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Cytotoxic and Cytotoxicity-Potentiating Effects of the Curcuma Root on L1210 Cell

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Abstract—A cytotoxic sesquiterpene against L1210 cell has been isolated from the root of *Curcuma domestica*. Its structure was identified as β-sesquiphellandrene. The cytotoxicity-potentiating substance was (+)-ar-turmerone. (+)-ar-Turmerone potentiated the cytotoxicity of β-sesquiphellandrene(5 fold in ED_{50} value) and an unknown sesquiterpene which was isolated from the root as well, and that of aurapten(6.3 fold) isolated from the unripe fruit of *Poncirus trifoliata*. Moreover, it potentiated the cytotoxic activities of MeCCNU 10 fold and cyclophosphamide 10 fold. Except the fact that all the effective cytotoxic substances possess relatively good lipophilicity, no relationship between structures of the cytotoxic substances and the cytotoxicity-enhancing effect of (+)-ar-turmerone could be observed.

Keywords—*Curcuma domestica* • β-sesquiphellandrene • (+)-ar-turmerone • cytotoxic sesquiterpene against L1210 • cytotoxicity potentiation

Ahn and coworkers[1] have undertaken studies on the cytotoxic activity of the medicinal plants against L1210 cell. They have selected fourty drugs, which have been claimed to be of anti-tumour in the Korean traditional medicine or in the folklore, and tested their activity. 28% of the drugs screened showed the activity. The root of *Curcuma domestica* (Zingiberaceae) belongs to one of the active drugs. The curcuma root has been used for treating chest pain, abdominal pain, liver pain, stomach pain, menorrhagia and jaundice in the oriental medicine. In the screening program mentioned above, it was found that the petroleum ether fraction of the root had a good cytotoxic effect on L1210 cell (ED_{50}=4.8 μg/ml). It will be reported here about cytotoxicity-directed isolation procedure from the root, structure identification and cytotoxicity-enhancing effect of an isolated sesquiterpene on some natural and synthetic cytotoxic substances against L1210 cell.

Materials and Methods

Cultivation and maintenance of L1210 cell

L1210 stock cell was grown in screw cap tubes (10×160 mm) at 37° and transfered twice a week.

Materials

The curcuma root was purchased from the market of the oriential drugs in Taejon-City and used after identification.

Silica gel 60 and precoated silica gel 60 GF-TLC plates were purchased from Merck AG, Darmstadt, West Germany. The solvents of industrial grade were three times distillated and then used for drug extraction. All other

reagents used were extra pure grades.

The antitumor agents (MeCCNU, cyclophosphamide, melphalane, adriamycin 5-fluorouracil and cisplatin) used for experiment of cytotoxicity potentiation were gift from the hospital pharmacy of Chungnam National University.

Aurapten, β-sesquiphellandrene, compound III, skullkapflavone II, panaxydol and acetylshikonine were the cytotoxic natural products against L1210 cell which had been isolated in our laboratory.

Fractionation of the drug extract

Refer to "results and discussion".

Determination of ED_{50} value on L1210 cell

Determined by the protocol of National Cancer Institute, USA[2].

Results and Discussion

The powered root was extracted with methanol. We have fractionated further the methanol extract with solvents and by means of silica gel chromatography, as shown in Fig. 1. β-sesquiphellandrene and an unknown substance have been isolated from the active fractions Fr-Ib and Fr-IIIa as the cytotoxic components. β-sesquiphellandrene was isolated from the root of *Zingiber officinale*, but isolated first time from the root of *Curcuma domestica*. Since the unknown substance showed a similar ^{1}H-NMR pattern to that of β-sesquiphellandrene, it must be a similar sesquiterpene. This substance was so unstable that a chromatography on silica gel was impossible.

The cytotoxic activities of all the fractions were weaker than the petroleum ether extract (ED_{50}=1.8 μg/ml). This observation suggests that a cytotoxicity-enhancing substance is contained in the petroleum ether extract. To confirm the cytotoxicity-enhancing effect of these fractions, we have mixed the fractions each other and observed the potentiated cytotoxicity of the mixed fractions (Table I). Consequently the fraction II potentiated the cytotoxic activities of Fr-Ib and Fr-III containing β-ses-

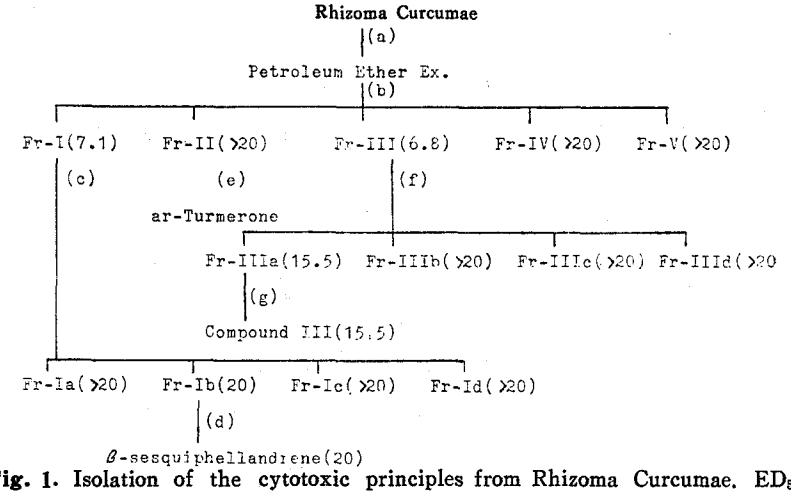

Fig. 1. Isolation of the cytotoxic principles from Rhizoma Curcumae. ED_{50} values(μg/ml) are in parenthesis.
(a); extracted with petroleum ether for 72 hrs, (b); chromatography on silica gel column(SC), benzene/acetone(1:0~3%, gradient, (c); chromatography on SC, n-hexane/acetone(1:0~1%, gradient), (d); chromatography on SC, benzene, (f); chromatography on SC, cyclohexane/ethyle acetate(98:2), (g); chromatography on SC, n-hexane/acetone(98:2).

Table I. Cytotoxicity enhancement of the fractions by Fr-II(ED_{50}>20)

Fractions	ED_{50}(μg/ml)	Fractions	ED_{50}(μg/ml)
Fr-III	6.8	Fr-IV	>20
Fr-V	>20	Fr-Ia	>20
Fr-Ib	20	Fr-Ic	>20
Fr-Id	>20	Fr-II+Fr-III	3.5
Fr-II+Fr-IV	17.8	Fr-II+Fr-V	>20
Fr-II+Fr-Ia	>20	Fr-II+Fr-Ib	7.3
Fr-II+Fr-c	>20	Fr-II+Fr-Id	>20

Table II. Enhancement of the cytotoxic activity of some cytotoxic substances by ar-turmerone.

Compounds \ Mol ratio*	3:1	2:1	1:1	1:2	1:3	0:1
β-Sesquiphellandrene	4.9**	5.1	5.2	8.2	8.5	20.0
Compound III	—	3.9	6.6	7.1	—	15.5
Aurapten	—	2.8	1.6	1.2	—	10.1
Skullkapflavone II	—	2.4	4.8	3.7	—	3.8
Panaxydol	—	0.031	0.028	0.03	—	0.03
Acethylshikonine	—	0.09	0.09	0.12	—	0.10
MeCCNU	—	0.71	0.28	0.48	—	2.81
Cisplatin	—	0.16	0.14	0.15	—	0.15
Mitomycine-C	—	0.11	0.12	0.09	—	0.10
Cyclophosphamide	—	1.31	1.81	1.73	—	12.7
5-Fluorouracil	—	0.08	0.09	—	—	0.15
Melphalane	—	0.15	0.15	0.14	—	0.31
Adriamycine	—	0.01	0.01	—	—	0.009

* Mol ratio=ar-turmerone:compound

** ED_{50} (μg/ml) values were the mean of duplicate experiments.

quiphellandrene and the compound III.

Chromatographing the fraction II on a silica gel flash column yielded a pure substance (compound II in Fig. 2). A comparison of the physical data revealed that this substance is (+)-ar-turmerone, which had been already isolated from the same drug[4,5,6,7].

Observation of the cytotoxicity-enhancing effect of ar-turmerone extended to other cytotoxic substances which have been isolated from the herbs or collected from drug stores(Table II). Among the natural products the cytotoxicities of β-sesquiphellandrene, compound III and aurapten were potentiated by mixing ar-turmerone. The cytotoxicity of aurapten was potentiated to a greatest extent(6.3 fold in ED_{50} value) by molar ratio of ar-turmerone/aurapten=1:1. The cytotoxicities of skullkapflavon II, panaxydol, acetyl shikonine, mitomycin C and adriamycin were not affected. Among the synthetic substances the potentiating effects on MeCCNU(10 fold by molar ratio of 1:1) and cyclophosphamide(10 fold by molar ratio of 2:1) are distinguished.

One can not extract any relationship between the structures of the cytostatics and the cytotoxicity-enhancing activity of(+)-ar-turmerone from these data. However, it is interesting that

the strongest effect was exhibited by the nonpolar neutral cytotoxic ones of the cytotoxic substances tested. This leads to a suggestion that (+)-ar-turmerone affects the cell permeability for the nonpolar substances.

Cyclophosphamide is another example supporting this suggestion. This cytotoxic substance is known to show weak *in vitro* cytotoxicity and become cytotoxic after chemical transformation in liver[8]. It was observed here that cyclophosphamide was weak *in vitro* cytotoxicity (ED_{50}=12.7 μg/ml) against L1210 cell as well (Table II). Thus it is a possible suggestion that the potentiation of the cyclophosphamide cytotoxicity by (+)-ar-turmerone was caused by accelerated transport of the cytotoxic substance into the cell and then by bioactivation inside it.

Recently it is reported that some noncytotoxic vasodilating drugs such as reserpine and verapamil exert cytotoxicity-potentiating effects. Reserpine is known to increase the uptake of *vinblastine in rat ascites hepatoma*[9]. *Verapamil* potentiated cytotoxic efficacy of some structurally unrelated cytostatics.[10~13] Scheid and coworkers[13] reported that verapamil reduces the mitotic indices of bleomycin and peplomycin synergistically in cultured human lympocytes. They suggested that the drug could make the cell membrane more permeable for the cytostatics.

Synergism, which is caused by the mutual action of the substances from same or from other herbs, is one of the important principles in Chinese medicine.

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