension

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To elucidate the relationship between the high concentration of taurine in platelets and platelet aggregation in patients with EPH gestosis (gestosis with edema, proteinuria and hypertension), platelet aggregation and the platelet release response (release of ATP and β -thromboglobulin) were studied in the washed platelet suspension (PS) obtained from normal pregnant or non-pregnant women and EPH gestosis patients. Platelet aggregation and platelet release response were significantly lower in EPH gestosis patients than in normal pregnant and non-pregnant women. Platelet aggregation, platelet release response induced by ADP and collagen and the aggregation induced by A23187 were inhibited in taurine-loaded PS from non-pregnant women. These results suggest that the decrease of platelet aggregation in EPH gestosis patients was caused by high concentrations of taurine in platelets, which may inhibit the intracellular Ca^{2+} movement and platelet release response. Therefore, taurine appears to have a protective effect against the hyper-coagulative state in EPH gestosis.

Key words : platelet, taurine, platelet aggregation, platelet release response, EPH gestosis

EPH gestosis (gestosis with edema, proteinuria and hypertension), which is a state of chronic intravascular coagulation (1), is accompanied by significant alterations in the hemostatic mechanism, but its etiology remains unclear. Such a hyper-coagulative state in EPH gestosis, the thrombocytopenia (2,3), the increase in plasma β -thromboglobulin (β -TG) (3,4) and the decrease in platelet aggregation (2,3,5) have been reported.

Taurine (2-aminoethanesulfonic acid), one of the sulfur-containing free amino acids, is widely distributed in almost all mammalian organs, and

its concentration is especially high in important organs and in body fluids (6). Some authors have reported the relationship between taurine and platelet aggregation in patients with essential hypertension and myocardial infarction; that is, taurine may play an important role as an anti-aggregative agent of platelet (7-9). Recently, it was reported that in EPH gestosis, the concentration of taurine in platelets and the uptake of taurine by platelets showed a marked increase (10).

To elucidate the relationship between taurine and platelet aggregation in EPH gestosis, we examined the taurine concentration in platelets,

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platelet aggregation and platelet release response using washed platelets suspension (PS).

Materials and Methods

Human subjects. In this study PS was obtained from 5 normal pregnant women (32–38 weeks of pregnancy), 5 patients with severe EPH gestosis (32–39 weeks) and 15 non-pregnant normal women, whose informed consent was obtained. The severity of EPH gestosis was categorized according to the gestosis index proposed by Organization Gestosis (11).

Preparation of PS. Venous blood was withdrawn by clean antecubital venopuncture into plastic syringe containing 0.1 volume of acid-citrate-dextrose solution (ACD, Terumo, Tokyo, Japan) (sodium citrate 2.30 w/v %, citrate acid 0.80 w/v %, glucose 2.20 w/v %, pH 4.4–5.5) as an anticoagulant. Platelet-rich plasma (PRP) was obtained by separation of the supernatant after centrifugation of whole blood at $120 \times g$ for 15 min at room temperature. PRP was centrifuged at $1500 \times g$ for 10 min at room temperature after the addition of 0.1 volume of 10 mM EDTA and 0.01 volume of 100 U/ml apyrase (grade V, Sigma Chemical Co., St. Louis, MO, USA), followed by the separation of plasma and the platelet pellet. The platelet pellet was resuspended in isotonic HEPES buffer (pH 7.4) containing 10 mM HEPES, 140 mM NaCl, 5 mM KCl, and 0.5 % bovine serum albumin. This suspension was referred to as PS. Number of platelet in PRP and PS were counted with an electric particle counter (Platelet Counter PL-100, Toa Medical Electronics, Kobe, Japan).

Preparation of taurine-loaded PS. Taurine solutions in 200 μ l saline were added to 1800 μ l of PRP to give final taurine concentrations of 6.25 mM, 25 mM and 50 mM. Saline was added to PRP as a control. These PRP were incubated at 37°C for 5 min to facilitate the taurine uptake by platelets. The incubation was terminated by chilling in an ice-cold water for 1 min. Taurine-loaded PS and control were obtained by centrifugation and resuspension in the same method as described above.

Measurement of platelet aggregation induced by ADP, collagen and Ca ionophore A23187. Platelet aggregation was measured turbidimetrically by recording light transmission at 660 nm using aggregometer (NBS Hema Tracer Model 601, Niko Bioscience, Tokyo, Japan). The degree of aggregation (%) was standardized by assuming the light transmission of 10 mM HEPES

buffer as 100 % and that of PS as 0 % and determined 3 min after the addition of stimulants. The aggregometer cuvette contained 170 μ l of PS and 10 μ l of fibrinogen (Baxter Dado, MO, USA) solution containing 20 U/ml of heparin and 10 μ l of 20 mM CaCl_2 (final concentration 1 mM). The cuvette was put in the aggregometer at 37°C and stirred at 1,000 rpm. Aggregation was induced by adding either ADP (Boehringer-Mannheim, Mannheim, Germany) solution to a final concentration of 0.5–1.5 μ M, or collagen (Hormon-Chemie, Munchen, Germany) solution to a final concentration of 0.5–1.25 μ g/ml.

When the aggregation was induced by adding 1 μ M A23187 (Sigma), either 10 mM HEPES buffer or the same buffer with 1 mM EDTA was added to 190 μ l of PS prior to activation of the platelets to get the different concentrations of extracellular Ca^{2+} in an aggregometer cuvette.

Assay of ATP and β -thromboglobulin. Three minutes after the addition of aggregation inducers the reaction was terminated by chilling in an ice-cold water bath and followed by adding 20 μ l of 50 mM EDTA. The mixture was centrifugated at $4,500 \times g$ for 5 min at 4°C. The resulting supernatant was separated and used for the assay of ATP and β -TG. ATP was determined by luciferase-luciferin method as described previously (12). Luminescence was detected by a lumicounter (Lumicounter ATP-237, Advantec Toyo Co., Tokyo, Japan). Released β -TG in the supernatant was measured with the radio-immunoassay (RIA) method (13) using β -Thromboglobulin Riapac (RIA-kits) (Amersham Internat, Little Chalfont, UK).

Analysis of taurine. Amino acid analysis was performed with a high performance liquid chromatography (HPLC) using PICO-TAG method (Waters Assoc. Milford, USA) (14) for the determination of taurine concentrations. PRP or PS (200 μ l) was added to 50 mM EDTA (20 μ l) followed by centrifugation at $600 \times g$ for 10 min at room temperature to separate the platelet pellet. The platelet pellet was resuspended in 200 μ l of physiological saline. The platelet suspension was subjected to freezing and thawing 3 times for complete platelet lysis. Samples were deproteinized by 8 % trichloroacetic acid. Amino acids were modified with phenylisothiocyanate to PTH-amino acids (15), and applied to the reversed-phase HPLC with a detector at 254 nm.

Statistical analysis. The results are presented as the mean $\pm$ SD. The significance of the differences was examined using Student's *t*-test.

Results

Concentration of taurine in platelets. The taurine concentration in platelets of EPH gestosis patients was 4.28 ± 0.43 pmoles/ 10^4 platelets, which was significantly higher than that of normal pregnant (3.28 ± 0.46 pmoles/ 10^4 platelets) or non-pregnant women (2.95 ± 0.44 pmoles/ 10^4 platelets) ($p < 0.001$, vs normal pregnant; $p < 0.01$, vs non-pregnant women). There was no significant difference in the taurine concentration of platelets between non-pregnant and normal pregnant women. Taurine concentrations in taurine-loaded PS were 3.35 ± 0.53 , 4.21 ± 0.58 , 5.36 ± 0.95 and 6.45 ± 1.71 pmoles/ 10^4

platelets when taurine-loading was performed in 0.0, 6.25, 25 and 50 mM taurine solutions, respectively. Thus, the higher the taurine concentration in the taurine-loading solution was, the higher it was in the taurine-loaded PS.

Platelet aggregation and platelet release response of ATP and β -TG induced by ADP and collagen. Aggregation of platelets from patients with EPH gestosis induced by ADP or collagen was significantly lower than that of platelets from normal pregnant or non-pregnant women. However, there was no significant difference in aggregation between normal pregnant women and non-pregnant women as shown in Table 1. ATP and β -TG release response of platelets from EPH gestosis patients induced by ADP or collagen were significantly lower than those of platelets from normal pregnant or non-pregnant women. No significant difference was seen between these responses of platelets from normal pregnant and non-pregnant women as shown in Table 2. Aggregation, ATP and β -TG release response induced by ADP or collagen in taurine-loaded PS were significantly decreased in a concentration-dependent manner as shown in Tables 3 and 4.

Platelet aggregation and release response of ATP and β -TG induced by calcium ionophore A 23187. Effect of calcium ionophore A 23187 as a platelet aggregation inducer was examined with PS in 10 mM HEPES buffer or in the same buffer containing 1 mM EDTA. Platelet aggrega-

Table 1 Platelet aggregation induced by ADP and collagen^a

Subjects	ADP (1 μ M)	Collagen (1 μ g/ml)
Non-pregnant	53.8 ± 21.2	57.6 ± 9.0
Normal pregnant	45.5 ± 23.3	51.3 ± 12.5
EPH gestosis	$18.2 \pm 10.0^*$	$26.2 \pm 21.4^*$

a: ADP- and collagen-induced aggregation of platelets obtained from blood samples from non-pregnant and normal pregnant women and from patients with EPH gestosis was examined as described under Materials and Methods. Aggregation was determined as % light transmission at 3 min after the addition of ADP or collagen taking 10 mM HEPES buffer as 100 % and platelet suspension as 0 %. Each value represents the mean $\pm$ SD of 5 experiments. Statistically significant difference was derived by Student's *t*-test: * $p < 0.05$, non-pregnant women versus EPH gestosis patients; No significant difference was seen in these measurements between non-pregnant women versus normal pregnant women.

Table 2 ATP and β -thromboglobulin (β -TG)-release response of platelets induced by ADP and collagen^a

Subjects	ATP release (nmoles/ 10^5 PLT)		β -TG release (ng/ 10^5 PLT)	
	ADP (1 μ M)	Collagen (1 μ g/ml)	ADP (1 μ M)	Collagen (1 μ g/ml)
Non-pregnant	9.71 ± 1.80	80.4 ± 19.2	43.3 ± 10.1	145.1 ± 26.6
Normal pregnant	7.73 ± 1.98	78.5 ± 21.3	40.4 ± 11.9	140.3 ± 30.5
EPH gestosis	$6.40 \pm 1.66^*$	$45.9 \pm 14.0^*$	$29.8 \pm 3.97^*$	$122.8 \pm 22.0^*$

a: ATP- and β -TG-release responses of platelets from blood samples obtained from non-pregnant and normal pregnant women and from patients with EPH gestosis were induced by ADP and collagen. ATP and β -TG released for 3 min after the addition of ADP or collagen were measured. Each value represents mean $\pm$ SD of 5 determinations. Statistically significance was derived by Student's *t*-test: $p < 0.05$, non-pregnant women versus EPH gestosis patients and normal pregnant women versus EPH gestosis patients; No significant difference was seen in these measurements between non-pregnant women versus normal pregnant women.

Table 3 Induction of aggregation of taurine-loaded platelet suspension by ADP and collagen^a

Concentration of taurine in platelets (pmoles/10 ⁴ PLT) (Concentration of taurine used for taurine-loading (mM))	ADP (0.5-1.5 μ M)	Collagen (0.5-1.25 μ g/ml)
3.35 (0.0)	100	100
4.21 (6.25)	74.4 $\pm$ 16.2**	70.5 $\pm$ 35.2*
5.36 (25)	63.0 $\pm$ 27.5**	70.7 $\pm$ 25.9**
6.45 (50)	57.6 $\pm$ 26.3**	63.3 $\pm$ 26.9**

a: Taurine-loaded platelet suspensions were prepared by incubating platelet-rich plasma with various concentrations of taurine solution for 5 min as described under Materials and Methods. Aggregation was determined as that in Table 1. Each value represents % of the control (taurine-unloaded platelet suspension was taken as 100 %) and expressed as mean $\pm$ S.D. of 10 determinations. Statistically significant difference was derived by Student's *t*-test: *, *p* < 0.01; **, *p* < 0.001, taurine loaded platelet suspension (PS) versus control.

Table 4 ATP and β -thromboglobulin (β -TG)-release response induced by ADP and collagen from taurine-loaded platelet suspension^a

Concentration of taurine in platelets (pmoles/10 ⁴ PLT) (Concentration of taurine used for taurine-loading (mM))	ATP release		β -TG release	
	ADP (0.5-1.5 μ M)	Collagen (0.5-1.25 μ g/ml)	ADP (0.5-1.5 μ M)	Collagen (0.5-1.25 μ g/ml)
3.35 (0.0)	100	100	100	100
4.21 (6.25)	70.8 $\pm$ 17.6**	45.5 $\pm$ 32.1*	78.2 $\pm$ 6.1*	70.1 $\pm$ 22.1*
5.36 (25)	60.4 $\pm$ 20.4**	43.3 $\pm$ 21.4**	70.4 $\pm$ 15.4**	71.6 $\pm$ 14.7**
6.45 (50)	38.9 $\pm$ 23.2**	42.1 $\pm$ 24.6**	51.9 $\pm$ 32.4**	58.8 $\pm$ 22.9**

a: ATP- and β -TG release response of taurine-loaded platelet suspensions were induced by ADP or collagen. and β -TG release for 3 min after the addition of ADP or collagen were determined. Each value represents % of the control (taurine-unloaded platelet suspension was taken as 100 %) and expressed as mean $\pm$ S.D. of 10 determinations. Statistically significant difference was derived by Student's *t*-test: *, *p* < 0.01; **, *p* < 0.001, taurine loaded platelet suspension (PS) versus control.

Table 5 Aggregation and release response of taurine loaded platelet suspension induced by A23187^a

Concentration of taurine in platelets (pmoles/10 ⁴ PLT) (Concentration of taurine used for taurine-loading (mM))	Aggregation		ATP response		β -TG response	
	HEPES buffer	HEPES buffer with EDTA	HEPES buffer	HEPES buffer with EDTA	HEPES buffer	HEPES buffer with EDTA
3.35 (0.0)	100	100	100	100	100	100
4.21 (6.25)	54.0 $\pm$ 32.7	92.7 $\pm$ 12.7	90.3 $\pm$ 41.7	104.3 $\pm$ 6.5	71.7 $\pm$ 29.9	110.7 $\pm$ 25.7
5.36 (25)	33.3 $\pm$ 25.0*	92.7 $\pm$ 11.2	80.5 $\pm$ 35.9	103.3 $\pm$ 6.5	64.5 $\pm$ 19.2	111.3 $\pm$ 7.0
6.45 (50)	30.6 $\pm$ 30.0*	91.3 $\pm$ 7.7	83.4 $\pm$ 41.5	94.5 $\pm$ 8.2	60.0 $\pm$ 23.5	106.6 $\pm$ 10.1

a: Aggregation was determined as that in Table 1 at 3 min after addition of 1 μ M A23187. Release of ATP and β -thromboglobulin (β -TG) for 3 min after the addition of 1 μ M A23187 was measured. Aggregation and release response were observed in 10mM HEPES or in 10mM HEPES with 1 mM EDTA. Each value represents % of the control (taurine-unloaded platelet suspension was taken as 100 %) and expressed as mean $\pm$ S.D. of 5 determinations. Statistically significant difference was derived by Student's *t*-test: *, *p* < 0.05, taurine loaded platelet suspension (PS) versus control.

tion (expressed as % of the control) induced by $1\mu\text{M}$ A23187 decreased significantly than the control in taurine-loaded PS with 25 and 50mM taurine solutions, when EDTA was not included in the incubation mixture. However, when 1mM EDTA was added to the reaction mixture, no significant difference was seen in the aggregation features between control and taurine-loaded PS. As shown in Table 5, both ATP and β -TG release responses induced by A23187 did not show a significant difference, even in the absence of 1mM EDTA, among platelets loaded with different concentrations of taurine.

Discussion

EPH gestosis is a state of chronic hypercoagulation and chronic intravascular coagulation (1,4). The characteristic pathology is the presence of fibrin-platelet thrombi in multiple organs. These thrombi are the result of one or more episodes of intravascular coagulation, and the placenta exhibits an increase of fibrin in the intravillous space (1). In patients with EPH gestosis, decreased platelet aggregation (2,3,5) (Table 1), lowered platelet counts (2,3), increased plasma levels of β -TG (3,4) and lower levels of 5-HT in platelets (5) were observed relative to such measurements in normal pregnant women.

Taurine is the most abundant free amino acid in mammalian organs and in body fluids (6). Taurine is also abundant in adult blood platelet (16,17), and its concentration was reported to be six times greater than that of any other amino acid (17). Otani *et al.* have recently reported that in EPH gestosis the concentration of taurine in platelets and the uptake of taurine into platelets showed a marked increase in parallel with the severity of EPH gestosis (10). While, taurine in the platelet is reported to inhibit the aggregation of platelets (7-9). We, therefore, investigated the effect of taurine concentration on platelet aggregation in EPH gestosis.

The aggregation of platelets was inhibited in the case of severe EPH gestosis, which showed higher concentration of taurine in platelets (10) (Table 1). In the present study, we have confirmed that the loading of exogenous taurine to PRP obtained from non-pregnant women inhibits the aggregation of platelets in a dose dependent manner (Table 3).

The mechanism by which an increase of taurine inhibits platelet aggregation is not clear at present. The platelet aggregation and release reactions are induced by both physiological agents (ADP, collagen, thrombin *etc.*) and non-physiological agents (A23187 *etc.*). When platelets are activated, substances such as ADP, ATP, Ca^{2+} , 5-hydroxytryptamine (5HT), from dense bodies, and β -TG, platelet factor-4 (PF-4), fibrinogen *etc.* from α -granules are released (18). These constituents act to accelerate platelet aggregation. Taurine in platelets has been reported to inhibit the ATP-release response of platelets (19). In the present study, PS from EPH gestosis patients and the taurine-loaded PS from non-pregnant women exhibited decrease release responses of ADP and collagen (Tables 2, 4). These findings seem to suggest that taurine inhibits not only the platelet aggregation but also the platelet release response.

Ca^{2+} is an important messenger of activation in platelets (20). In the activated platelets the elevation of cytoplasmic Ca^{2+} concentration ($[\text{Ca}^{2+}]$) is produced by the supplement from the dense tubular systems, the platelet membrane and the influx from extracellular Ca^{2+} . In the heart, taurine antagonizes the calcium paradox and retards the development of lesion formation in calcium overload myopathy (21), and in central and peripheral nervous systems, taurine acts as a modulator of membrane excitability by inhibiting the release of other neurotransmitters and by decreasing mitochondrial release of Ca^{2+} (22). Taurine in platelets prevents the release of serotonin and ATP which are implicated in platelet aggregation (19,23).

A23187 is reported to be a Ca^{2+} -independent

activator for platelets, that is, A23187 directly changes membranous transmission, increases Ca^{2+} influx, and consequently $[\text{Ca}^{2+}]$ is elevated (24). The platelet aggregation induced by A 23187 was inhibited by taurine-loaded in the presence of extracellular Ca^{2+} , but it was not inhibited in the absence of extracellular Ca^{2+} as examined in the presence of 1 mM EDTA (Table 5). Therefore, taurine might inhibit Ca^{2+} influx into platelets.

Results in the present study seem to suggest that platelets in EPH gestosis positively augment the uptake of taurine, and increased intra-platelet taurine inhibits the platelet aggregation by decreasing the release reaction and Ca^{2+} influx. Thus, taurine might function to maintain the homeostasis in EPH gestosis which is a hypercoagulative state. Further studies are needed to elucidate the precise relationship between platelet taurine and platelet aggregation in EPH gestosis.

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