「Production of bioactive furanonaphthoquinones by callus cultures of the Brazilian medicinal plant, *Tabebuia avellanedae*」

ブラジル原産*Tabebuia avellanedae*カルスによるがん細胞増殖抑制活性ナフトキノン類の生産

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【目的】

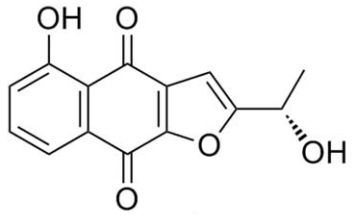
最近我々は、ブラジル原産*Tabebuia avellanedae*（ノウゼンカズラ科）の内皮にごくわずかに含まれる強力ながん細胞増殖抑制活性やがん予防活性をもつナフトキノンNQ801のグラムスケールでの化学合成[1]に成功した。一方で、我々は、多種類の化学薬品を用いない環境にも配慮したNQ801の大量生産にも興味を持っている。そこで、*T. avellanedae*の葉より誘導されたカルス組織を用いたNQ801の生産を試みた。

【方法･結果】

エタノール（70%, 1分）と次亜塩素酸ナトリウム（1%、10分）で滅菌したT. avellanedaeの葉を2,4ージクロロフェノキシ酢酸（2,4ーD, 1ppm ）とキネチン（1ppm）を含むMS寒天培地（pH 5.95～6.05）に移し、25℃、暗条件で培養した。約一週間でカルス化が観察されると同時に、MS寒天培地が黄色化し、NQ801を含むナフトキノン類の生産が示唆された。培養開始2週間以降、5日置きに培地をクロロホルムで抽出、クロロホルム層を水洗後、濃縮して得られた抽出物をHPLCならびにLC/MSで分析した。興味深いことにNQ801が主成分としてNMRでも明確に分かるほど生産され、それは、培養期間とともに増加していることが示された。また、NQ801の類縁体と推測されるいくつかのキノン類の生産も観察された。さらに、生物活性とともに植物ホルモン種の違いによる生産性の変化についても調べたので、それらの結果も併せて報告する。

■English abstract

The Bignoniaceaeous plant, *Tabebuia avellanedae* Lorentz ex Griseb is native to South America from Brazil to north Argentina and has been known as a useful medicinal plant since the Incan Era. Findings of the antitumor activity of an alcoholic extract of the stem bark of this plant and efforts to find clinically acceptable antitumor compounds led to the isolation of (−)-5-hydroxy-2-(1'-hydoxyethyl)naphtho[2,3-b]furan-4,9-dione (1) that shows potent antitumor and cancer chemopreventive activity.
Naphthoquinone 1 can be obtained from the inner bark of only wild *T. avellanedae* plants of over 20 years old. Furthermore, the yield of 1 from the inner bark of the tree is very low (less than 0.001%). However, artificial propagation of this plant is very difficult. These facts promoted us to develop a naphthoquinone-producing cell line.
The seedings (leaf disks) of *ca* 10 mm in length were transferred to the MS medium supplemented with 2,4-D (1ppm) and Kinetin (1ppm) and incubated at 25 ˚C in the dark for one week to give callus tissues. After an incubation period of two to four weeks, the MS medium was extracted with chloroform. The chloroform phase was washed with water, dried over sodium sulfate and concentrated in reduced pressure to yield a yellowish residue. Analysis of the residue by HPLC and LCMS showed the production of 1 and its analogues in addition to unidentified compounds. Furthermore, the presence of 1 was also confirmed by the comparison of the 1H-NMR spectrum of the extract with that of the authentic 1.
We report herein the production of 1 by callus cultures of *T. avellanedae*. We also describe the anti-proliferative activity of the extract against several human tumor cell lines.



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1) Bioorg. Med. Chem., 2009, *17*, 6286−6291.

「Search for anti-inflammatory compounds using the SENCAR mouse-induced SST cells」

SENCARマウス由来SST細胞を利用した抗炎症活性化合物の探索

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【目的】

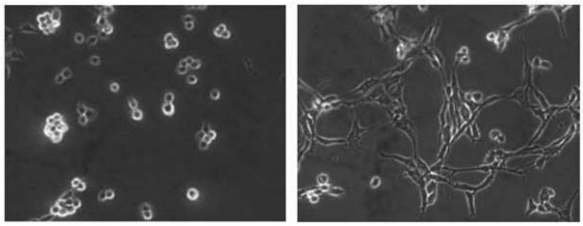
炎症は腫れや痛みなどの症状を引き起こすばかりでなく、がんやアレルギーならびに生活習慣病を引き起こす原因でもある。最近では、動脈硬化や糖尿病といった生活習慣病が炎症メディエーターによって引き起こる慢性の炎症によるものだということがわかってきた。このため、生活習慣病予防や進行の抑制のためにも炎症をいかに抑えていくかが重要になる。抗炎症活性を調べる方法としては、マクロファージ様細胞株RAW264.7細胞などを用いるいくつかのアッセイ法が知られている。今回、我々は以前から抗炎症活性試験・抗腫瘍活性試験で用いていたSENKER mouseの皮膚から樹立したSST細胞（SENKER mouse skin transformed cells）を用いるアッセイを試みた。

【結果】

シャーレ底部に密着したSST細胞をUVB10分間照射またはホットプレートで1分間加熱後、48h培養し細胞の形態変化を観察した。また、SST細胞にインドメタシンを200μg/ml添加して上記で述べた方法で実験を行い、細胞の形態変化を観察した。

【考察】

SST細胞はUVB照射や加熱の刺激により細胞傷害を引き起こし、紡錘状から丸状に形態変化を引き起こした。一方で、インドメタシンを添加したSST細胞は形態変化が抑制された。以上の結果から、SST細胞の形態変化を指標として抗炎症活性を評価できることがわかった。



Damaged SST cells　　SST cells

■English abstract

Inflammation causes not only the symptom such as puffiness and swelling but also allergy diseases and lifestyle diseases. Recent findings indicate that chronic-inflammation attributed to mediator of inflammation causes lifestyle diseases such as arteriosclerosis and diabetes.

Therefore it is of importance to suppress inflammation for preventing lifestyle diseases and controlling the development of the diseases. In general, RAW264.7 macrophages are used to examine anti-inflammatory activity. Recently, we have developed a new assay method using the SENCAR mouse transformed (SST) cells. A SENCAR mouse is a model animal sensitive to inflammation SST cells were cultured in dishes at density of 1.0×10^5cells/ml. When the cells were confluent, they were exposed to UVB irradiation for one minute or heated on hotplate at 80°C for ten minutes. Then the dishes were further incubated at 37°C for 48 h after the addition of indomethacin. Morphological changes of the cells were observed under a microscope to examine effect of anti-inflammation.

Damaged SST cells by heating and UVB irradiation were morphologically changed from spindle cells to spherical cells. On the other hand, the morphological change of SST cells treated with indomethacin was suppressed. Therefore, it has been found that the morphological change of SST cells is useful for estimating anti-inflammatory activity. Herein we report the isolation of some anti-inflammatory compounds from the alcoholic extract of the Brazilian medicinal plant, *Tabebuia avellanedae*.