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The Inhibitory Effect of Taheebo Extract on Histamine Release from Rat Peritoneal Mast Cells

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Abstract:

The effect of hot water extract of *Tabebuia avellanedae* (Taheebo) in rat peritoneal mast cells during stimulation of histamine release was studied. Taheebo at concentrations of over 5μg/ml caused an decrease in histamine release stimulated by C48/80 in a dosage-dependent manner. In the Ca^{2+} free medium, Taheebo caused a decrease in histamine release too. The effect of Taheebo was suppressed by high concentrations of C48/40 competitively. Taheebo at concentrations of over 10μg/ml caused an decrease in histamine release stimulated by concanavalin A in a dosage-dependent manner, but the inhibition was non-competitive. These results suggested that Taheebo caused a decrease in the histamine release, which led to a decrease in the allergy symptoms.

Keywords: Tabebuia Avellanedae, Taheebo, Mast cells, Histamine release

Introduction

Taheebo tea is brewed from the bark of Tabebuia Avellanedae, a Bignoniaceae family tree, of which the essence has been used traditionally as a drug for many diseases among people from Brazil. [1] Recently, Ueda *et al.* presented a remarkable report that an extract of Taheebo tea, Naft Fran Dion (NFD), suppressed activation of early expression of TPA-inducible Epstein-Barr (EB) virus and inhibited activity of tumor promoters *in vitro*. [2] In Brazil, Taheebo is considered to have an anti-inflammatory effect; however, it is just empirically recognized. [1] In this short article, the author evaluated the effect of Taheebo on the release of histamine, a chemical mediator that induces type I allergy, using peritoneal mast cells of rats.

Methods

1. Isolation and preparation of rat peritoneal mast cells

Male Wistar rats (8 weeks old, 200 to 230 grams) were decapitated and exsanguinated under ether anesthesia, injected with 10 ml of N-2-Hydroxyethylpipcrazine-N'-2-ethanesulfonic acid (HEPES) buffer medium (154 mM NaCl, 5.6 mM KCl, 1.0 mM $MgSO_4$, 1.0 mM $CaCl_2$, 10 mM glucose, 0.1% BSA, and 20 mM HEPES (pH 7.4)) in the abdominal cavity, and the intra-abdominal fluid was collected subsequent to 2-minute massage. [3] The collected liquid was purified using Percoll gradient (Pharmacia, Uppsala, Sweden). [4] The obtained cells were rinsed three times with HEPES buffer medium at 80 x g at 2°C for 4 minutes to prepare the cell suspension of 2.5 x 10^6 cells/ml and were used as the mast cell fraction. The purity of the purified mast cells was 91± 4%, and the viability was 95± 4%.

2. Preparation of Taheebo tea extract

Taheebo tea produced from natural inner bark of *Tabebuia Avellanedae*, a Bignoniaceae family tree, was provided by Taheebo Japan Co., Ltd. The raw material tree was logged by a Nogueira Chagas in the Federative Republic of Brazil. Distilled water was poured to Taheebo 5 g to make 1,000 ml solution, which was boiled for 15 minutes and filtered with paper filter. The filtrate was used as the stock solution and was diluted with distilled water as needed to use in each test. The concentration of the Taheebo stock solution was defined as 5 g/1,000 ml = 5 mg/ml.

3. Release and determination of histamine

To HEPES buffer medium, Taheebo extract and compound 48/80 (C48/80), or Taheebo extract and concanavalin A (Con A) plus phosphatidylserine were added to constitute 0.8 ml solution, which was preincubated at 37°C for 5 minutes, and then 0.2 ml of the above-mentioned mast cell fraction was added to the solution to evaluate the histamine-releasing activity at 37° C, for 5 minutes for C48/80 stimulation and for 15 minutes for Con A stimulation. Then the test solution was ice-cooled for 5 minutes, centrifuged at 300 x g at 2°C for 5 minutes. The supernatant was used for determination of histamine. For all the operations of histamine release test, plastic tubes and pipettes were used.

Histamine released from mast cells was determined by the modified Shore's method developed by Komatsu *et al.* [5] To the test solution containing histamine released from mast cells, 0.25 ml of 2N PCA was added, and the solution was centrifuged at 1500 x g for 10 minutes. To the supernatant, 3.5 ml of n-butanol chloroform solution (3:2) and 0.27 ml of 5N NaOH were added, and the mixture was shaken for 5 minutes. Following 500 x g centrifugation for 2 minutes, 3 ml of the organic layer was moved to a separate tube, to which 1.2 ml of 0.1N NaCl and 3 ml of n-heptan were added. After the tube was shaken for 5 minutes and centrifuged at 500 x g for 2 minutes, 1 ml of the HCl layer was taken to a separate tube, to which 120 μl of 1N NaOH and 100 μl of 0.2% *o*-phthalaldehyde were added. Subsequent to 40 minute ice cooling and addition of 50 μl of 0.8N HCl, the fluorescence intensity of the test solution was determined at excitation wavelength of 360 nm and fluorescence wavelength of 440 nm.

Results

1. Effect of Taheebo on histamine release induced by C48/80 stimulation

Histamine release from mast cells induced by 0.175 μg/ml C48/80 stimulation was suppressed in a dose-dependent manner by Taheebo of 1 μg/ml or more concentration (Fig. 1). In the figure, histamine release is expressed by the percentage of the total, and the data represents mean ± SD. With the stimulation by 0.175 μg/ml C48/80, histamine release from mast cells was 26.3 ± 1.2% (n = 4). When Taheebo of 1, 5, 10, 20, 40, and 50 μg/ml concentration was added to the solution containing 0.175 μg/ml C48/80, histamine release was suppressed to 24.2 ± 1.1, 12.5 ± 1.1, 8.3 ± 0.6, 4.1 ± 0.6, 1.0 ± 0.5, and 0.5 ± 0.4%, respectively.

When seen by the time course, histamine release at 15, 30, 45, 60, and 300 seconds after the start of stimulation was 21 ± 1.5, 22.5 ± 1.5, 23.5 ± 2.0, 23.5 ± 2.0, and 25.5 ± 2.1%, respectively, and in the presence of 5 μg/ml Taheebo, it was 16.0 ± 1.0, 10.8 ± 0.8, 11.0 ± 1.5, 10.0 ± 1.3, and 10.5 ± 1.8%, respectively (Fig. 2).

When the concentration of C48/80 was increased to 0.35 μg/ml or more, suppression of histamine release seen by addition of 5 μg/ml Taheebo was nullified (Fig. 3). With C48/80 of 0.2, 0.25, 0.3, 0.35, and 0.4 μg/ml concentration, histamine release was 25.8 ± 1.9, 27.6 ± 1.6, 28.5 ± 1.3, 29.7 ± 2.0, 35.3 ± 1.8, and 35.3 ± 1.9%, respectively, and in the presence of 5 μg/ml Taheebo, it was 14.9 ± 2.0, 20.2 ± 2.4, 24.8 ± 1.0, 27.3 ± 1.5, 35.5 ± 2.0, and 35.3 ± 1.9%, respectively.

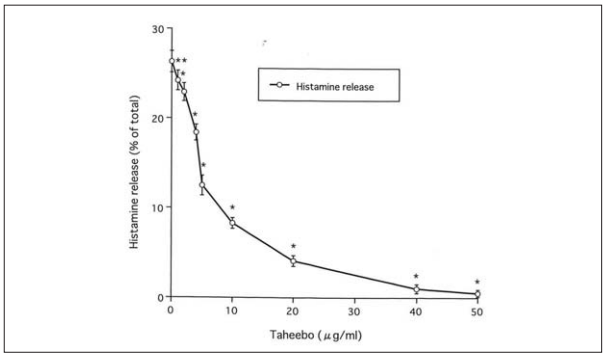

Fig.1 Effect of Taheebo on 0.175μg/ml C48/80 induced histamine release. The cells were incubated at 37℃ for 5 min. The data represent the mean± S.D for 4 experiments. Significant difference from the control:**p<0.05, *p<0.01

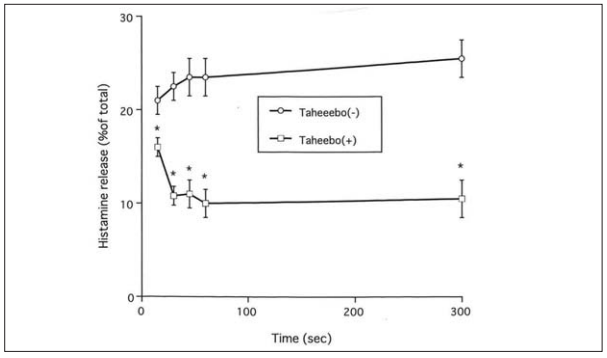

Fig.2 Incubation time on C48/80 induced histamine release in the absence (○)or presence (□) of 5μg/ml Taheebo. *p<0.01

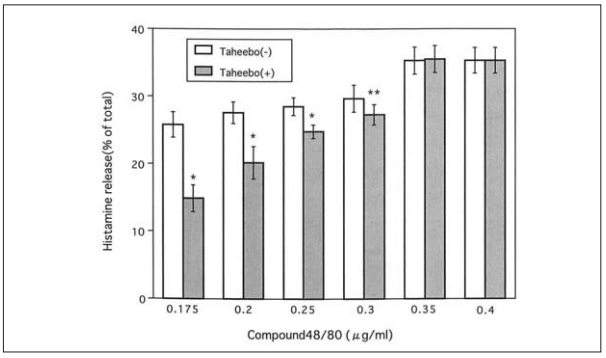

Fig.3 Effect of various C48/80 concentrations in the absence(opencolumns) and presence of 5μg/ml Taheebo(hatched columns). The cells were incubated at 37℃ for 5 min. The data represent the mean±S.D for 4 experiments. Significant difference from the control : **p<0.05, *p<0.01

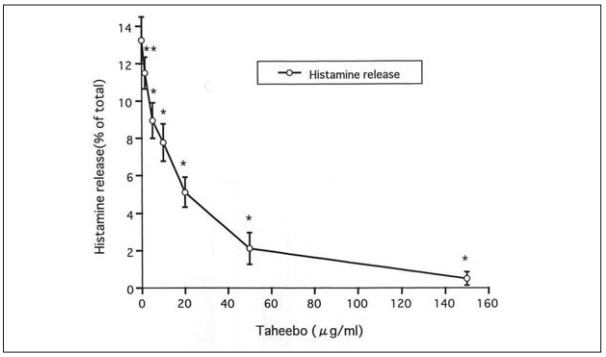

Fig.4 Effect of Taheebo on 0.175μg/ml C48/80 induced histamine release in Ca++ free medium. The cells were incubated at 37℃ for 5 min. The data represent the mean±S.D for 4 experiments. Significant difference from the control: **p<0.05, *p<0.01

Also, in the Ca++ free medium, Taheebo caused a decrease in histamine release in a dose-dependent manner (Fig. 4). With the stimulation by 0.175 μg/ml C48/80, histamine release from mast cells was 13.3 ± 1.2% (n = 4) in the Ca++ free medium. When Taheebo of 5, 10, 20, 50, and 150 μg/ml concentration was added to the medium containing 0.175 μg/ml C48/80, histamine release was suppressed to 9.0 ± 0.9, 7.8 ± 1.0, 5.1 ± 0.8, 2.1 ± 0.8, and 0.5 ± 0.3%, respectively.

When seen by the time course, histamine release at 30, 45, 60, and 300 seconds after the start of stimulation was 12.0 ± 0.8, 13.3 ± 1.0, 13.3 ± 0.8, and 13.2 ± 1.0%, respectively, and in the presence of 10 μg/ml Taheebo, it was 16.0 ± 1.0, 10.8 ± 0.8, 11.0 ± 1.5, 10.0 ± 1.3, and 10.5 ± 1.8%, respectively (Fig. 5).

When the concentration of C48/80 was increased to 1.0 μg/ml or more, suppression of histamine release seen by addition of 10 μg/ml Taheebo was nullified (Fig. 6). With C48/80 of 0.2, 0.4, 0.6, 0.8, and 1.0 μg/ml concentration, histamine release was 17.0 ± 1.0, 20.1 ± 1.1, 24.8 ± 1.4, 30.0 ± 1.5, and 30.0 ± 1.2%, respectively, and in the presence of 10 μg/ml Taheebo, it was 12.1 ± 0.9, 15.2 ± 1.0, 20.7 ± 1.2, 27.0 ± 1.3, and 30.1 ± 1.5%, respectively.

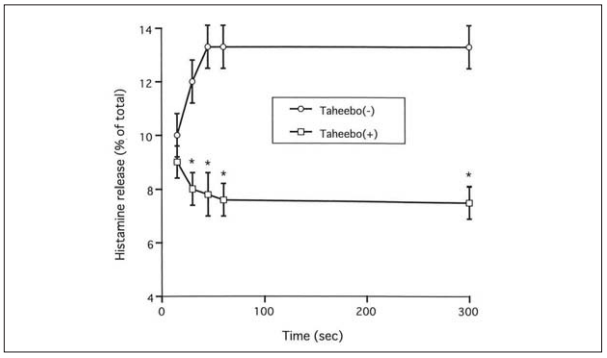

Fig.5 Incubation time on 0.175μg/ml C48/80 induced histamine release in Ca++ free medium in the absence(○) or presence (□) of 10μg/ml Taheebo. The data represent the mean±S.D for 4 experiments. Significant difference from the control: *p<0.01

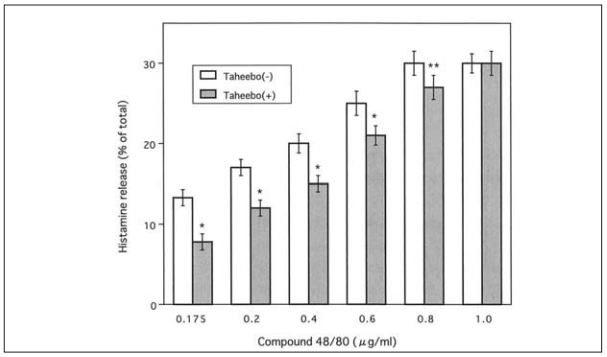

Fig.6 Effect of various C48/80 concentrations in Ca++ free medium in the absence (open columns) and presence of 10μg/ml Taheebo(hatched columns). The cells were incubated at 37℃ for 5 min. The data represent the mean±S.D for 4 experiments. Significant difference from the control: **p<0.05, *p<0.01

2. Effect of Taheebo on histamine release induced by Con A stimulation

Histamine release from mast cells induced by 10 μg/ml Con A stimulation was suppressed in a dose-dependent manner by Taheebo of 10 μg/ml or more concentration (Fig. 7). With the stimulation by 10 μg/ml Con A, histamine release from mast cells was 11.3 ± 0.8%. When Taheebo of 10, 20, 50, 250, 500, and 600 μg/ml concentration was added to the solution containing 10 μg/ml Con A, histamine release was suppressed to 8.5 ± 0.8, 7.9 ± 0.7, 7.5 ± 0.6, 2.8 ± 0.5, 0.5 ± 0.3, and 0.3 ± 0.2%, respectively.

When seen by the time course, histamine release at 1, 5, 10, 15, 30, 45, and 60 minutes after the start of stimulation was 1.65 ± 0.5, 4.3 ± 0.5, 7.7 ± 1.0, 11.0 ± 1.1, 15.9 ± 1.3, 17.4 ± 1.1, and 17.7 ± 1.0%, respectively, and in the presence of 50 μg/ml Taheebo, it was 0.44 ± 0.3, 2.7 ± 0.5, 4.4 ± 0.8, 6.4 ± 1.1, 9.0 ± 0.8, 9.2 ± 0.9, and 9.5 ± 1.0%, respectively (Fig. 8).

When the concentration of Con A was increased up to 200 μg/ml in the presence of 50 μg/ml Taheebo, suppression of histamine release seen by addition of Taheebo was still observed (Fig. 9).

Furthermore, when the concentration of phosphatidylserine was increased up to 40 μg/ml in the presence of 50 μg/ml Taheebo and 10 μg/ml Con A, suppression of histamine release seen by addition of Taheebo was still observed (Fig. 10).

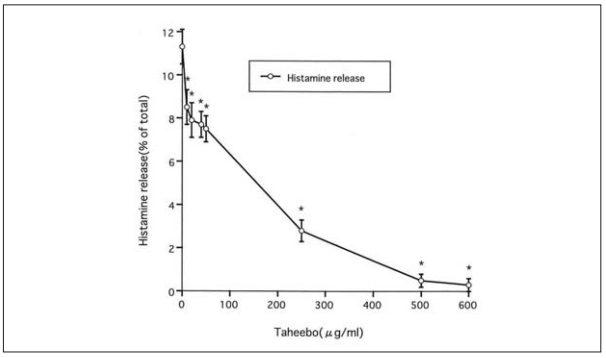

Fig.7 Effect of Taheebo on 10μg/ml conA and 8μg/ml phosphatidylserine induced histamine release. The cells were incubated at 37℃ for 15min. The data represent the mean±S.D for 4 experiments. Significant difference from the control:*p<0.01

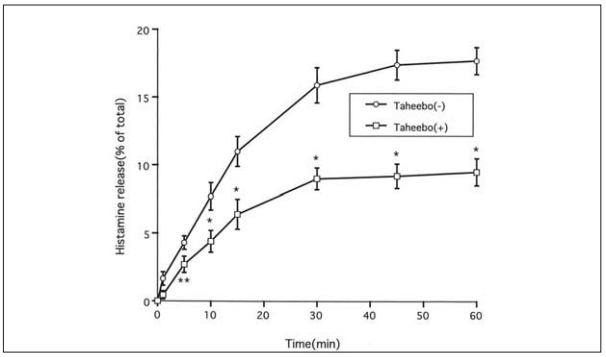

Fig.8 Incubation time on 10μg/ml conA and 8μg/ml phosphatidylserine induced histamine release in the absence (○)or presence (□) of 50μg/ml Taheebo. The data represent the mean±S.D for 4 experiments. Significant difference from the control: **p<0.05, *p<0.01

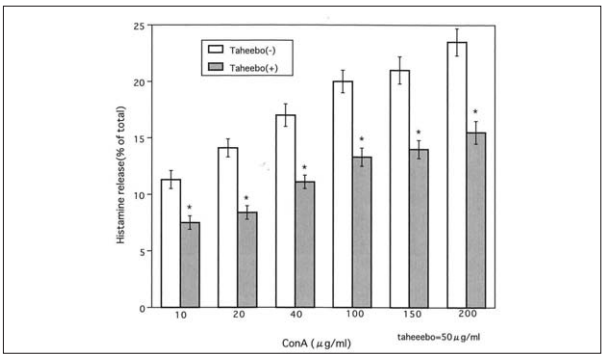

Fig.9 Effect of various conA concentrations in the absence (open columns) and presence of 50μg/ml Taheebo(hatched columns). The cells were incubated at 37℃ for 15 min. The data represent the mean±S.D for 4 experiments. Significant difference from the control:*p<0.01

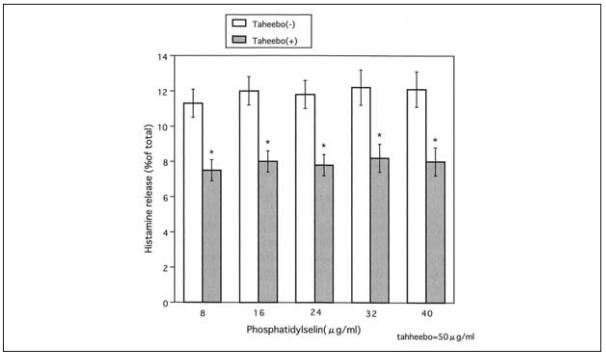

Fig.10 Effect of various phosphatidylserine concentrations in the absence (open columns) and presence of 50μg/ml Taheebo (hatched columns). The cells were incubated at 37℃ for 5 min. The data represent the mean± S.D for 4 experiments. Significant difference from the control:*p<0.01

Discussion

In vivo, histamine release from mast cells is induced by cross-linking of two neighboring IgE-receptors on cells by antibodies. Some agents that induce histamine release *in vitro* include Con A and anti-IgE antibodies[6] act in the IgE-receptor-mediated histamine release, and compound 48/80 (C48/48) [7] and A23187 [8] act in the non-IgE-receptor-mediated histamine release.

In this article, effect of Taheebo essence extracted from *Tabebuia Avellanedae* dust on C48/48- or Con A-induced histamine release from rat mast cells was evaluated.

In the normal or less concentration for drink, Taheebo suppressed both C48/48- and Con A-induced histamine release from rat mast cells in a dose-dependent manner.

Because Taheebo's inhibitory effect on C48/80-induced histamine release was nullified when the concentration of C48/80 was increased, one can presume that the agent in Taheebo having an inhibitory effect on histamine release may act in an opposing manner to C48/80. In addition, because such nullification was observed both in the presence and absence of the extracellular fluid Ca^{2+}, one can presume that Taheebo had inhibitory effect on histamine release due to other cause than its inhibition of inflow of extracellular Ca^{2+}.

At IgE-receptor-mediated stimulation by Con A, inhibitory effect of Taheebo on histamine release was not nullified by increased concentration of Con A. Based on this, one can presume that the site of action of the agent in Taheebo having inhibitory effect on histamine release would not be the IgE-receptor.

These findings suggest that Taheebo suppresses inflammation response by inhibiting histamine release from mast cells and thus alleviates the symptoms of type I allergy.

Acknowledgements

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Literature

1) Accorsi WR. Taheebo - A miracle tree and leaves. The Kobe Shimbun Sogo Shuppan Center. 1988:48.
2) Ueda S, Umemura T, Dohguichi K, et al. Production of anti-tumorpromoting furanonaphthoquinones in *Tabebuia Avellanedae* cell culture. Phytochemistry 1994; 36: 323-325.
3) Otsubo N. Study on histamine release from mast cells. The Journal of the Iwate Medical Association. 1991; 43: 179-186.
4) Wells E, Mann J. Phosphorylation of a mast cell protein in response to treatment with anti-allergic compounds: implications for the mode of action of sodium cromoglycate. Biochem Pharmacor 1983; 32: 837-842.
5) Komatsu M. Studies on histamine in bronchial asthma: I. Japanese Journal of Allergology. 1987; 27: 67-74.
6) Ishizuka T, Ishizuka K. Triggering of histamine release from rat mast cells by divalent antibodies against IgE-receptor. J Immunol 1978;120:800-805.
7) Lagunoff D, Martin TM. Agents that release histamine rom mast cells. Ann Rev Pharmacol Toxicol 1983; 23: 331-351.
8) Lichitenstein LM. The mechanism of basophil histamine release induced by antigen and by the calcium ionophor A23187. J Immunol 1975; 114: 1692-1699.

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南米産樹木茶タヒボ抽出物のヒスタミン遊離抑制作用

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（要旨）
タベブイア・アベラネダエ粉末から抽出されたタヒボエキスの,ラット腹腔内肥満細胞からのヒスタミン遊離に対する作用を*in vitro* において調べた. 肥満細胞をcompound 48/80 またはconcanavaline A で刺激した際,通常の飲用濃度のタヒボエキスはどちらの刺激に対しても,濃度依存性にヒスタミン遊離を抑制した. タヒボエキスはcompound 48/80による刺激に対し,競合的阻害を示し,concanavaline Aによる刺激や,phosphatidylserine に対しては非競合的阻害を示した. 以上より,タヒボエキスはヒスタミンによるアレルギー反応を抑制することが示唆された

キーワード:タベブイア・アベラネダエ,タヒボ,肥満細胞,ヒスタミン遊離

緒　　言

南米産樹木茶タヒボ(TAHEEBO)は,ノウゼンカズラ科の学名*Tabebuia avellanedae* の樹皮の熱水抽出物で,南米ブラジルでは民間治療薬として種々の疾患に対して広く用いられている[1].近年,上田らによりタヒボ茶抽出物ナフトフランジオンがTPA誘発EBウイルス初期発現の活性化を抑制し,*in vitro* で発癌プロモーター活性を阻害することが報告され注目を集めた[2].また,原産地ブラジルではタヒボが抗炎症作用を有することが経験的に知られているだけであるので[1],今回,I型アレルギーの症状を引き起こすchemical mediatorのヒスタミンを含むラット腹腔内肥満細胞からのヒスタミン遊離に対するタヒボの作用を調べた.

方　　法

1. ラット腹腔内肥満細胞の分離,調製

Wistar系雄性ラット(8週齢,200g～230g)をエーテル麻酔下にて断頭,放血し,Hepes Buffer Medium [154 mM NaCl, 5.6 mM KCl, 1.0 mM $MgSO_4$, 1.0 mM $CaCl_2$, 10 mM Glucose, 0.1% BSA, 20 mM Hepes (pH 7.4)] 10 ml を腹腔内に注射し,2分間マッサージ後,腹腔内液を採取し[3],Parcoll gradient (Pharmacia, Uppsala, Sweden)を用いて純化した[4].得られた細胞をHepes Buffer Medium にて80×g,2℃,4分間にて3回洗浄し,細胞数を2.5×10^6cells/mlに調整し,肥満細胞画分として用いた.精製後の肥満細胞のpurityは91±4%,viabilityは95±4%であった。

2. タヒボ茶抽出物の調製

ブラジル連邦共和国ノゲイラ・シャーガス社が伐採したノウゼンカズラ科・*Tabebuia Avellanedae* の純正内部樹皮を原料とする樹木茶TAHEEBOをタヒボジャパン(株)より恵与を受けた. タヒボ(TAHEEBO)5gに蒸留水を加え1000mlとし,15分間沸騰させ,濾紙にて濾過した液を原液とし,必要に応じて蒸留水で希釈して実験に用いた. タヒボの濃度表示はこの原液を5g/1000ml＝5mg/mlとした.

3. ヒスタミン遊離及び定量

Hepes Buffer Medium にタヒボ抽出物, compound 48/80(C48/80), もしくはconcanavalin A (conA), phosphatidylserineを加え0.8mlとし,37℃,5分間preincubationを行い,これに上記肥満細胞画分0.2mlを加え, 37℃, C48/80刺激は5分間,conA刺激は15分間ヒスタミン遊離反応を行った.その後,5分間氷冷し,300×g,2℃,5分間遠心し,その上清をヒスタミン定量に用いた.ヒスタミン遊離実験操作はすべてプラスチック製試験管およびプラスチック製ピペットを用いた.

肥満細胞から遊離したヒスタミンは小松によるShoreらの改良法で定量を行った[5].肥満細胞から遊離したヒスタミンを含む反応液に2N PCAを0.25ml加え,1500×g,10分間遠心し,その上清にn-ブタノール:クロロホルム=3:2の溶液を3.5ml,および5N NaOH 0.27 ml を加え5分間振とうした.500×g,2分間遠心後,有機層3mlを別の試験管に移し,この有機層に0.1N NaCl,

1.2ml,n-ヘプタン3mlを加え,5分間振とうを行い,500×g,2分間遠心後,HCl層を別の試験管に1ml取り,1N NaOH 120μl, 0.2% *o*-Phthalaldehyde 100μlを加え,氷冷中に40分間放置し,その後,0.8N HCl 50μlを加え,励起波長360nm, 蛍光波長440nmで蛍光量を測定した.

結　　果

1. C48/80 刺激によるヒスタミン遊離に対するタヒボの作用

0.175μg/ml C48/80による肥満細胞からのヒスタミン遊離は1μg/ml以上の濃度のタヒボにより濃度依存性に抑制された(図1). ヒスタミン遊離量は% of total で表し,数値はmean±S.D.とした. 0.175μg/ml C48/80による肥満細胞からのヒスタミン遊離は26.3±1.2%(n=4)であり,同濃度C48/80存在下でタヒボ,1, 5, 10, 20, 40, 50μg/mlにより,24.2±1.1, 12.5±1.1, 8.3±0.6, 4.1±0.6, 1.0±0.5, 0.5±0.4%とそれぞれヒスタミン遊離は抑制された.

また,反応開始後,15秒,30秒,45秒,60秒,300秒でヒスタミン遊離量はそれぞれ,21±1.5,22.5±1.5,23.5±2.0, 23.5±2.0,25.5±2.1%であり,5μg/mlタヒボ存在下では16.0±1.0,10.8±0.8,11.0±1.5,10.0±1.3,10.5±1.8%であった(図2).

C48/80濃度を0.35μg/ml以上に上昇させると5μg/mlタヒボによるヒスタミン遊離抑制は解除された(図3). C48/80濃度0.2, 0.25, 0.3, 0.35, 0.4μg/mlにおいてヒスタミン遊離量はそれぞれ,25.8±1.9,27.6±1.6,28.5±1.3,29.7±2.0,35.3±1.8,35.3±1.9%であり,5μg/mlタヒボ存在下では,14.9±2.0, 20.2±2.4, 24.8±1.0, 27.3±1.5, 35.5±2.0, 35.3±1.9%であった.

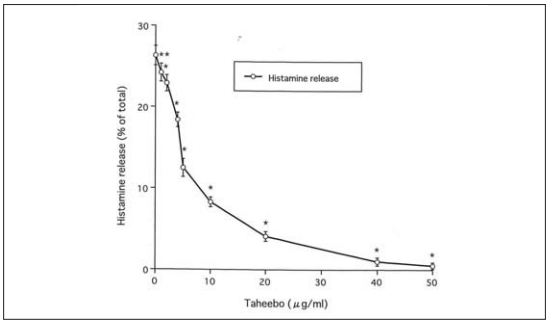

図1　0.175μg/ml C48/80刺激によるヒスタミン遊離に対するタヒボの作用。細胞は37℃で5分間培養した。データは4回の実験の平均±標準偏差値を表している。対照との有意差を認めた(** p <0.05、* p <0.01)。

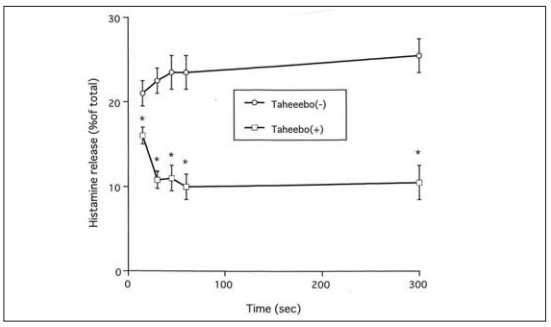

図2　培養時間でみたC48/80刺激によるヒスタミン遊離量。○:5μg/mlタヒボ無添加。□:5μg/mlタヒボ存在下(* p <0.01)。

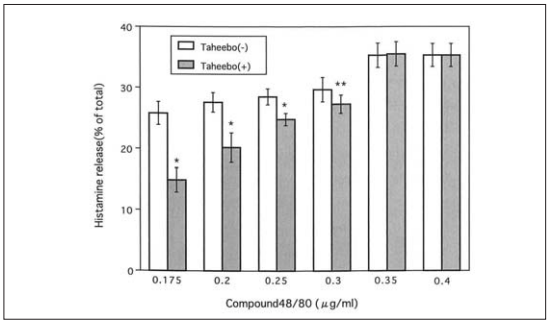

図3　さまざまな濃度のC48/80刺激下の作用を、5μg/mlタヒボを添加した場合(網掛け棒グラフ)と添加しない場合(白棒グラフ)とで調べた。細胞は37℃で5分間培養した。データは4回の実験の平均±標準偏差値を表している。対照との有意差を認めた(** p <0.05、* p <0.01)。

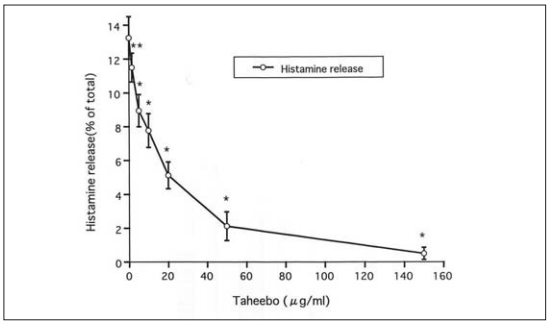

図4　0.175μg/ml C48/80刺激によるヒスタミン遊離に対するタヒボの作用をCa++を除いた反応液中で調べた。細胞は37℃で5分間培養した。データは4回の実験の平均±標準偏差値を表している。対照との有意差を認めた(** p <0.05、* p <0.01)。

Ca++ を除いた反応液中においてもタヒボはヒスタミン遊離を濃度依存性に抑制した(図4). Ca++ を除いた反応液中において,0.175μg/ml C48/80による肥満細胞からのヒスタミン遊離は13.3±1.2%(n=4)であり,同濃度C48/80存在下でタヒボ,5,10,20,50,150μg/mlにより9.0±0.9,7.8±1.0,5.1±0.8,2.1±0.8,0.5±0.3%とそれぞれヒスタミン遊離は抑制された.

また,反応開始後,30秒,45秒,60秒,300秒でヒスタミン遊離量はそれぞれ,12.0±0.8, 13.3±1.0, 13.3±0.8, 13.2±1.0%であり,10μg/mlタヒボ存在下では16.0±1.0,10.8±0.8, 11.0±1.5,10.0±1.3,10.5±1.8%であった(図5).

C48/80濃度を1.0μg/mlに上昇させると10μg/mlタヒボによるヒスタミン遊離抑制は解除された(図6). C48/80濃度0.2,0.4,0.6,0.8,1.0μg/mlにおいてヒスタミン遊離量はそれぞれ,17.0±1.0,20.1±1.1,24.8±1.4,30.0±1.5,30.0±

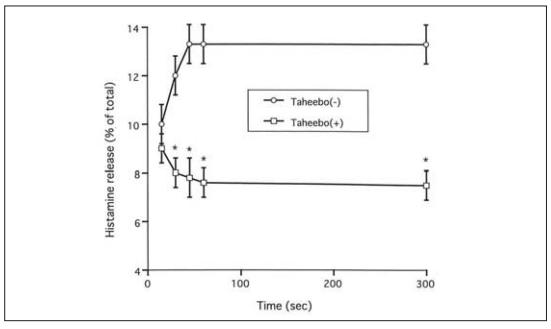

図5　培養時間でみた、Ca++を除いた反応液中における0.175μg/ml C48/80刺激によるヒスタミン遊離量。○:10μg/mlタヒボ無添加。□:10μg/mlタヒボ存在下。データは4回の実験の平均±標準偏差値を表している。対照との有意差を認めた(* p <0.01)。

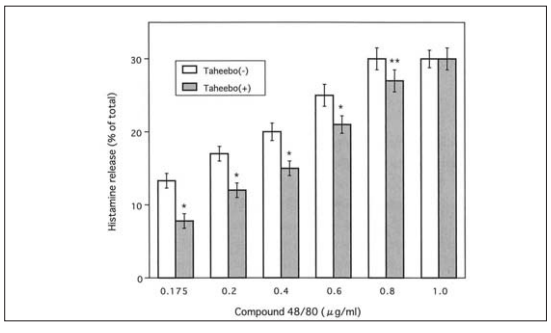

図6　さまざまな濃度のCa++を除いた反応液中におけるC48/80刺激下の作用を、10 μg/mlタヒボを添加した場合(網掛け棒グラフ)と添加しない場合　(白棒グラフ)とで調べた。細胞は37℃で5分間培養した。データは4回の実験の平均±標準偏差値を表している。対照との有意差を認めた(** p <0.05、* p <0.01)。

1.2%であり,10μg/mlタヒボ存在下では,12.1±0.9, 15.2±1.0,20.7±1.2,27.0±1.3,30.1±1.5%であった.

2. conA 刺激によるヒスタミン遊離に対するタヒボの作用

10μg/ml conAによる肥満細胞からのヒスタミン遊離は10μg/ml以上の濃度のタヒボにより濃度依存性に抑制された(図7). 10μg/ml conAによる肥満細胞からのヒスタミン遊離は11.3±0.8%であり,同濃度conA 存在下でタヒボ,10, 20, 50, 250, 500, 600μg/mlにより8.5±0.8, 7.9±0.7, 7.5±0.6, 2.8±0.5, 0.5±0.3, 0.3±0.2%とそれぞれヒスタミン遊離は抑制された.

また,反応開始後,1, 5, 10, 15, 30, 45, 60分でヒスタミン遊離量はそれぞれ,1.65±0.5, 4.3±0.5, 7.7±1.0, 11.0±1.1, 15.9±1.3, 17.4±1.1, 17.7±1.0%であり,50μg/mlタヒボ存在下では,0.44±0.3,2.7±0.5, 4.4±0.8, 6.4±1.1, 9.0±0.8, 9.2±0.9, 9.5±1.0%であった(図8).

50μg/mlタヒボ存在下で, conA濃度を200μg/mlまで上昇させてもタヒボによるヒスタミン遊離抑制は解除されなかった(図9).

さらに,50μg/mlタヒボ,10μg/ml conA 存在下で, phosphatidylserine濃度を40μg/mlまで上昇させてもタヒボによるヒスタミン遊離抑制は解除されなかった(図10).

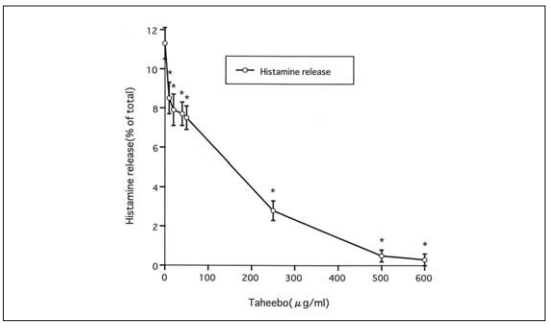

図7　10μg/ml Con Aと8 μg/mlホスファチジルセリンの刺激によるヒスタミン遊離に対するタヒボの作用。細胞は37℃で15分間培養した。データは4回の実験の平均±標準偏差値を表している。対照との有意差を認めた(* p <0.01)。

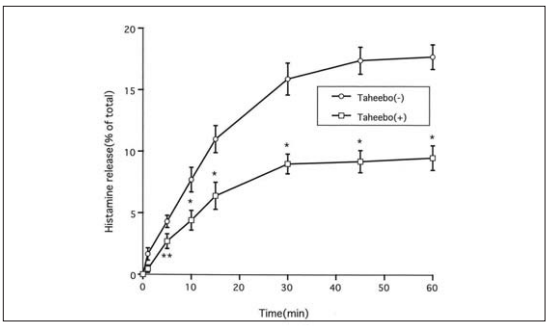

図8　培養時間でみた10 μg/ml Con Aと8 μg/mlホスファチジルセリンの刺激によるヒスタミン遊離量。○:50 μg/mlタヒボ無添加。□:50μg/mlタヒボ存在下。データは4回の実験の平均±標準偏差値を表している。対照との有意差を認めた(** p <0.05、* p <0.01)。

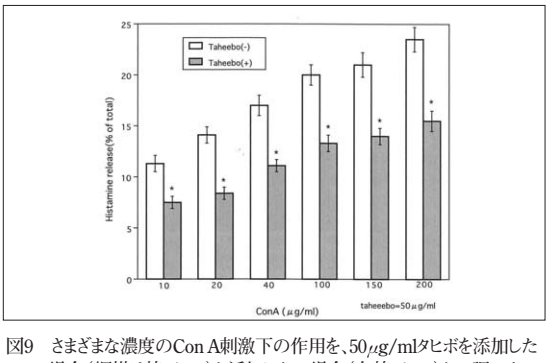

図9　さまざまな濃度のCon A刺激下の作用を、50μg/mlタヒボを添加した場合(網掛け棒グラフ)と添加しない場合(白棒グラフ)とで調べた。細胞は37℃で15分間培養した。データは4回の実験の平均 ±標準偏差値を表している。対照との有意差を認めた(* p <0.01)。

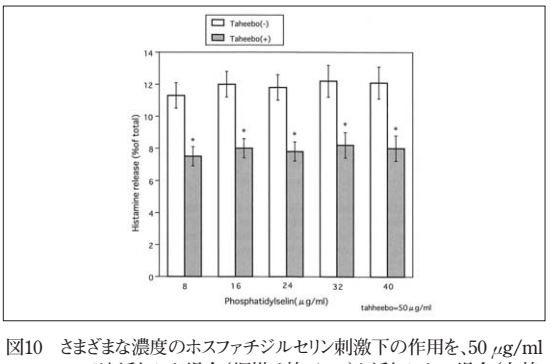

図10　さまざまな濃度のホスファチジルセリン刺激下の作用を、50 μg/ml タヒボを添加した場合(網掛け棒グラフ)と添加しない場合(白棒グラフ)とで調べた。細胞は37℃で5分間培養した。データは4回の実験の平均± 標準偏差値を表している。対照との有意差を認めた(* p <0.01)。

考　　察

生体内において肥満細胞からのヒスタミン遊離は細胞上の隣接する2つのIgE受容体が抗体による架橋されることによって引き起こされる.試験管内においてヒスタミン遊離を起こす物質としては,IgE受容体を介する物質としてconA,抗IgE抗体[6],IgE受容体を介さないものとしてcompound 48/80(C48/48)[7],A23187[8]等が知られている.

本報告では,C48/48,conAによるラット肥満細胞からのヒスタミン遊離に対するタベブイア・アベラネダエ粉末から抽出されたタヒボエキス(タヒボ)の作用を調べた.

通常の飲用濃度以下のタヒボは,C48/48またはconAによるラット肥満細胞からのヒスタミン遊離をともに濃度依存性に抑制した.

C48/80刺激時のタヒボのヒスタミン遊離抑制は,C48/80濃度を上昇させることにより解除されたことより,C48/80の作用部位においてタヒボ中のヒスタミン遊離抑制作用を有する物質はC48/80と競合的にヒスタミン遊離阻害を示すと考えられる.また,この解除は細胞外液Ca^{2+}存在下,非存在下の両方において観察されたことより,タヒボは細胞外からのCa^{2+}の流入を阻害したためにヒスタミン遊離抑制を示したものではないと考えられる.

IgE受容体を介するconAによる刺激に対するタヒボのヒスタミン遊離抑制は,conA濃度を上昇させることにより解除されなかったことより,タヒボ中のヒスタミン遊離抑制作用を有する物質の作用部位はIgE受容体ではないと考えられる.

以上より,タヒボは肥満細胞からのヒスタミン遊離を抑制することによって炎症反応を抑制し,I型アレルギーの症状を軽減することが示唆された.

謝　　辞

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参　考　文　献

1)ウォルター・ラダメス・アコーシ.奇跡の葉木　タヒボ.神戸新聞総合出版センター.　1988:48.

2)Ueda S, Umemura T, Dohguichi K, et al. Production of anti-tumorpromoting furanonaphthoquinonesin *Tabebuia Avellanedae* cell culture. Phytochemistry　1994; 36: 323-325.

3) 大坪憲弘.　肥満細胞からのヒスタミン遊離に関する研究.　岩手医誌.　1991; 43: 179-186.

4) Wells E, Mann J. Phosphorylation of a mast cell protein in response to treatment with anti-allergic compounds: implications for the mode of action of sodium cromoglycate. Biochem Pharmacor 1983; 32: 837-842.

5)小松道俊.　気管支喘息におけるHistamineに関する研究(第1報).　アレルギー.　1987;27:67-74.

6)Ishizuka T, Ishizuka K. Triggering of histamine release from rat mast cells by divalent antibodies against IgE-receptor. J Immunol 1978;120:800-805.

7)Lagunoff D, Martin TM. Agents that release histamine rom mast cells. Ann Rev Pharmacol Toxicol 1983; 23: 331-351.

8)Lichitenstein LM. The mechanism of basophil histamine release induced by antigen and by the calcium ionophor A23187. J Immunol 1975; 114: 1692-1699.