widely used in the study of conditioned avoidance responding. Our experimental conditions being limited, we used an ordinary voltage device in this study. In this case, the learning acquisition performance of control rats appeared too high to be convenient to the study of cognition-enhancing drugs, however, GSLS still showed the facilitation on learning and memory.

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人参茎叶皂甙对大鼠单路回避行为的影响

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提要 用穿梭箱法，研究多次 ip 人参茎叶皂甙(GSLS)对大鼠学习和记忆的影响. GSLS 10, 30, 60 mg·kg^{-1} 以钟形方式缩短回避潜伏期从而易化了学习和记忆获得. GSLS 30 mg·kg^{-1} 增加回避次数和/或缩短回避潜伏期从而改善了东莨菪碱(0.8 mg·kg^{-1} sc)遗忘和环己酰亚胺(2.5 和 5 mg·kg^{-1} ip)遗忘.

关键词 皂甙类；回避学习；记忆；东莨菪碱；环己酰亚胺；获得；保持 (心理学)；人参

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Inhibitory effect of triptolide on colony formation of breast and stomach cancer cell lines

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ABSTRACT Triptolide (Tri) is a diterpenoid triepoxide isolated from *Tripterygium wilfordii* Hook F. The effects of Tri on the colony formation of breast cancer cell lines MCF-7 and BT-20, stomach cancer cell lines MKN-45, MKN-7, and KATO-III, and promyelocytic leukemia cell line HL-60 were reported. Using Hamburger-Salmon's double layer agar technique with certain modifications, cancer cells were cultured in 0.3% agar in a highly humidified atmosphere of 5% CO_2 at 37℃ for 14-21 d. Colonies were counted on d 14 (occasionally d 21) with the colony analyzer system CA-7A. Of the 5 solid tumor cell lines tested, 4 showed duminished colony formation in soft agar by >70% of control value in Tri 10^{-8} mol·L^{-1} (continuous exposure). The magnitudes of the inhibitory effect of Tri on most breast and stomach cancer cell lines were similar to that on the leukemia cell line HL-60. IC_{50} were 0.504-1.22 μg·L^{-1}. The clinically achievable peak

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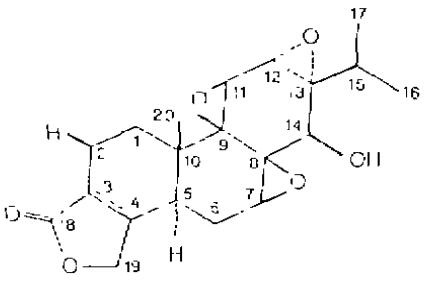
plasma concentration (PPC) of Tri was estimated as 0.15 mg · L^{-1}, being 72–126 times higher than the IC_{70} of the cancer cell lines except KATO–III. The results suggest that Tri might have a potential therapeutic effect on some types of solid tumors, e.g., breast and stomach cancers.

KEY WORDS triptolide; colony-forming units assay; cell line; cultured tumor cells; breast neoplasms; stomach neoplasms; leukemia

Triptolide (Tri), a diterpenoid triepoxide isolated from *Tripterygium wilfordii* Hook F, showed significant effect against the L1210 and P388 leukemias in mice and *in vitro* against cells derived from human carcinoma of the nasopharynx (KB), and was recommended as an antileukemic agent[1,2], but no published clinical report against leukemia has been seen and little is known about its effect on tumor stem cells. This urged us to study whether it is active against other types of tumor cells. The inhibitory effects of Tri on the colony formation of human breast cancer cell lines MCF–7 and BT–20 and stomach cancer cell lines MKN–45, MKN–7, and KATO–III are reported.



Triptolide

MATERIALS AND METHODS

Tumor cell lines Six human tumor cell lines maintained in our division were used in the clonogenic assay. They were breast cancer cell lines MCF–7 and BT–20, stomach cancer cell lines MKN–45, MKN–7, and KATO–III, and promyelocytic leukemia cell line HL–60. The cell lines except BT–20 were propagated in RPMI1640 medium (Nissui Pharmaceutical Co), supplemented by 10% heat–inactivated fetal calf serum (Boehringer Mannheim GmbH, Germany), penicillin 100 units · ml^{-1} and streptomycin 100 μg · ml^{-1} (RPMI–FCS) in a highly humidified atmosphere of 5% CO_2 at 37℃. BT–20 cells were propagated in Eagle minimum essential medium (EMEM, Grand Island Biological Company, GIBCO, New York, USA), with the same supplements mentioned above.

The cell lines were subcultured routinely once a week, but three times a week for HL–60. The cell cultures were ascertained free of mycoplasma contaimination by the immunofluorescent examination.

Clonogenic assay The clonogenic assay system was a modification of the double agar method[3]. Viability of the tumor cells was evaluated by trypan blue dye exclusion test. One ml of tumor cell suspension in medium–FCS with 0.3% agar (Bacto–agar, Difco, Detroit, MI, USA) was layered onto 1 ml of prepared underlayer in a 35 mm Falcon plastic Petri dish (Becton Dickinson & Co, Oxnard, CA, USA). The medium–FCS was RPMI–10% FCS for MCF–7, MKN–45, MKN–7, and KATO–III, RPMI–15% FCS for HL–60, and EMEM–10% FCS for BT–20. The underlayer contained 0.5% agar in the same medium–FCS. For HL–60, after agar solidification, 0.1 ml of GCT conditioned medium (GIBCO, NY, USA) was added onto the surface of the agar of each dish. After plating, the tumor cells were inspected under the inverted microscope to confirm that there was no tumor cell clump in the Petri dish and then incubated at 37℃ in 5% CO_2 in a highly humidified incubator for 14–21 d. The plating efficiency (PE) of MKN–45 was about 35%. For the other cell lines, PE were 2–20%.

Drugs Tri was obtained from the Fujian

Provincial Institute of Medical Sciences, Fuzhou, China. It was isolated from *Tripterygium wilfordii* Hook F and was well identified as pure triptolide. Aqueous solution of Tri was prepared with the addition of some propylene glycol (PG) to promote solubilization. The concentration of PG in the stock solution of Tri 1 mmol · L^{-1} was 12% (vol / vol). Standard anticancer drugs employed as positive controls were vincristine, vinblastine, and vindensine. In each experiment only one of them was used. These standard agents and PG solutions without any drug were tested simultaneously in the same clonogenic assay as the positive and vehicle control, respectively. Two drug exposure schedules were used: 1-h exposure prior to plating and continuous exposure in agar. The stock solutions of drugs were stored at −20℃. Immediately before use, the stock solution was diluted with medium-FCS. For 1-h exposure tumor cells were incubated with or withour the drug for 1 h at 37℃ in medium-FCS. The cells were washed with the same medium and prepared for culture. For continuous exposure the drugs were incorporated into the top layer agar for culture. Each test was performed in triplicate, and a wide range of drug concentrations were tested: 0.1, 1, 10, 100, 1 000, 10 000 nmol · L^{-1}.

Evaluation of the results After 14-21 d (usually 14 d) of incubation, the colonies were counted by a computerized image analyzer (colony analyzer system CA-7A, Oriental Instrument Ltd, Tokyo). The images >60 μm in diameter (an aggregation of about 50 cells) were regarded as colonies. The % of colony survival = [($\bar{x}$ of colony counts in 3 test dishes) / ($\bar{x}$ of colony counts in 6 control dishes)] × 100. Experiment was repeated at least 3 times. A 70% reduction in colony survival at a relatively low dose of drug was considered significant[4-6]. Then compared the 70% inhibitory concentration (IC_{70}) with the estimated clinically achievable peak plasma concentration (PPC) and calculated the ratio PPC / IC_{70}. IC_{70} and IC_{50} values were determined graphically from a dose-response curve for each cell line. Since the acute ip LD_{50} of Tri in mice was reported to be 0.9 mg · kg^{-1} [7], the PPC was estimated as 0.15 mg · L^{-1} by the following formula[8]:

$$\lg(PPC) = 0.755 \times \lg(\text{mouse } LD_{50}) - 0.788$$

Morphologic examination of colonial cells was performed employing colonies from representative plates with HE stain.

RESULTS

Tri showed a significant suppressive effect on the colony formation of MCF-7, BT-20, MKN-45, MKN-7, and HL-60 cell lines at 10^{-8} mol · L^{-1} when the cells were continuously exposed to the drug (Tab 1). But for stomach cancer cell line KATO-III, the IC_{70} was around 10^{-7} mol · L^{-1}. IC_{50}, IC_{70}, and PPC / IC_{70} of each cell line are shown in Tab 2. All PPC / IC_{70} values were >10 (72-126) with the exception of KATO-III (8.7).

In 1-h exposure schedule, IC_{70} was around 10^{-6} mol · L^{-1} for most cancer cell lines tested and even higher for KATO-III (Tab 1).

Vehicle control test showed that the same PG concentrations as those used in Tri 10^{-6}-10^{-8} mol · L^{-1} solutions did not significantly suppress colony formation of cancer cell lines tested in both 1-h and continuous exposure assays.

Standard anticancer drugs, vincristine, vinblastine and vindensine, showed suppressive effects on the colony formation in continuous exposure schedule. The representative results are presented in Tab 3.

DISCUSSION

The present study showed that Tri had a suppressive effect on the colony formation of

Tab 1. Survival % of colony ($\bar{x}\pm$ SD) of cancer cell lines after continuous or 1-h exposure to triptolide.

Cell line	Exp time	Concentration of triptolide, nmol · L^{-1} 0.1	1	10	100	1 000	10 000
MCF-7	Cont	88.1 ± 36.0	57.8 ± 21.5	4.3 ± 2.7	2.7 ± 1.3	2.8 ± 2.1	2.7 ± 1.7
	1-h	102.0 ± 22.6	91.6 ± 19.0	85.6 ± 34.3	75.9 ± 24.9	4.5 ± 1.6	4.3 ± 2.0
BT-20	Cont	110.9 ± 17.1	73.0 ± 16.9	5.1 ± 1.1	4.3 ± 2.6	5.6 ± 2.1	4.3 ± 1.3
	1-h	99.3 ± 7.8	109.5 ± 0.5	96.7 ± 10.6	63.7 ± 27.3	5.6 ± 3.0	4.1 ± 2.1
MKN-45	Cont	101.0 ± 4.4	102.7 ± 12.7	4.5 ± 2.0	4.7 ± 2.0	4.5 ± 1.4	3.1 ± 0.7
	1-h	91.5 ± 23.6	104.5 ± 1.5	90.5 ± 3.7	96.0 ± 22.8	9.2 ± 3.8	3.6 ± 1.6
MKN-7	Cont	95.0 ± 20.8	77.9 ± 28 9	8.6 ± 4.9	5.2 ± 2.8	1.5 ± 0.7	2.7 ± 0.3
	1-h				85.5 ± 14.6	7.8 ± 3.3	6.9 ± 2.4
KATO-III	Cont	101.2 ± 10.6	97.2 ± 8.6	84.3 ± 20.9	5.1 ± 3.2	2.3 ± 1.4	1.9 ± 1.4
	1-h	112.0 ± 11.0	114.4 ± 15.0	106.3 ± 16.2	109.1 ± 17.0	58.2 ± 24.1	2.0 ± 0.7
HL-60	Cont	97.7 ± 3.3	76.1 ± 28.0	15.5 ± 5.0	7.2 ± 3.5	11.5 ± 4.3	4.5 ± 1.9

Exp time: exposure time. Cont: continuous.

Tab 2. IC_{50}, IC_{70}, and PPC / IC_{70} values of human cancer cell lines in continuous exposure to triptolide (PPC = 0.15 mg · L^{-1}).

Cell line	IC_{50}, μg · L^{-1}	IC_{70}, μg · L^{-1}	PPC / IC_{70}
Breast cancer			
MCF-7	0.504	1.19	126
BT-20	0.774	1.55	97
Stomach cancer			
MKN-45	1.22	1.96	77
MKN-7	0.9	1.76	85
KATO-III	9.54	17.3	8.7
Leukemia			
HL-60	0.9	2.09	72

solid tumor cell lines tested, and the magnitude of the effect on most of the breast and stomach cancer cell lines tested was similar to that on the leukemia cell line HL-60 in continuous exposure schedule (Tab 1). Clonogenic assay is an accepted technique for disease-oriented information about *in vitro* activity, yielding results that correlated closely with the clinical response[3-6,9-13]. In the last decade more and more authors employed colonogenic assay of established human tumor cell lines to evaluate new antitumor drugs *in vitro*[4-6,10-13]. PPC is an important pharmacokinetic parameter in antitumor drug research. The PPC of a drug, that may have a

Tab 3. Survival % of colony ($\bar{x}\pm$ SD) of cancer cell lines after continuous or 1-h exposure to vincristine.

Cell line	0.01	Concentration of vinblastine, nmol · L^{-1} 0.1	1	10	100	1 000	10 000
MCF-7							
Cont	99.8 ± 6.2	45.5 ± 20.5	5.1 ± 2.8	6.2 ± 3.4	1.9 ± 1.0	1.2 ± 0.09	2.5 ± 0.45
1-h			113.5 ± 5.5	74.3 ± 4.0	59.7 ± 4.6	6.2 ± 3.3	4.5 ± 2.4
MKN-45							
Cont		39.3 ± 2.0	4.1 ± 2.0	3.3 ± 1.0	2.9 ± 1.0	1.9 ± 0.5	2.7 ± 1.0
1-h		92.6 ± 13.0	92.6 ± 6.8	88.0 ± 14.0	49.4 ± 4.0	4.9 ± 1.3	5.8 ± 1.0

Cont: continuous exposure. 1-h: 1-h exposure.

potential therapeutic effect on certain tumors, must be at least 10 times higher than its IC_{70} (or IC_{50}) in clonogenic assay[5,9,12,13]. Our results showed that the PPC of Tri was 72–126 times higher than IC_{70} of the cancer cell lines tested except for KATO–III. The ratios PPC / IC_{70} of solid tumor cell lines tested (77–126) were even a little higher than that of leukemia cell line HL–60 (72). These suggest that Tri might have a potential therapeutic value for some types of solid tumor and worth further studying. Some compounds from plants, e.g., harringtonin[14], which had been thought to be only active against leukemia, was later proved to have a dramatic activity against some types of solid tumor cells. Probably, it is also the case with Tri. Of course, a lot of work have to be done before any definitive conclusion could be drawn. Anyway, we think these data are interesting especially if we take into account the fact that there are at present few satisfactory drugs in the chemotherapy of stomach cancer.

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雷藤甲素对乳癌和胃癌细胞系集落形成的抑制作用

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提要 以集落形成试验测试癌细胞对雷藤甲素的敏感性. 在持续接触条件下, 检测过的 5 个乳癌和胃癌细胞系中有 4 个, 雷藤甲素 1×10^{-8} mol·L^{-1} 可抑制其软琼脂集落形成 70%以上, 强度与对人白血病细胞系 HL–60 的相近 IC_{50} 为 0.504–1.22 μg·L^{-1}. PPC 的估计值为 0.15 mg·L^{-1}. PPC / IC_{70} 在该 4 细胞系为 77–126, 略高于 HL–60 的 72. 提示该药可能对某些实体瘤细胞也有一定作用.

关键词 雷藤甲素; 集落形成单位测定; 细胞株; 培养的肿瘤细胞; 乳腺肿瘤; 胃肿瘤; 白血病