Biotherapy
バイオセラピー

Antlmetastatic Effect of Hot-Water Extract of TAHEEBO, Tabebuis Avellanedae Grown in South America

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Summary

The antitumor effect of TAHEEBO extract at a distance was examined in a double grafted tumor system, in which mice received simultaneous intradermal inoculations of Meth-A in both right (10^6 cells) and left (2×10^5 cells) flanks and were then injected with 0.1 mℓ of TAHEEBO extract (25 μg/mℓ naphthoquinones in water) in the right tumor on days 3, 4 and 5. The extract inhibited the growth of both the right tumor and the left, non-treated tumor. Immunosuppressive acidic protein (IAP) was produced by activated macrophages and neutrophils. IAP in serum of TAHEEBO extract-treated mice was measured as a marker protein of activated macrophages and neutrophils. IAP in serum was increased transiently soon after intradermal injection of 0.1 mℓ of TAHEEBO extract. The effect of TAHEEBO extract on *in vitro* invasion of murine RL♀-1 leukemia cells was studied using Biocoat Matrigel Invasion Chamber (Becton Dickinson Labware). TAHEEBO extract inhibited invasion of RL♀-1 cells for 24hr incubation. Antimetastatic effect of TAHEEBO extract on the spontaneous liver metastasis of RL♀-1 tumor in BALB/c mice was then examined. Intratumoral administration of this extract (0.1 mℓ × 3d) dose-dependently decreased the number of metastatic nodules.

Key words: Biological response modifier (BRM), Intratumoral administration, Immunosuppressive acidic protein (IAP), Invasion, TAHEEBO
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Introduction

We have confirmed that the administration of various biological response modifiers (BRMs) to primary tumors can cure not only the primary tumor itself, but also distant metastatic tumors, using a double grafted tumor system in mice that we developed. In other words, in cases of primary tumor alone, the surgical resection of the tumor is an appropriate procedure. However, in cases with distant metastatic tumors, particularly in the presence of metastatic microtumors that are macroscopically unidentifiable, the surgical resection of the tumor will not lead to the treatment of the cancer, but rather lead to the initiation of the proliferation of metastatic lesions. Thus, the treatment of metastatic lesions is a significant issue in cancer therapy.

Recently, it has been reported[1] that the consumption of at least 10 cups of green tea, which contains catechin as the major ingredient, can prevent lung cancer. We have studied the antimetastatic effect of a hot-water extract of TAHEEBO (TAHEEBO tea), which contains naphthoquinones as the major ingredient and is widely consumed in Brazil, South America. TAHEEBO tea is a hot-water extract from the bark of the NOUZENKAZURA tree whose scientific name is Tabebuia avellanedae. The major ingredient of TAHEEBO tea is the naphthoquinones shown in Fig. 1. Ueda et al.[3] reported that a naphthoquinone extracted from TAHEEBO tea suppresses the TPA-induced activation of the early antigen expression of EB virus and activates anti-tumor promoters in vitro.

We investigated further the effect of TAHEEBO extract on in vitro invasion associated with cancer metastasis. Then, the metastasis-inhibitory activity of the TAHEEBO tea extract was examined in the spontaneous lung metastasis model of Colon 26 tumor and the spontaneous liver metastasis model of RL male 1 tumor in BALB/c mice.

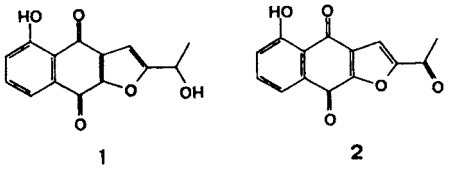
Ⅰ. Materials and Methods

1. Mice and tumor cells

Male BALB/c mice at 7 weeks of age were purchased from Japan S L C. The mice received intradermal inoculations of BALB/c Meth-A fibrosarcoma as a solid tumor. RL male 1 cells derived from BALB/c lymphoma, and Colon 26 cells derived from BALB/c colon carcinoma, were used as tumor cells with metastatic properties.

2. TAHEEBO tea

TAHEEBO, a bark tea, whose raw material is the pure inner bark of Tabebuia avellanedae (NOUZENKAZURA family), and is harvested by Noguera Chagas Co., Federative Republic of Brazil, was provided by TAHEEBO Japan Co., Ltd. Hot-water extracts of TAHEEBO, obtained by boiling 15 g of TAHEEBO tea in 900 mL of water either for 5 minutes or for 30 minutes, were used in the experiment. 5-min boiled and 30-min boiled hot-water extracts contained 25 mg/mL and 75 mg/mL of naphthoquinones, respectively.



furanonaphthoquinone

Fig. 1 TAHEEBO (*Tabebuia avellanedae*)

3. Double grafted tumor system

In this system, BALB/c mice receive simultaneous intradermal inoculations of Meth-A in both the right (10^6 cells) and left flanks (2×10^5 cells). From the third day when the tumor in the right flank (assumed to be the primary tumor) becomes large enough to be palpable, intratumoral administration of the drug is then started for 3 consecutive days. On the other hand, the non-treated distant tumor in the left flank (assumed to be a metastatic lesion) is observed for reduction in size. As the control, a "single tumor system" was prepared by intradermally inoculating 2×10^5 cells of Meth-A only into the left flank, followed by subcutaneous administration of the drug in the right side on the third day. This control system was included in order to exclude the direct cure of the left tumor by the drug delivered through the blood flow.

4. Evaluation of the anti-tumor effect

The tumor diameter was measured daily following the tumor inoculation. The tumor size was expressed in $\sqrt{}$ (major axis)$\times$ (minor axis) (mm). The judgment was made based on the tumor size and the tumor weight on the 21st day. The comparisons in tumor size and in tumor weight were performed by a t-test.

5. Measurement of serum IAP (immunosuppressive acidic protein) level

The serum IAP level following the intradermal administration of the drug in BALB/c mice was measured by the single radial immunodiffusion method (SRID).

6. Measurement of the invasion

A BIOCOAT matrigel invasion chamber (Becton-Dickinson Labware) was used as a model for the basement membrane, which is an extracellular matrix underneath the cells that line the blood vessels. As a tumor cell line, RL male 1 was used. Cell suspension ($1\text{-}2\times10^5$/200μmL) was placed on the matrigel-coated filter membrane that is contained in the upper chamber, and then the drug was added. In the lower compartment, 500μmL of FCS-RPMI-1640 was added. After incubation at 37℃ for 24 to 72 hours, the number of cells that had invaded the lower chamber through the filter with a pore size of 8μm was counted.

Table 1 Antitumor effect of "TAHEEBO" in the "double grafted tumor system" and "single tumor system"

Group	Right tumor (10^6 cells)		Left tumor (2×10^5 cells)	
	Tumor free /tested	Tumor weight (g±SD)	Tumor free /tested	Tumor weight (g±SD)
"Double-tumor" control	0/7	5.1±1.1	0/7	2.3±0.8
TAHEEBO (0.1 ml×3)	3/7	1.1±1.4	1/7	1.9±0.9
"Single-tumor" control	—	—	0/7	1.4±1.0
TAHEEBO (0.1 ml×3)	—	—	0/7	1.1±0.7

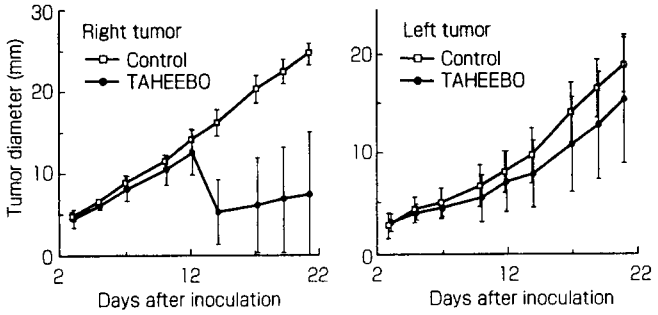


Fig. 2 Antitumor effect of TAHEEBO

7. Colon 26-induced spontaneous lung metastasis model

1×10^5 cells of Colon 26 were inoculated subcutaneously into BALB/c mice. Then, intratumoral administration of the TAHEEBO tea extract was given for three consecutive days (on the 10th, 11th and 12th days) after inoculation, followed by oral administration of 0.1 mL for the subsequent 10 days. On the 21st day after inoculation, the number of lung metastatic nodules was determined.

8. RL male 1-induced spontaneous liver metastasis model

1×10^6 cells of RL male 1 were inoculated subcutaneously in BALB/c mice. Then, intratumoral administration of the TAHEEBO tea extract was given for three consecutive days (on the 10th, 11th and 12th days) after inoculation. On the 19th day after inoculation, the number of liver metastatic nodules was determined.

Ⅱ. Experiment results

1. Anti-tumor effect of intratumorally administered TAHEEBO in the "double grafted tumor system"

From the 3rd day after inoculation of 0.1 mL of the tumor cell suspension, three administrations of the 5-min extract of TAHEEBO tea were given to the right tumor for three consecutive days. As a result, as shown in Table 1 and Fig. 2, the growth of the left and right tumors was suppressed. The right tumor disappeared in 3 out of 7 animals, and also the left tumor, which received no intratumoral administration, disappeared in 1 out of 7 animals. On the other hand, in the "single tumor system," the 3 subcutaneous administrations of 0.1 mL of the TAHEEBO tea extract to the right side did not suppress the growth of the left tumor. In other words, it is suggested that the administration of TAHEEBO tea extract to the primary tumor can lead to the cure of distant tumors through the body's immune functions, which is caused not by the direct action of TAHEEBO tea on the distant tumor, but by the action of the leukocyte and cytokine systems of the host[4].

Table 2 Induction of IAP in serum by TAHEEBO

Treatment	Control	TAHEEBO
Mouse 1	80	210
Mouse 2	55	210
Mouse 3	55	225
Mean IAP (μg/ml)	63.3	215**
SD	11.8	7.1

One day after intradermal injection of TAHEEBO (0.1 ml), serum IAP levels in BALB/c mice were assayed.
Significant difference from the control: **$p<0.01$

2. Induction of IAP in serum by administration of TAHEEBO tea extract

The results from Section 1 described above suggested the possibility of the involvement of immunological competence in the growth inhibition of the left tumor. Therefore, the production of immunosuppressive acidic protein, IAP, which is produced by activated neutrophils and macrophages, was investigated[4]. As a result of the investigation on serum IAP by the 3-day intradermal administration of 0.1 mL of TAHEEBO tea 5-min boiled hot-water extract, the induction of IAP was observed as shown in Table 2.

3. Inhibitory action of TAHEEBO tea extract on cancer cell invasions

Regarding the suppression of the growth of the right tumor in Section 1, it was highly likely that the TAHEEBO tea extract would directly act on the tumor cells. Therefore, the inhibitory action of the TAHEEBO effect on in vitro invasion was investigated. The RL male 1 cells derived from BALB/c lymphoma, were incubated for 24 hours with the 5-min boiled and 30-min boiled hot-water extracts of TAHEEBO. As a result, dose-dependent inhibition of the cell invasion was observed, as shown in Fig. 3.

4. Effect of orally administered TAHEEBO on Colon 26-induced spontaneous lung metastasis

Since the results of the investigation in Section 1 suggested that TAHEEBO might suppress the metastasis also in spontaneous metastasis models, the following experiment was performed. Colon 26 cells were inoculated subcutaneously in BALB/c mice. Then, intratumoral administration of 0.1 mL of the 5-min boiled TAHEEBO tea extract was given for three consecutive days from the 10th day after inoculation when the tumor became sufficiently large. In addition, 10 oral administrations of 0.1 mL of the TAHEEBO tea extract were given from the 8th to 12th day and 14th to 18th day after the tumor inoculation. On the 21st day after inoculation, the number of nodules metastasized to the lungs was determined. The number of nodules was decreased from 8.4± 5.9 to 3.6± 3.4, showing significant suppression of metastasis in animals receiving concomitant oral treatment.

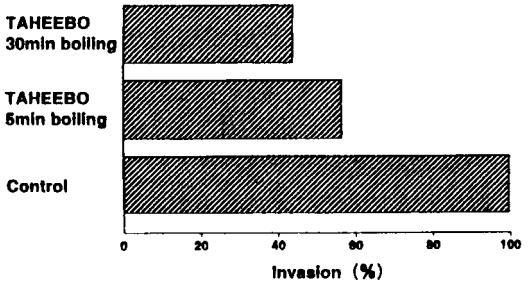


Fig. 3 Inhibitory action of TAHEEBO on RL♀-1 cell invasion

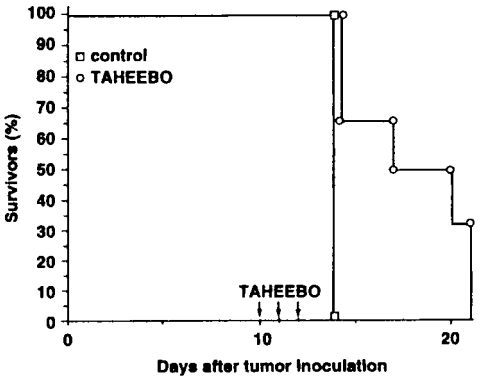


Fig. 4 Survival curves of BALB/c mice treated intratumorally with TAHEEBO in Colon 26 tumor

5. Life-prolonging effect of TAHEEBO intratumorally administered to Colon 26-treated mice

Colon 26 cells were inoculated subcutaneously in BALB/c mice. Then, intratumoral administration of 0.1 mL of the 5-min boiled TAHEEBO tea extract was given for three consecutive days from the 10th day after inoculation. As a result, a life-prolonging effect was observed as shown in Fig. 4.

6. Effect on RL male 1-induced spontaneous liver metastasis

The results from the investigation of Section 3 suggested that TAHEEBO might also prevent spontaneous liver metastasis in vivo. Therefore, the following experiment was conducted. RL male 1 cells were inoculated subcutaneously in BALB/c mice. Then, intratumoral administration of 0.1 mL of the 5-min boiled TAHEEBO tea extract was given for three consecutive days from the 10th day when the tumor became sufficiently large. On the 19th day, the weight and size of the primary tumor, the weight of the metastasized liver, and the number of metastatic nodules were determined. As a result, TAHEEBO significantly suppressed liver metastasis.

Then, this experiment was repeated using 5-min and 30-min boiled extracts of TAHEEBO tea. As shown in Fig. 5, naphthoquinones significantly and dose dependently suppressed liver metastasis of RL male 1 tumor.

Table 3 Anti-metastatic effect on RL ♂-1 tumor by intratumoral admini stration of TAHEEBO in BALB/c mice

	No. of liver metastasis[1] (Nodules)	
Treatment	Control	TAHEEBO[2]
Mouse 1	250	250
2	250	98
3	71	134
4	250	250
5	42	66
6	250	250
7	94	22
Mean±SD	172.4±90.6	152.9±89.7*

Significant difference from the control: * $p<0.05$

1) : No. of liver metastatic colonies was counted 19 days after RL ♂ 1 (2×10^6) s.c. inoculation.

2) : TAHEEBO (0.1 m*l*) was injected i.t. 10, 11 and 12 days after tumor inoculation.

Ⅲ. Discussion

We investigated the most effective administration method for BRM. As a result, we found that the administration of BRM to the primary tumor in the ♂double grafted tumor system♀ can cure not only the primary tumor, but also distant metastatic tumors[5].

The results obtained this time revealed that the TAHEEBO extract suppresses the growth of right and left tumors in the "double grafted tumor system" (Table 1 and Fig. 2), and induces IAP in serum (Table 2). These results suggest that macrophages and neutrophils are activated by the administration of TAHEEBO tea and are introduced into tumors, which triggers the series of cascade reactions involving cytokines and leukocytes, resulting in the suppression of the growth of distant metastatic tumors[6].

On the other hand, it was considered possible that the TAHEEBO tea extract would also directly act on tumor cells. Therefore, its inhibitory action on in vitro invasion was investigated. As a result, marked inhibition of cell invasion was observed as shown in Fig. 3. Metastatic cancer cells possess enzymatic activities capable of degrading the components of the basal membrane, such as laminin, collagen, fibronectin, and heparan sulfate proteoglycan. Regarding this, it is suggested, from this invasion study, that these enzymatic activities may be suppressed by the TAHEEBO tea extract and therefore TAHEEBO may also be involved in the in vivo suppression of metastasis.

Therefore, the in vivo inhibitory effect of the TAHEEBO tea extract was investigated in the spontaneous metastasis model. As a result, the TAHEEBO tea extract inhibited the metastasis of the RL male 1 tumor as shown in Table 3 and Fig. 5. The details of the inhibitory mechanism will be further investigated.

This research was partially supported by a research grant of Sendai Microbiology Laboratories.

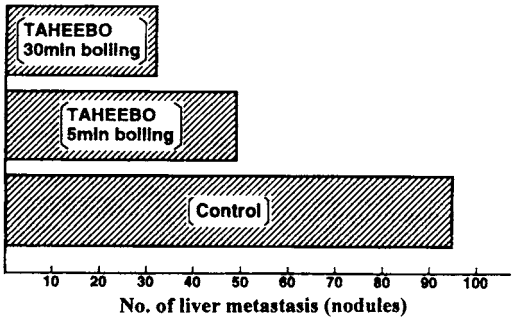


Fig. 5 Anti-metastatic effect on RL ♂-1 tumor by intratumoral administration of TAHEEBO

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バイオセラピー

南米産樹木茶タヒボ抽出物の抗腫瘍効果

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要旨　南米産樹木茶タヒボの熱水抽出物（有効成分としてナフトキノンを含む）の転移抑制活性についてわれわれが考案したマウス“二重移植腫瘍系”で解析した。BALB/c マウスに Meth-A 腫瘍細胞を右側腹皮内に 10^6，左側腹皮内に 2×10^5 個接種し，3 日目から 3 日間，水 900 m*l* にタヒボ茶 15 g を加えて 5 分間沸騰させた熱水抽出物（このなかに 25 μg/ m*l* のナフトキノンが含まれる）を 0.1 m*l* ずつ右側腫瘍内に投与した結果，左側遠隔転移腫瘍の増殖を抑制した。その作用機序として，タヒボ茶の投与が血中に免疫抑制酸性蛋白である IAP を誘導し，Mϕ ならびに好中球を活性化していた。次に転移抑制機序の一つとして腫瘍細胞の浸潤抑制活性について調べた結果，タヒボ茶の処理はマウスリンパ腫 RL♀-1 細胞の浸潤を阻害した。そこで *in vivo* における自然転移モデルで転移抑制活性を調べた結果，タヒボ茶抽出物の 3 回の腫瘍内投与は RL♀-1 の肝転移を濃度依存的に阻害した。

はじめに

各種 BRM を原発腫瘍内に投与すると原発腫瘍のみならず，遠隔転移腫瘍まで治癒させるもののあることを，われわれが考案したマウス“二重移植腫瘍系”で明らかにしてきた。すなわち腫瘍がまだ原発巣しかない場合，手術して摘出すれば問題はないが，すでに遠隔転移巣がある場合，特に肉眼で確認できない微小転移巣がある場合は，手術によって原発巣を摘出すると転移巣が増殖を開始し，癌治療には結び付かない。すなわち，転移巣の治療が癌治療の大きな課題といえる。

最近カテキンを主成分とする緑茶の 1 日 10 杯以上の飲用が肺癌の予防につながるとの報告がでた[1)]。そこで南米ブラジルで広く飲用されているナフトキノンを主成分とする TAHEEBO（タヒボ茶）の熱水抽出物の転移抑制効果について検討を加えたので報告する。タヒボ茶はノウゼンカズラ科の学名 *Tabebuia avellanedae* の樹皮の熱水抽出物でその主成分は Fig. 1 に示すようなナフトキノンである。上田らはタヒボ茶抽出物ナフトキノンが TPA 誘発 EB ウイルス初期抗原の活性化の抑制を示し *in vitro* で抗発癌プロモーター活性を有する成分であることを報告している[3)]。

癌転移に関与している *in vitro* 浸潤能に及ぼすタヒボ茶抽出物の効果について検討を加え，次に Colon 26 の自然肺転移モデルと RL♀-1 の自然肝転移モデルを使ってタヒボ茶抽出物の BALB/c マウスにおける転移抑制活性を調べた。

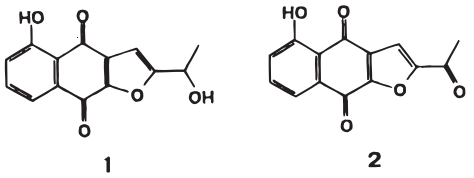
I. 材料と方法

1. マウスと腫瘍細胞

日本エスエルシー（株）より購入した 7 週齢 BALB/c 雄マウスを使用した。腫瘍は BALB/c マウスと同系の Meth-A 線維芽肉腫細胞を皮下に接種し固形腫瘍として使用した。転移能のある腫瘍細胞として BALB/c マウスリンパ腫由来 RL♀-1 細胞，BALB/c マウス結腸癌由来 Colon 26 細胞を使用した。

2. タヒボ茶

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。タヒボ茶（TAHEEBO）15 g を 900 m*l* の水に入れ 5 分間沸騰させた熱水抽出物ならびに 30 分間沸騰させた熱水抽出物を実験に供した。5 分間沸騰させた熱水抽出物には 25 μg/m*l* の，30 分間沸騰させた熱水抽出物には 75 μg/m*l* のナフトキノンが含まれていることがわかっている。



フランナフトキノン

図1　TAHEEBO（タベブイア・アベラネダエ）

3. 二重移植腫瘍系

BALB/c マウスの右側腹皮内に 10^6 個，左側腹皮内に 2×10^5 個の Meth-A 細胞を同時に移植し，右側の大きな腫瘍（原発巣と想定）が指で触れるようになる 3 日目より腫瘍内に薬剤を 3 日間連日投与することにより治療し，治療していない左側の遠隔腫瘍（転移巣と想定）の退縮を観察する系である。対照として左側腹皮内にだけ 2×10^5 接種し 3 日目に右側皮下に薬剤を投与する“single tumor system”を作り，薬剤が血流を介して左側腫瘍を直接治癒させるものと区別した。

4. 抗腫瘍効果の評価

腫瘍接種後経日的に腫瘍径を測定し，√長径×短径（mm）で腫瘍の大きさを表し，21日目の腫瘍重量（g）とともに判定した。腫瘍の大きさならびに腫瘍重量の比較にはt検定を行った。

5. 血清IAP（immunosuppressive acidic protein）値測定

BALB/cマウスに薬剤を皮内注射した時の血清IAP値をsingle radial immunodiffusion（SRID）法を用い，測定した。

6. 浸潤能測定

血管内皮細胞の下側に存在する細胞外基質である基底膜のモデルとしてBIOCOAT matrigel invasion chamber（Becton-Dickinson Labware）を用い，腫瘍細胞としてはRL♀-1を用いた。フィルター上面にmatrigelがコートされているチャンバー上室に細胞浮遊液（$1\sim2\times10^5/200\mu l$）を入れ，これに薬剤を添加した。下室には10％FCS/RPMI-1640 $500\mu l$を入れ，37℃で24～72時間インキュベートした後，孔径$8\mu m$のフィルターを通過して下室に浸潤した細胞数を計測した。

Table 1 Antitumor effect of "TAHEEBO" in the "double grafted tumor system" and "single tumor system"

Group	Right tumor (10^6 cells)		Left tumor (2×10^5 cells)	
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TAHEEBO (0.1 ml×3)	3/7	1.1±1.4	1/7	1.9±0.9
"Single-tumor" control	—	—	0/7	1.4±1.0
TAHEEBO (0.1 ml×3)	—	—	0/7	1.1±0.7

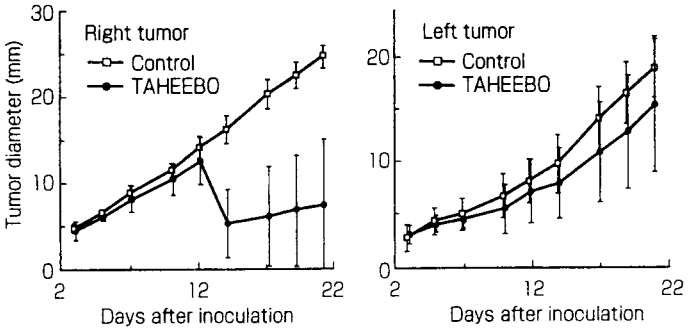

Fig. 2 Antitumor effect of TAHEEBO

7. Colon 26自然肺転移モデル

BALB/cマウスの皮下に1×10^5個のColon 26細胞を接種し，タヒボ茶抽出物を接種後10，11，12日目の3日間腫瘍内投与し，さらに10日間0.1 mlずつ経口投与し，21日目に肺転移結節数を算定した。

8. RL♀-1自然肝転移モデル

BALB/cマウスの皮下に1×10^6個のRL♀-1細胞を接種し，タヒボ茶抽出物を接種後10，11，12日目の3日間腫瘍内投与し，19日目に肝転移結節数を算定した。

II. 実験結果

1. 腫瘍内投与による"二重移植腫瘍系"における抗腫瘍効果

タヒボ茶5分間抽出物を0.1 ml腫瘍移植後3日目から3，4，5日目の3回右側腫瘍内に投与したところ，Table 1，Fig. 2に示すように左・右の腫瘍の増殖を抑制した。右側腫瘍で，7匹中3匹で腫瘍がなくなり，投与していない左側腫瘍も7匹中1匹で腫瘍が消失した。一方"single tumor system"でタヒボ抽出物を0.1 mlずつ3回右側皮下に投与しても左側腫瘍の増殖を抑えることはできなかった。すなわちタヒボ茶抽出物は原発腫瘍内に投与することにより体の免疫機能が働いて遠隔腫瘍も治癒させることができ，この時直接タヒボ茶が遠隔腫瘍に達して作用しているのではなく，原発腫瘍内に投与することにより，宿主の白血球系とサイトカイン系が働き遠隔腫瘍を治癒させていることが示唆された[4]。

Table 2 Induction of IAP in serum by TAHEEBO

Treatment	Control	TAHEEBO
Mouse 1	80	210
Mouse 2	55	210
Mouse 3	55	225
Mean IAP (μg/ml)	63.3	215**
SD	11.8	7.1

One day after intradermal injection of TAHEEBO (0.1 ml), serum IAP levels in BALB/c mice were assayed.
Significant difference from the control: **$p<0.01$

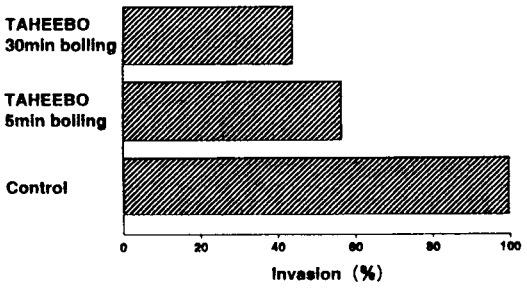

Fig. 3 Inhibitory action of TAHEEBO on RL⇕-1 cell invasion

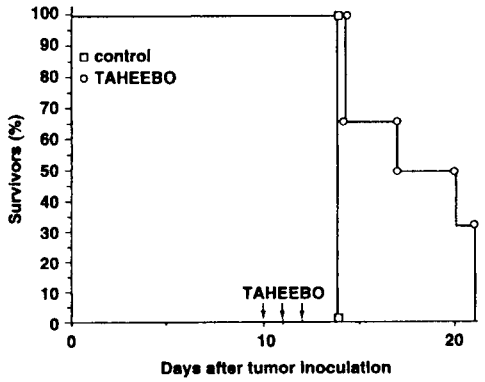

Fig. 4 Survival curves of BALB/c mice treated intratumorally with TAHEEBO in Colon 26 tumor

2. タヒボ茶抽出物投与による血清IAPの誘導

1. の結果から左側腫瘍の増殖抑制に免疫能が働いている可能性があるので，活性化好中球ならびにマクロファージが産生することが知られている免疫抑制蛋白IAPの産生に関して検討した[4]。タヒボ茶5分間熱水抽出物0.1 mlを3日間皮内投与した時の血清IAP値を調べた結果Table 2に示すようにIAPの誘導が認められた。

3. タヒボ茶抽出物の癌細胞浸潤阻害

1. の右側腫瘍の増殖抑制に関してタヒボ茶抽出物が腫瘍細胞に直接働いている可能性が高いので，*in vitro*の浸潤阻害能について検討した。BALB/cマウスリンパ腫由来RL⇕-1細胞にタヒボ茶抽出物5分間煮沸抽出物ならびに30分間煮沸抽出物を24時間処理したところFig. 3に示すように濃度依存的に浸潤を阻害した。

4. Colon 26自然肺転移に対するタヒボ茶抽出物の経口投与による効果

1. の結果から自然転移モデルでも転移を抑制している可能性があるので以下の実験を行った。Colon 26細胞をBALB/cマウスの皮下に接種し，腫瘍が大きくなった10日目から3日間腫瘍内にタヒボ茶5分間抽出物を0.1 mlずつ投与し，さらに腫瘍接種8～12日目，14～18日目まで10回タヒボ茶抽出物を0.1 mlずつ経口投与し，21日目の肺に転移した結節数を調べたところ，結節数が8.4±5.9から3.6±3.4に減少し，経口投与併用群で有意に転移を抑制していた。

5. Colon 26接種マウスのタヒボ茶抽出物腫瘍内投与による延命効果

BALB/cマウスにColon 26細胞を皮下接種し，接種後10日目より3日間腫瘍内にタヒボ茶5分間熱水抽出物を0.1 mlずつ投与したところ，Fig. 4のごとく，延命効果が認められた。

6. RL⇕-1自然肝転移に対する効果

3. の結果からRL⇕-1の*in vivo*における自然肝転移も予防する可能性があるので，以下の実験を行った。RL⇕-1細胞をBALB/cマウスの皮下に接種し，腫瘍が大きくなった10日目から3日間，タヒボ茶5分間煮沸抽出物を0.1 mlずつ腫瘍内に投与し，19日目に原発腫瘍重量・大きさと転移した肝臓の重量と転移結節数を算定した。その結果Table 3に示すように有意に肝転移を抑制していた。

次にタヒボ茶抽出物の5分間煮沸抽出物と30分間煮沸抽出物で同様の実験を行ったところFig. 5のごとくナフトキノンの濃度依存性にRL⇕-1の肝転移を有意に抑制していた。

Table 3 Anti-metastatic effect on RL⇕-1 tumor by intratumoral admini stration of TAHEEBO in BALB/c mice

	No. of liver metastasis[1] (Nodules)	
Treatment	Control	TAHEEBO[2]
Mouse 1	250	250
2	250	98
3	71	134
4	250	250
5	42	66
6	250	250
7	94	22
Mean±SD	172.4±90.6	152.9±89.7*

Significant difference from the control: *$p<0.05$
1) : No. of liver metastatic colonies was counted 19 days after RL⇕1 (2×10^6) s.c. inoculation.
2) : TAHEEBO (0.1 ml) was injected i.t. 10, 11 and 12 days after tumor inoculation.

III. 考　察

今までわれわれはBRMの最も効果的な投与法を検討した結果，“二重移植腫瘍系”の原発腫瘍内にBRMを投与することによって原発腫瘍のみならず，遠隔転移腫瘍まで治癒させることを見いだしてきた[5]。

今回タヒボ茶抽出物が“二重移植腫瘍系”で左右腫瘍の増殖を抑制し，(Table 1, Fig. 2) 血清IAPを誘導すること (Table 2) からタヒボ茶を投与することにより，マクロファージと好中球が活性化し，腫瘍内に好中球とマクロファージが誘導され，一連のサイトカイン・白血球カスケード反応が起こり，遠隔転移腫瘍の増殖が抑制されることが示唆された[6]。

一方タヒボ茶抽出物が腫瘍細胞にも直接働いている可能性があるので，まず *in vitro* における浸潤阻害について調べたところ Fig. 3 に示すように著明に浸潤阻害がみられた。転移性癌細胞が基底膜成分である laminin, collagen, fibronectin, heparan sulfate proteoglycan などを分解する酵素活性をもっているのに対し，タヒボ茶抽出物がそれらの酵素活性を抑制していることが考えられ *in vivo* における転移抑制にも関与していることが示唆された。

そこで *in vivo* における自然転移モデルでのタヒボ茶抽出物による抑制効果を調べたところ Table 3, Fig. 5 に示すように RL⇕-1 の転移を抑制していることがわかった。今後さらにその詳細な阻害機構に検討を加えたい。

本研究の一部は（財）仙台微生物研究所の研究助成金により行った。

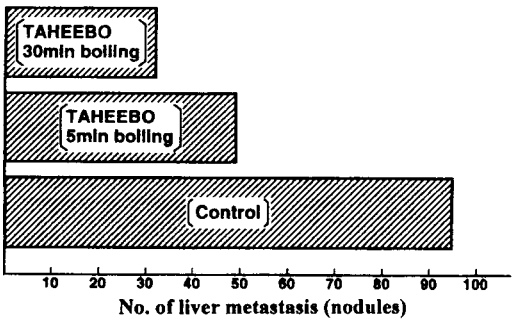

Fig. 5 Anti-metastatic effect on RL⇕-1 tumor by intratumoral administration of TAHEEBO

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