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원 저

Suppressive Effect of *Curcuma Zedoaria Roscoe on Pulmonary* Metastasis of B16 Melanoma Cells.

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Objective : We examined the antimetastatic effect of *Curcuma zedoaria Roscoe* (CZ) on pulmonary metastasis of B16 cells.

Methods : For 6 weeks, Zedoariae Rhizoma made from dried CZ were dissolved in distilled water and administered to mice 2 weeks before they were injected with B16 melanoma cells. Mice were given CZ at doses of 250 and 500 mg/kg, and were compared for lung weight, survival days, and NO production.

Results : Intake of CZ throughout the experiment extended the average survival time. Intake after B16 cell injection slightly prolonged survival time, but intake before B16 cell injection did not influence life span.

We examined the effect of CZ on macrophage function by measuring NO production. After the macrophages were given CZ for 6 weeks, the amount of NO generated by the macrophages stimulated with LPS in culture medium increased. NO generated by the macrophages also served as a cytotoxic factor against B16 melanoma cells. B16 melanoma-conditioned medium reduced NO production by macrophages. However, CZ treatment reversed the reduction in NO production by the conditioned medium significantly.

Conclusion : These findings may suggest that macrophage function-modulating activity by CZ appears to underlie its antimetastatic activity, which leads to a decrease in the number of lung metastatic surface nodules and the extension of life span.

Key Words: *Curcuma Zedoaria Roscoe*, B16 melanoma cells, lung cancer

Introduction

The lung, as well as the liver, is one of the organs most frequently involved in metastatic deposits from primary tumors[1]. Tumor cell metastasis is a complex, multistep process that involves cell separation from the primary tumor, entry into the vascular and lymphatic systems, transport to and arrest within the microcirculation of distant organs, and extravasation[2]. The emergence of metastasis in organs distant from the primary tumor is the most devastating aspect of cancer. From this point of view, various inhibitors, such as inhibitors of angiogenesis and matrix metalloproteinase, are presently being developed as novel therapeutic drugs. Several traditional and herbal medicines, such as Keishi-ka-kei-to, Juzen-taiho-to, Shimotsu-to, Unsei-in, Hochu-ekki-to, Shosaiko-to, and Shichimotsu-koka-to have been so far reported to exhibit an an-timetastatic effect[3,4]. Among them, Juzen-taiho-to reduces liver

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metastasis by colon 26-L5 carcinoma cells as well as pulmonary metastasis by B16-BL6 melanoma cells, and its antimetastatic effect appears likely to be mediated by the ac-tivation of macrophages and/or T cells in the host immune system[5]. Keishi-ka-kei-to is also able to inhibit the pul-monary metastasis of B16 melanoma cells and its effect is in part due to the stimulation of CD8+ T cells[3]. Shosaiko-to suppresses pulmonary metastasis induced by Lewis lung carcinoma cells, and the enhanced number of peritoneal macrophages and elevated binding of C3 cleavage products to macrophages after Shosaiko-to treatment may be related to its antimetastatic effects[6]. However, there have been few studies of herbal medicines in the metastatic setting.

Zedoary (*Curcuma zedoaria* ROSCOE, Zingiberaceae) has been extensively cultivated as a vegetable, spice, and perfume in South and Southeast Asian countries. The rhizome of C. zedoaria (Zedoariae Rhizoma) is widely used as a stim-ulant, stomachic, carminative, diuretic, anti-diarrheal, anti-emetic, anti-pyretic, and depurator, and also to clean and cure ulcers, wounds, and other skin disorders in India and South-east Asian countries. In Japanese and Chinese traditional medicines, Zedoariae Rhizoma (Japanese name, Gajutsu; Korean name, Bongchul) which is listed in the Korean Pharmacopoeia, has been known to exhibit stomachic and emmenagogue-like effects. In particular, this natural medicine is prescribed in various Chinese preparations used for the treatment of Ohyul or Oketsu syndrome, which is thought to be caused by blood stagnation. It was already reported that the Zedoariae Rhizoma extract exhibited potent vasorelaxant and hepatoprotective activities[7-10].

Previous studies have centered around its gastrointestinal effects. The zedoary or its extracts have been shown to inhibit the intestinal transit of charcoal meal in mice, prevent stress ulcer inmice,andprotect theliver fromoxidative injuryinduced by CCl4[11]. Zedoary contains essential oils including sesquiterpenes and monoterpenes[12]. Dehydrocurdione is the major sesquiterpene in zedoary. Helenalin, a sesquiterpene lactone from Arniae flos, has been reported to have an antiinflammatoryactivity[13], while the antiinflammatoryeffect of zedoary or that of dehydrocurdione has not been studied so far. In the present study, we investigated the antitumor properties of the zedoary of Kyungju origin and was stored at -20℃ until use.

CZ, which is used to treat human tumor and atherosclerosis, has antimetastatic effects. However, in addition to its antimetastatic effect, the pharmacological and biological actions of CZ have not been thoroughly investigated to date. In a preliminary study, CZ showed radical scavenging activity by increasing superoxide dismutase activity and decreasing xanthine oxidase activity in the brain. CZ is able to quench superoxide anion as determined by electron-spin resonance analysis in vitro. Considering that reactive oxygen species play an aggravating role in the stimulation of metastasis, CZ is expected to suppress tumor metastasis. Pharmacological and biochemical studies indicate that immunomodulating activity is found in many Korean herbal medicines, which rarely produce side effects even after long-term administration.

Taken together, it is worth studying the antimetastatic effect of herbal medicines. Therefore, in the present study, we examined the antimetastatic effect of CZ using a mouse pulmonary metastasis model.

Materials and Methods

1. Animals

Male C57BL/6 mice were purchased from Charles River (Hino, Japan). They were housed in a temperature-controlled room (at 23±2℃) with lighting from 06:00 to 18:00 under specific pathogen-free conditions. The humidity was automatically maintained

at 50±10%. They received a commercial diet (DEA-3, Daehan Experimental Animals Co. Ltd., Seoul, Korea) and water *ad libitum*. All mice were 8 weeks of age when tumor cells were injected intravenously.

2. Tumor Cells

B16 (C57BL/6 mice, melanoma) cells were obtained from Korea Collections for Type Culture, Korea Research Institute of Bioscience and Biotechnology (Daejon, Korea). B16 melanoma cells were cultured in MEM-Eagle's salt medium (nonessential amino acids, Irvine Scientific Co., Santa Ana, CA, U.S.A.) supplemented with 10% fetal calf serum (FCS) with penicillin 100 U/ml and streptomycin 100 mg/ml (Life Technologies Inc., Grand Island, NY, U.S.A.).

3. Preparation of CZ

CZ (human daily dose) was pre-pared as follows. 25.0 g was added to 500 ml of water, decocted for 1 h, and concentrated to 250 ml. This decoction was lyophilized to give 5.3 g of extract. In this extract, dhydrocurdione (a major sesquiterpene in zedoary) and helenalin (a sesquiterpene lactone) were contained as the main components (1.80 and 1.32, respectively). All of these extracts were kindly provided by Kyungju Oriental Medical Hospital, Dongguk University (Kyungju, Korea).

4. Pulmonary Metastasis Model

Male C57BL/6 mice were injected intravenously with $1.3x10^5$ or $2.4x10^5$ cells/mouse of cultured B16 melanoma cells. Mice injected with $1.3x10^5$ cells were killed 21 d after injection of B16 melanoma cells and the lung was removed. The number of metastatic colonies in the lung were macroscopically counted and their weights were measured. On the other hand, mice injected with $2.4x10^5$ cells were allowed to live until they succumbed to the metastatic tumor burden in the lung. Various concentrations of CZ were dissolved in distilled water and mice received them ad libitum as described in the figure legends.

5. Preparation of Thioglycollate-Eliciting Peritoneal Macrophages

Male C57BL/6 mice received CZ dissolved in their daily intake of drinking water at various doses for 5 weeks and were injected intraperitonealy with 2 ml of 3% thioglycollate 4 d before the last day of the experiment. Peritoneal macrophages were harvested from abdominal cavity and maintained in RPMI1640 supplemented with 10%

FCS. Resulting macrophages were seeded at a concentration of $1.3x10^6$ cells/ml and incubated in the presence or absence of lipopolysaccharide (LPS) 5.0 ㎍/ml for 20 h. Nitric oxide (NO) production was determined by measuring the accumulation of nitrite in the incubation medium using Griess reagent[14].

6. Co-culture Experiment

Murine peritoneal macrophages ($1.3x10^6$ cells/ml) were co-cultured with B16 cells ($2.4x10^5$ cells/ml) in the Transwell system separated by a polyethylene terephthalate permeable support (Cell Culture & Insert, Becton Dickinson, Rahway, NJ, U.S.A.). To examine the effect of NO produced by macrophages on the viability of B16 cells, macrophages were seeded in the cell-culture inserts, while B16 melanoma cells were cultured in 24 well plates for 24 h before the start of co-culture. Following 20 h stimulation of macrophages with LPS 5.0 ㎍/ml, the concentration of NO which infiltrated into the culture medium of B16 cells and the viability of B16 cells were determined by the Griess and the MTT methods[15], respectively.

7. Preparation of conditioned medium(CM)

B16 melanoma cells ($1.6x10^5$ cells/ml) were maintained

in MEM-Eagle's salt medium supplemented with 10% FCS for 24 h. Then B16 melanoma-conditioned medium was prepared by incubation of B16 melanoma cells in serum-free MEM-Eagle's salt medium for 12 h. The conditioned medium was centrifuged at 1000 rpm for 5 min to remove cellular components and stored at -80℃ until use. Control conditioned medium was prepared according to this procedure but without cells.

8. Statistical Analysis

Data are expressed as mean ± S.E. with the number of animals. Statistical significance was de-termined by non-paired Student's *t*-test, Mantel-Cox log-rank test, or Dunnett's test using Stat Light software. *p* values less than 0.05 were considered significant.

Results

1. Antimetastatic effect of CZ on pulmonary metastasis of B16 cells

To evaluate the effect of CZ on pulmonary metastasis, we first examined the effect of CZ on the number of lung nodules and the weight of the lung in pulmonary metastasis model mice injected with B16 cells. The intake of CZ and B16 cells injection were scheduled as described in the legend to Table. 1. At 21 d after B16 cells injection, mice were killed and lung weight and the number of lung surface nodules were measured (Table. 1A, B). The weight of the lung increased significantly in the control group, whereas the intake of CZ, especially at 6 times the human daily doses (250 and 500 mg/kg), significantly reduced the lung weight compared with the control group (Table. 1A). Similarly, about 351 lung nodules were formed in the lungs of control mice, but the intake of CZ at doses of 250 and 500 mg/kg significantly decreased the number of lung nodules to about 150 and 154, respectively.

2. Effect of CZ on pulmonary metastasis of B16 cells

We examined the effect of daily intake of CZ dissolved in drinking water and the duration of the intake of CZ on the life span of C57BL/6 mice (Table. 2). Mice were given CZ at a dose of 250 mg/kg according to three different pro-tocols. In the first group, mice were given CZ for 2 weeks starting 10 days before B16 melanoma cells injection (Table. 2A). In the second group, mice were given CZ from 5 d after B16 melanoma cells injection until all mice succumbed to the metastatic tumor burden in the lung (Table. 2B). In the third group, mice were given CZ throughout the

Table 1. Antimetastatic Effect of CZ on Pulmonary Metastasis of B16 Cells

(A)

Spe Lung weight (g)		
Normal		0.143±0.01
Control		0.215±0.02**
CZ (mg/kg)	50	0.197±0.02
	250	0.175±0.015#
	500	0.177±0.02#

(B)

Surface nodules (number)		
Control		351±43
CZ (mg/kg)	50	186±53
250	150±16#	
500	154±22#	

Male C57BL/6 mice were injected intravenously with $1.3x10^5$ of B16 cells, and CZ at the indicated doses was given orally from 10 d before B16 cell injection. Twenty-eight days after B16 cell injection, mice were killed and lung weight (A) and surface nodule number (B) were determined. Open column, normal C57BL/6 mice (n=6), hatched column, control C57BL/6 mice (n=6) injected with B16 cells and given water instead of CZ; closed column, C57BL/6 mice injected with B16 cells and given CZ at the indicated doses (n=5). Each column represents the means±S.E. of mice in each group. Statistical significance was determined by the nonpaired Student's *t*-test.
**, $p<0.01$ significantly difference vs. normal mice. #, $p<0.05$ significantly difference vs. control mice.

Table 2. Effect of CZ on Pulmonary Metastasis of B16 Cells

	Days after i.v. injection of B16 cells										
	20	25	30	35	40	45	50		60		70
Survival ratio (%)											
Control	100	100	85	40	10	10	0				
CZ	100	88	67	43	35	0					

(A) 10 d before B16 cell injection (n=10)

Survival days: control, 43.5±1.3; CZ, 41.3±1.7

	Days after i.v. injection of B16 cells									
	20	25	30	35	40	45	50	55	60	70
Survival ratio (%)										
Control	100	100	85	40	10	10	0			
CZ	100	100	100	86	35	35	25	10	0	

(B) 5 d after B16 cell injection (n=10)

Survival days: control, 43.5±1.2; CZ, 47.5±2.1

	Days after i.v. injection of B16 cells										
	20	25	30	35	40	45	50	55	60	65	70
Survival ratio (%)											
Control	100	100	85	40	10	10	0				
CZ	100	100	80	80	55	40	30	30	30	10	0

(C) 10 d before B16 cell injection until mice succumbed to the metastatic tumor burden in the lung (n=10)

Survival days: control, 43.5±1.1; CZ, 55.4±2.3. Control vs CZ group showed a significance difference (p<0.05).

Table 3. Dose-Dependent Effect of CZ on NO Production by Peritoneal Macrophages

	△ NO_2 (μM)	
Control	32.5±2.3	
가) CZ (mg/kg) 50		36.8±3.1#
250	38.4±1.7##	
500	40.3±2.3##	

Male C57BL/6 mice received CZ, which was dissolved in the daily intake of drinking water at the indicated doses for 6 weeks and were intraperitonealy injected with 3% thioglycollate. Four days later, macrophages were prepared from the abdominal cavity. Macrophages at a concentration of $1.3x10^6$ cells/ml were incubated in the presence or absence of LPS 5.0 ßð/ml for 20 h. NO production was determined by measuring The accumulation of nitrite in the culture medium by the Griess method. Each column rep-resents mean±S.E. of 5 mice. Statistical significance was determined by the nonpaired Student' s *t*-test. #, p<0.05; ##, p<0.01, significantly difference vs. control mice.

experimental period (Table. 2C). In the control group, all mice had died by 45 d after B16 cells injection, and the average length of survival was 43.5±1.3 d. However, the intake of CZ throughout the experiment significantly extended the average survival time to 55.4 ±2.3 d (Table. 2C). The intake after B16 cell injection slightly prolonged survival time and intake only for 2 weeks before B16 cell injection did not influence the life span.

3. Dose-dependent effect of CZ on NO production by peritoneal macrophages and effect of NO generated by macrophages on viability of B16 cells

Macrophages are known to be involved in prevention of metastasis. We therefore examined the effect of CZ on macrophage function by measuring NO production. C57BL/6 mice received CZ for 6 weeks and thioglycollate-eliciting peritoneal macrophages were harvested as described in Materials and Methods. Following the stimulation of macrophages with LPS 5.0 ㎍/ml for 20 h, the amount of NO generated in

culture medium was determined (Table. 3). Al-though antimetastatic activity shown by CZ was most effective at 5 times the human daily dose, CZ dose dependently enhanced NO production by macrophages with no effect on body weight during the experiment. However, the intake of CZ for 1 or 2 weeks did not influence NO production by macrophages (data not shown). As NO is known to act as cytotoxic mediator against cells or bacteria, we examined whether NO generated by macrophages prepared from CZ-treated mice served as a cytotoxic factor against B16 melanoma cells. Macrophages and B16 melanoma cells were cocultured by maintaining macrophages in cell-culture inserts and B16 melanoma cells in 24-well plates. We then assessed whether NO generated by macrophages following LPS stimulation infiltrated into B16 melanoma cell culture and decreased the viability of B16 melanoma cells. LPS stimulation increased NO concentration in B16 melanoma cell culture, and the NO concentration in B16 melanoma cell culture was much higher in the co-culture with macrophages prepared from CZ-treated mice than with those from control mice (Table. 4A). The viability of B16 melanoma cells was reduced in co-culture with macrophages following LPS stimulation, and the decrease in the viability was larger in the co-culture with macrophages prepared from CZ-treated mice (Table. 4B).

Table 4. Effect of NO Generated by Macrophages on Viability of B16 Cells

(A)

		△ NO_2 (μM)
B16		0.35±0.02
B16/normal macrophages		6.8±0.7**
B16/macrophages+CZ(mg/kg)	50	10.3±1.1*
	250	9.6±1.7*
	500	9.8±1.5

(B)

		Viability (%)
B16		100±5.3
B16/normal macrophages		89.6±5.4*
B16/macrophages+CZ(mg/kg)	50	82.4±0.8#
	250	82.6±3.5#
	500	80.3±5.3

B16 cells at a concentration of $1.3x10^5$ cells/ml were co-cultured with peritoneal macrophages ($1.3x10^6$ cells/ml) as described in Materials and Methods. Following the stimulation of macrophages with LPS 5.0 ug/ml for 20 h, NO, which was infiltrated from the insert in which macrophages were cultured into B16 culture, was determined by the Griess method (A), and the viability of B16 cells was determined by the MTT method (B). B16, B16/normal macrophages and B16/macrophages prepared from mice treated with CZ(50, 250 and 500 mg/kg, respectively) were assayed. Each data reepresents mean±S.E. of 4 wells. Statistical significance was determined by Dun-nett°Øs test using Stat Light software. *, $p<0.05$ and **, $p<0.01$ vs. B16, respectively. #, $p<0.05$ vs. B16/normal macrophages.

Table 5. Reversal Effect of CZ on Suppression of NO Production by B16 Melanoma-conditioned Medium

		△ NO_2 (μM)
CCM		33.4±2.5
75% B16 CM		9.3±0.1**
75% B16 CM+CZ(mg/kg)	50	10.9±0.9#
	250	12.3±0.6##
	500	15.6±1.3##

Male C57BL/6 mice received CZ, which was dissolved in daily intake of drinking water at the indicated doses, for 5 weeks. Thioglycollate-eliciting macrophages and B16 melanoma-conditioned medium were prepared as described in Materials and Methods. Macrophages ($1.4x10^6$ cells/ml) derived from control mice (75% B16 CM) or CZ-treated mice (75% B16 CM+CZ) were incubated with 75% B16 melanoma-conditioned medium (CM) for 20 h in the presence or absence of LPS 5.0 ㎍/ml. As a control, control macrophages were incubated with control conditioned medium (CCM) for 20 h in the presence or absence of LPS 5.0 ßð/ml. NO levels in the medium were determined by the Griess method. Each data represents the difference between NO production in the presence and absence of LPS as mean ±S.E. of 4 mice. Statistical significance was determined by Dunnett' s test using Stat Light software.
**, $p<0.01$ vs. CCM. #, ##, $p<0.05$ and $p<0.01$, respectively, vs. 75% B16 CM.

4. Reversal effect of CZ on suppression of NO production by B16 melanoma-conditioned medium

It is known that tumor cells effectively suppress macrophage functions to escape the host immunosurveillance system[16]. NO production by macrophages was reduced by co-culture with B16 melanoma cells, although data were not shown. We therefore tested the effect of B16 melanoma-conditioned medium on NO production and the inhibitory effect of CZ. Macrophages were prepared from C57BL/6 mice that received water containing various doses of CZ for 6 weeks and incubated in 75% B16 melanoma-conditioned medium for 20 h in the presence or absence of LPS. B16 melanoma-conditioned medium markedly decreased NO production in control macrophages compared with the control conditioned medium. In contrast, the reduction in NO production by B16 melanoma-conditioned medium was dose de-pendently reversed by CZ treatment (Table. 5). The viability of macrophages were not affected by treatment with 75% B16 melanoma-conditioned medium.

Discussion

In the present study, we evaluated the effect of the Herbal medicine CZ on pulmonary metastasis of B16 melanoma cells and found that the long-term intake of CZ reduced the number of pulmonary metastatic nodules and extended the life span significantly. The most effective dose of CZ was 250 mg/kg, which corresponds to 5 times the human daily dose, and intake throughout the experimental period pro-longed the life span significantly.

The process of metastasis involves a series of sequential steps in which malignant cells are released from the primary tumor, disseminate to distant sites via lymphatic and/or circulatory systems, arrest within the microcirculation of distant organs, extravasate, and proliferate in target organs. The metastasis model used in the present study represents only the process following transport of tumor cells by the circulatory system to target organs. Because intake for only 2 weeks before tumor injection did not affect the life span, early events just after tumor injection in this model, such as tumor cell aggregation, adhesion to endothelial cells, and invasion under the endothelium, may not be influenced by CZ treatment. On the other hand, we found that the intake of CZ for 5 weeks, not 1 or 2 weeks, enhances NO production by macrophages and that CZ reverses the suppression of NO production by B16 melanoma cells.

If the enhanced NO production by macrophages is involved in the antimetastatic effect of CZ, long-term intake should be necessary to reduce metastasis and extend the life span. Recent accumulating evidence indicates that the role of NO in metastasis appears to depend on the cells that produce NO and the subtypes of NO synthase (NOS). For example, NO, which is produced by endothelial NOS, appears likely to promote metastasis by maintaining vasodilator tone in the blood vessels in and around the melanoma[17]. NO produced by tumor cells appears to regulate the metastatic potential of tumor cells themselves, as evidenced by the finding that melanoma cells with high inducible NOS (iNOS) activity have lower metastatic potential than those with low iNOS activity. NO produced by iNOS of macrophages is considered to play an important role in tumor cytotoxicity by macrophages[18]. In addition, interferon-γ-mediated suppression of tumor growth and metastasis resulted from stimulation of a high level of NO production by macrophages[19]. Recent results indicate that host-derived NO may differentially modulate tumor progression and

metastasis, which depends on the NO sensitivity of tumor cells[20]. In the present study using co-cultured B16 melanoma cells and macrophages, NO levels in B16 melanoma cell culture and cytotoxicity by NO were increased by CZ treatment, but not dose dependently. This may be explained by the limited permeation of NO through macrophage layers cultured confluently in cell-culture insert or by polarity existing in the re-lease of NO from macrophages. However, NO produced by macrophages, especially macrophages derived from CZ-treated mice, was sufficiently cytotoxic against B16 melanoma cells. This result indicates that the antimetastatic effect shown by CZ may be partly explained by the stimulation of NO production by macrophages.

We found a substance in B16 melanoma-conditioned medium which had a molecular weight lower than 3000 Da and suppressed NO production by macrophages (unpublished data). NO production was reduced by treating macrophages with B16 melanoma-conditioned medium in the present study. However, the reduction in NO production was reversed in macrophages derived from CZ-treated mice. Transform-ing growth factor-β, interleukin-10, prostaglandin E2 , and phosphatidylserine have been so far reported to inhibit NO production by macrophages[21]. Although the substance in B16 melanoma-conditioned medium appears to be different from them, CZ may protect the signaling pathway leading to NO production from inhibitory substance in B16 melanoma-conditioned medium. That is, the antimetastatic effect shown by CZ may also be explained by the protection of macrophage function from suppression by metastatic tumor cells.

CZ showed radical-scavenging activity by increasing superoxide dismutase activity and decreasing xanthine oxidase activity in the liver. Reactive oxygen species (ROS), which are generated by tumor or endothelial cells, are known to play important roles in tumor invasion and metastasis. Since the cell adhesion molecules such as P-selectin are involved in the recruitment of metastatic tumor cells to a target organ and also increases with the exposure of pancreatic tumor cells to ROS and expression of VLA-4 on B16 melanoma cells also increases in response to hydrogen peroxide[22], scavenging of ROS may lead to a reduction in metastasis. This was evidenced by the fact that catalase or superoxide dismutase effectively suppresses metastasis in an experimental metastasis model[23]. CZ may effectively prevent metastasis by scavenging ROS, although CZ is not likely to prevent tumor adhesion to endothelial cells as described above.

In the present study, the antimetastatic activity was highest at 5 times the human daily dose of CZ (250 mg/kg), whereas NO production by macrophages was enhanced dose dependently by CZ treament. This indicates that the antimetastatic activity results from various effects of CZ, including the enhancement of NO production by macrophages, reversal of NO production suppressed by B16 melanoma cells, radical scavenging *etc*.

In conclusion, CZ shows antimetastatic activity against pulmonary metastasis of B16 melanoma cells, and that activity is at least in part due to stimulation of NO production by macrophages and the reversal of NO production by macrophages suppressed by B16 melanoma cells. The detailed mechanism underlying the antimetastatic effect of CZ is currently under investigation. An antimetastatic agent with the ability to enhance macrophage function has never been developed, so CZ should be a potential candidate for a new antimetastatic agent or an auxiliary to other antimetastatic agents.

Conclusion

The zedoary(ZedoariaeRhizoma) made from the

dried rhizome of Curcuma zedoaria *Roscoe* (CZ) originally cultivated in Kyungju and is a herbal drug used as an aromatic stomachic. CZ is a traditional Korean herbal medicine, which have analgesic and anticoagulative effects, so used in China to treat tumor and atherosclerosis[24]. We investigated the inhibitory effect of CZ on experimental pulmonary metastasis of B16 melanoma cells. The intake of CZ at doses of 250 and 500 mg/kg for 6 weeks from 2 weeks before tumor inoculation significantly reduced the number of metastatic surface nodules in the lung and extended the life span. When the duration of CZ intake was examined, survival time was not affected by preintake before B16 melanoma cell inoculation and was slightly extended by postintake after B16 melanoma cell inoculation, although the life span was prolonged by intake throughout the experiment. To address the mechanism underlying the antimetastatic effect of CZ, we studied whether CZ modulated macrophage function, which is involved in killing tumor cells. The intake of CZ for 6 weeks dose dependently increased nitric oxide (NO) production by macrophages following stimulation with lipopolysaccharide. The elevated NO was found to serve as a cytotoxic mediator against B16 melanoma cells in co-culture with macrophages. On the contrary, B16 melanoma-conditioned medium reduced NO production by macrophages. However, CZ treatment reversed the reduction in NO production by the conditioned medium significantly. These findings may suggest that macrophage function-modulating activity by CZ appears to underlie its antimetastatic activity, which leads to a decrease in the number of lung metastatic surface nodules and the extension of life span.

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