2011년 2월

석사학위논문

어성초 추출물이 인체 전립선암 세포주 PC-3에 미치는 영향

조선대학교 보건대학원

대체의학과

나 백 희

어성초 추출물이 인체 전립선암 세포주 PC-3에 미치는 영향

Effects of *Houttuynia cordata Thunb.* Extracts on PC-3 Human Prostate Cancer Cells

2011년 2월

조선대학교 보건대학원

대체의학과

나 백 희

어성초 추출물이 인체 전립선암 세포주 PC-3에 미치는 영향

Effects of *Houttuynia cordata Thunb.* Extracts on PC-3 Human Prostate Cancer Cells

지도교수 문 경 래

이 논문을 대체의학 석사학위신청 논문으로 제출함

2010년 10월

조선대학교 보건대학원

대체의학과

나 백 희

나백희의 대체의학 석사학위 논문을 인준함

위원장 조선대학교 교수 박 상 학 (인)

위 원 조선대학교 교수 서 재 홍 (인)

위 원 조선대학교 교수 문 경 래 (인)

2010 년 11 월

조선대학교 보건대학원

CONTENTS

LIST OF FIGURES

ABSTRACT

Effects of *Houttuynia cordata Tunb.* Extracts on PC-3 Human Prostate Cancer Cells

Na, Baek-Hee
Advisor: Prof. Moon, Kyung Rye, M.D., Ph.D.
Department of Alternative Medicine
Graduate School of Health Science
Chosun University

Objective : This study aimed to investigate the cytotoxicity and chemoprevention of *Houttuynia cordata Thunb.* on PC-3 human prostate cancer cells.

Methods : MTS assay was performed to estimate cell viability whether cytotoxicity was induced with hot water and methanol extracts of *HCT* or not. And Western blot analysis was used to evaluate whether COX-2 expression and iNOS expression were controlled.

Results : Methanol extracts of *HCT* significantly suppressed the cell survival rate in a dose dependant manner. Especially, it significantly inhibited about 50% of the cell viability at the low density of 200㎍/㎖ with treatment for 48 hours. The hot water extracts of *HCT* had no significant effect on cell viability.

Methanol extracts of *HCT* suppressed about 60% of COX-2 protein expression at the low concentration of 100㎍/㎖. However, iNOS expression

was elevated abundantly in methanol extracts. The hot water extracts of *HCT* reduced COX-2 and iNOS expressions in a dose dependent manner.

Conclusion : Methanol extracts of *HCT* that are micromolecules could be developed into a chemopreventive medicine since it significantly suppressed cell viability of PC-3 at a low density of 200㎍/㎖. However, abundant iNOS expression in methanol extracts in this experiment implicated the possibility to promote a cancer-inducing effect. Therefore, further studies associated with its safety would be necessary. In order to identify the chemopreventive effect of *HCT*, reexperiment with isolated and purified *HCT* would be needed. Moreover, additional studies would be required to verify the mechanism of action of *HCT*.

Key words: *Houttuynia cordata Thunb.,* PC-3 human prostate cancer cells, MTS, COX-2, iNOS

Ⅰ. INTRODUCTION

Prostate cancer is a very most common cancer in male worldwide.[1] In the United States, prostate cancer is leading the second cause of cancer deaths among males following lung cancer.[2] The incidence of prostate cancer in South Korea was relatively low in the past, but the rates are growing every year due to the increase of aged population and changes of dietary patterns. According to the annual report of the Korea Central Cancer Registry announced on 21. Dec. 2009, the incidence of prostate cancer in 2007 came in 7th among total cancers and 5th among male cancers in Korea.[3]

The risk factors of prostate cancer are known to include age, hormones, family history, exposure to harmful materials.[3] Prostate cancer mainly occurs in male aged over 50 and elder men are more vulnerable to the outbreak of prostate cancer. Prostate cancer has a long latency period and is asymptomatic without pain before showing its dysuria. It is well known that to prevent the attack of prostate cancer, it is essential to avoid high-fat dietary pattern and to reduce intake of meat related to excessive secretion of male hormones.

In clinical practice, surgery, hormonal therapy, radiotherapy and cytotoxic chemotherapy are used for treating prostate cancer patients. The treatment is carried out by adopting one of those therapies or in combination with one another in certain cases.

When prostate cancer is initially limited to prostate, hormonal therapy is effective for patients. However, prostate cancer is an invasive malignant tumor, and it easily metastasizes beyond the prostate into other organs.[4] Advanced prostate cancer is resistant to hormone therapy. This type of

advanced prostate cancer is strongly tolerant to current chemotherapies. And it more easily metastasizes into other organs, even into bones than androgen-dependent prostate cancer.[5] Bone metastasis risks fractures which are fatal to aged patients, and it results in the primary cause of death in prostate cancer.[6] Moreover, for patients with hormone-refractory prostate cancer after hormonal ablation therapies, any kind of treatment can not be effective. Current anticancer therapies effectively inhibit the proliferation of cancer cells and induce apoptosis and cancer cells death. However, those therapies are also cytotoxic to normal cells.[7] Therefore, the development of new nontoxic treatment, that is, more effective alternatives such as phytotherapy utilizing natural phytochemicals are urgently needed for patients.[8][9]

Recently, it was reported that excessive free radicals could cause cancers[10] and could cause oxidative stress in the body, promote mutation of DNA and aging, and have an influence on the outbreak of cancer.[11] Many researchers have investigated the constituents of dietary foods and plants and their biologically active effects as well. They have focused mainly on their chemopreventive or cancer-preventive properties. In addition, they have suggested that better diet or daily intake of foods possessing antioxidative properties and therapeutic effects might prevent, postpone and reduce the incidence of cancer.[12][13]

Chemoprevention has become recognized as an effective approach to the reducing cancer morbidity and mortality by inhibiting precancerous events before the occurrence of clinical disease.[14] Recently, traditional herbal medicine, phytotherapy and folk medicine practice have received increasing attention. Researchers have verified and characterized not only active phytochemicals in medicinal herbs but also their mechanisms of action systematically. These studies have provided the rationale for their efficacy and have contributed to transforming herbal practices into evidence-based

medicine.[2]

Houttuynia cordata Tunb.(HCT) is a perennial herbaceous plant belonging to the Saururaceae family. It is widely distributed in Korea, Japan, southern China and Southeast Asia,[15] and also has been cultivated a lot in some regions.

HCT is a traditional edible and medicinal herb used over thousands of years not only for diuresis and detoxification but also for treating a variety of diseases such as cough, bronchitis, pneumonia, uteritis, dropsy, dysentery, and eczema in folk medicine practice.[16] It is called *Ursungcho* or *Yakmomil* in Korea which means literally "fishy-smell herb" or "medicinal herb." It contains its unique physiologically active properties. It contains polyphenols[17] and volatile oil.[18][19] and also its major phytochemical components are composed of flavonoids[20] and alkaloids[21] and so on.

Recently, many studies have reported that *HCT* extracts exhibited a variety of biological and pharmalogical activities including antiviral activity,[22][23][24][25][26][27] antibacterial activity,[28] antiallergic activity.[29] Besides, *HCT* has been demonstrated to have the antioxidant action,[30] antiinflammatory ability,[31][32] and antimutagenic effect.[33]

Recent studies have payed more attention to the antitumor effect of *HCT.* With regard to anticancer activities, the effect of *HCT* in human colon adenocarcinoma cell line HT-29[34] and the effect of in human primary colorectal cancer cells[35] have been reported.

Considering the results of recent studies, it is presumed that *HCT* might have possibility of the chemopreventive effect on prostate cancer. However, to date, the effects of *HCT* on prostate cancer have not yet been studied.

Therefore, this study aimed at investigating whether *Houttuynia cordata*

Tunb. exerted cytotoxicity and chemopreventive effect on PC-3 human prostate cancer cells.

In this study, the suppressive effects of *HCT* methanol extracts and hot water extracts on PC-3 human prostate cancer cells were investigated. These two extracts were treated on PC-3 human prostate cancer cells and examined by time and concentration. The cell viability was measured by MTS assay. And COX-2 expression and iNOS expression were estimated by Western blot analysis. By examining whether *HCT* extracts had cytotoxicity on PC-3 human prostate cancer cells and it could suppress the proliferation of PC-3 cells, this study intended to investigate the potentiality of chemoprevention on prostate cancer.

Ⅱ. MATERIALS AND METHODS

1. Materials

Houttuynia cordata Tunb. used for the study was obtained from the Pokwang Usungcho Farming Association Inc. in Posung, Jeonnam, Republic of Korea. The whole plants were dried completely in the shade with cutting slices and then ground down to fine power.

2. Reagents

Methanol reagent in the first degree was used. 1% penicillin, and streptomycin were purchased from Sigma Company in the USA. Dulbeco's Modified Eagle's Medium(DMEM), containing 5% fetal bovine serum(FBS), (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS), ELISA microplate reader(Molecular Devices, Sunnyvale, CA, USA)

3. Cell culture and chemical treatment

The PC-3 human prostate cancer cells were obtained from the American Tissue Culture Collection (ATCC)(Manassas, VA). The human prostate cancer cells were cultured in DMEM containing 5% fetal bovine serum(FBS) and 100 U/ml each of penicillin and streptomycin in an atmosphere containing 5% CO_2.

4. Extraction of *Houttuynia cordata Tunb. (HCT)*

Hydrothermal extracts was drawn out through these processes. The first process was that 1.3 liters of the distilled water was added to the dried *HCT*(100g). Then it was heated in a 1.3 liter sized earthen pot preparing a medicinal decoction (DWP-5000 M) for 3 hours and extracted. After centrifugal separation of the output, only the upper layer was given a decompression filtration through a twofold filter paper(Whatman NO.1). One liter of methyl alcohol was poured into the dried total *HCT*(100g). After 24 hours, the impurities were filtered out using a filter paper. The sample was obtained by hot-water and lyophilization.

5. MTS assay

The effect of extracts of *HCT* on cell viability was estimated using the Cell Titer 96 Aqueous. One Solution Cell Proliferation Assay Kit (Promega, Madison, WI) according to the manufacturer's instructions. Cells were seeded in a 96-well plate and then incubated with different time dependent concentrations of extracts of *HCT*. MTS solution quantified in an ELISA microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 492 nm and 690 nm background.

6. Western blot analysis

After treating with extracts of *HCT*, cells were harvested and disrupted. The protein supernatant fractions were subjected to SDS-PAGE(sodium dodecyl sulfate polyacrylamide gel electrophoresis) and then transferred to membranes and blocked with 5% skim milk followed by hybridization with the indicated antibodies. Protein bands with the horse radish peroxidase-conjugated secondary antibody were observed with a chemiluminescence detection kit.

7. Statistical analysis

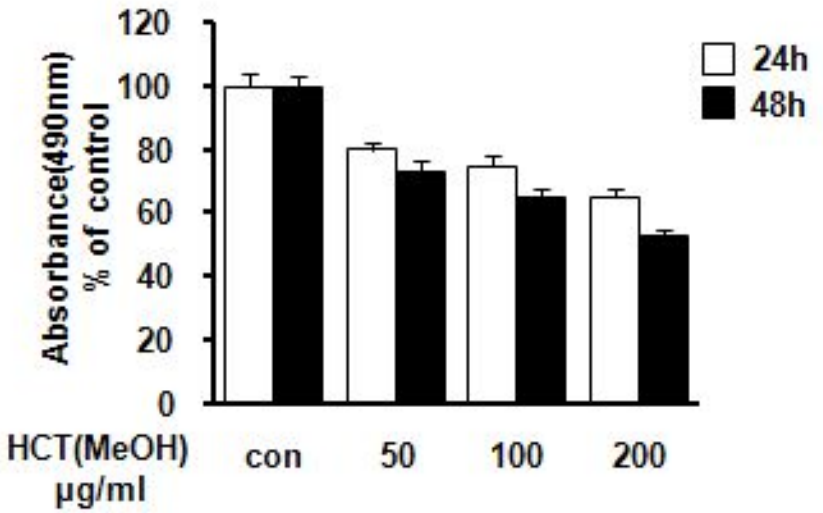
Data are represented as mean±S.D. of at least 3 independent experiments performed in triplicate. Data were assessed for statistical significance using Student's t-test. The minimum level of significance was set at $p < 0.05$.

Ⅲ. RESULTS

1. Effects of *HCT* extracts on PC-3 cell viability by MTS assay (Methanol extracts)

The MTS assay was performed to assess the effects of *HCT* (methanol extracts) on cell viability of PC-3 human prostate cancer cells. *HCT* extracts showed suppressive effects on the cell viability of PC-3 human prostate cancer cells compared to the non-treated ones.

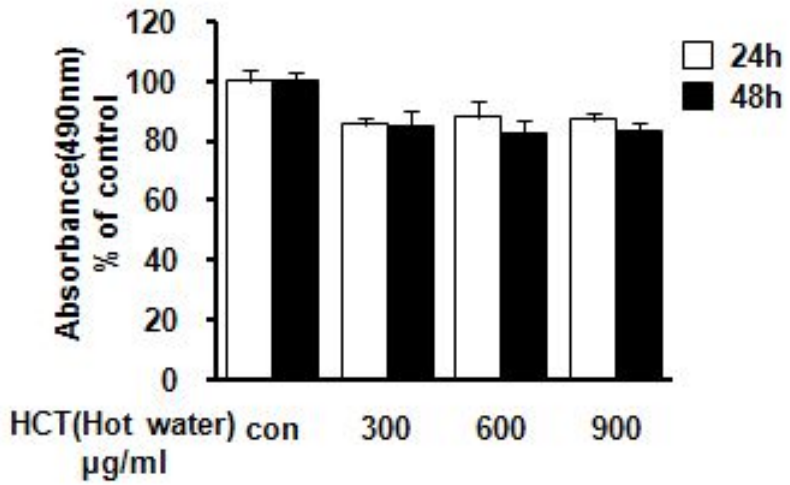
As shown in Figure 1, with incubation for 48 hours, the cell viability significantly decreased depending on concentration. Especially, it was significantly suppressed about 50% at the low density of 200㎍/㎖ after treatment for 48 hours.



<Fig. 1> Effects of *HCT* (methanol extracts) on the viability of PC-3 human prostate cancer cells. The cells were incubated for 24 and 48 hours with midium according to the concentration (0, 50, 100, 200 ㎎/㎖) of *HCT*. The cell viability was estimated by MTS assay. Each bar represents the means ± SD of three independent experiments.

2. Effects of *HCT* extracts on PC-3 cell viability by MTS assay (Hot water extracts)

The MTS assay was performed to estimate the effects of the hot water extracts of *HCT* on cell viability of PC-3. As shown in Figure 1, however, *HCT* hot water extracts did not significantly reduce PC-3 human prostate cancer cells irrespective of time and concentration. The macromolecule extracts, the hot water extracts of *HCT*, had no significant effect on cell viability.

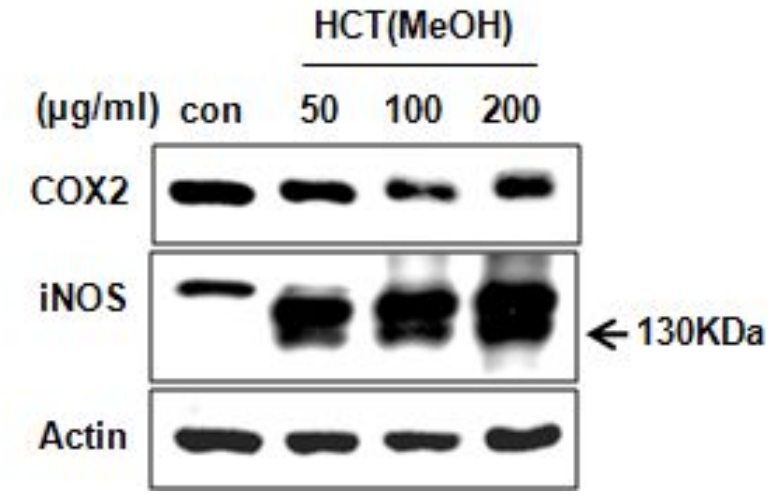


<Fig. 2> Effects of *HCT* (hot water extracts) on the cell viability of PC-3 cells after cultivation with *HCT* hot water extracts for 24 and 48 hours. The cells were incubated for 24 and 48 hours with midium of *HCT*. The cell viability was determined by MTS assay. Each bar represents the mean ± SD of three independent experiments.

3. Effects of *HCT* on COX-2 expression and iNOS expression by Western blot analysis (Methanol extracts)

Western blot analysis of PC-3 human prostate cancer cell lines was performed to assess COX-2 expression and iNOS protein expression. A representative experiment with CO_2-incubation of *HCT* was performed on PC-3 human prostate cancer cells for 24 hours.

As shown in Fig. 3, COX-2 expression was suppressed about 60% at the low density of 100㎍/㎖. In contrast, elevated iNOS expression suggested to promote a cancer-inducing effect. Therefore, reexperiment with isolated and purified *HCT* is required at the density of 900~1000㎍/㎖ of about 5 times concentration.

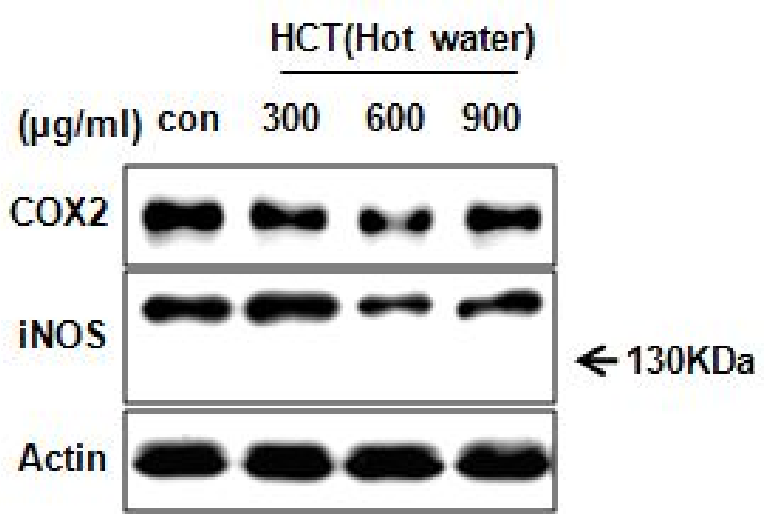


<Fig. 3> Inhibitory effects of *HCT* on COX-2 expression and iNOS expression (methanol extracts) in PC-3 human prostate cancer cells. The cells were pre-treated for 24 hours with either phosphate-buffered saline or *HCT* at indicated concentrations, and then stimulated for 24 hours with 5 ㎎/㎖ of *HCT*. Actin was probed to determine the evenness of the loading protein extracts form each treatment.

4. Effects of *HCT* on COX-2 expression and iNOS expression by Western blot analysis (Hot water extracts)

Western blot analysis of PC-3 human prostate cancer cells was performed to assess COX-2 expression and iNOS expression with hot water extracts of *HCT*. A representative experiment with CO_2-incubation of *HCT* was performed on PC-3 human prostate cancer cells for 24 hours.

As shown in Fig. 4, *HCT* reduced COX-2 expression and iNOS expression in a dose dependent manner. At the density of 600㎍/㎖, the hot water extracts of *HCT* reduced about 80% of COX-2 expression and about 65% of iNOS expression, respectively.



<Fig. 4> Effects of *HCT* on COX-2 expression and iNOS expression (hot water extracts) in PC-3 human prostate cancer cells. The cells were pretreated for 24 hours with either phosphate buffered saline or *HCT* at indicated concentrations, and then stimulated for 24 hours of *HCT*.

The COX-2 and iNOS protein expressions were determined by Western blot analysis. Similar results were observed in three separated experiments.

Ⅳ. DISCUSSION

Houttuynia cordata Tunb.(HCT) has been used as a traditional medicinal herb for a long time to treat a lot of diseases. Recently, *HCT* has been demonstrated to have a variety of biological and pharmalogical activities such as antiviral, antibacterial, antifungal, and antiallergic activities. In addition, *HCT* has been reported to exhibit the antioxidant action, antiinflammatory ability, and antimutagenic effect.

The main phytochemical components of *HCT* are composed of flavonoids and alkaloids. The flavonoid quercetin is known to have a lot of activities. Quercetin has been reported to reduce cell proliferation and HA(hyaluronic acid) emission in orbital fibroblasts of Graves' Ophthalmopathy(GO).[36] The flavonoid glycoside, isoquercitrin (3,3',4',5,7-pentahydroxyflavone-3 beta-O-glucoside) from Waldsteinia fragarioides(Rosaceae) had anti-HSV(herpes simplex type 1 virus).[24] Quercitrin and quercetin-3-O-β-D-galactopy-ranoside in *HCT* exhibited 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activation effectively with the higher level (with IC_{50} of 31 and 63㎛, respectively) than vitamin E(with IC_{50}, 80㎛).[37]

Previous studies showed that quercetin 3-ß-D-glucoside (isoquercetrin) in *HCT* had anti-HSV activity[38] and quercetin 3-D-galactoside (hyperin) in *HCT* could inhibit not only herpes viruses but also SARS and HBV(hepatitis B virus).[23] Chlorogenic acid in *HCT* had anti-pyretic and anti-HSV(herpes simplex type 1 virus) effects as well as anti-adenovirus effect.[25]

The epidemic influenza A virus had been cured more effectively with (Q3R)(quercetin 3-rhamnoside) of flavonoid components in *HCT* than oseltamivir at the initial stage of virus infection.[27] *HCT* injection had inhibited directly PrV(pseudorabies herpes viruses) in vitro.[26]

Houttuynin (decanoyl acetaldehyde), a beta-dicarbonyl compound in the volatile oil of *HCT* has been reported to be the major antibacterial constituent. Houttuynin was found in human plasma and had antibacterial, antimutagenic, and antioxidative activities.[19)]

The polyphenols in *HCT* have been reported to exhibit radical-scavenging activation effectively and might treat diseases related to excess free radicals.[27)]

The antioxidative and antiinflammatory activations are basically precedent conditions on the antitumor activation. The polyphenols in *HCT* had antioxidative capacity as well as antimutagenic capacity under OFO(oxidized frying oil) feeding-induced oxidative stress in a dose dependant manner in vivo. The water extracts of *HCT* had the stronger antimutagenic capacity than the methanol extracts.[33)]

According to the report of Shin et al., *HCT* had antiinflammatory effects corresponding to corticosteroids or NASAIDs by suppressing signaling activity. *HCT* inhibited not only tumor-necrosis factor(TNF)-α-NO but also COX 2 - PGE2 pathways. *HCT* decreased the secretion of NO and PGE2. *HCT* inhibited also carrageenan-induced inflammatory action in vivo.[38)]

Tang et al. reported that the ethanol extracts of *HCT* induced apoptosis at the density of 450㎍/㎖ via a mitochondria-dependent pathway in human colon adenocarcinoma HT-29 cells and the IC_{50} of *HCT* for 72 hours on HT-29 cells was the concentration of 435㎍/㎖. With treatment of *HCT* on HT-29 cells, reactive oxygen species(ROS) production was increased and mitochondria membrane potential(MMP) was reduced through the activation of caspase-cascades.[34)]

Lai et al. demonstrated that the ethanol extracts of *HCT* induced

cytotoxicity and apoptosis in three human primary colorectal cancer cells via a mitochondria-dependent signaling pathway, and the IC_{50} was lower than 435㎍/㎖(289.62, 321.09, and 296.41㎍/㎖, respectively). *HCT* suppressed proliferation of cancer cells in a concentration-dependent manner. At the concentration of 250㎍/㎖ after 24 hour treatment, an apoptotic characteristic chromatin condensation was shown. With treatment of *HCT,* ROS production was activated and the MMP was suppressed in human primary colorectal cancer cells.[35)]

There are two main goals to treat cancers. One is inhibiting cell proliferation or inducing cell cycle arrest and apoptosis. The other is inhibiting survival signaling pathways in cancer cells.[39)] Apoptosis is the best major mechanism through which apoptotic and autophagic cell death are induced at exposure to chemotherapy or radiotherapy.[19)] Phytochemicals with anticancer properties become increasingly payed attention to treat cancer.[40)]

COX-2(Cyclooxygenase-2) is an enzyme that regulates different inflammatory mediators activity such as prostaglandins. COX-2 activates tumor growth and metastasis. The COX-2 expression is elevated in many carcinomas and its potential role to cancer progress is acted on several pathways.[41)]

It is well known that iNOS (inducible nitric oxide synthase) expression is promoted by inflammatory reaction or stress. The iNOS is induced by not only bacterial components like LPS (lipopolysaccharide) but also various cytokines.[20)] In the research of antibacterial activities against the intracellular pathogen salmonella, iNOS expression was not affected by *HCT* alone. In that experiment, *HCT* treatment did not have direct relation with iNOS expression, but *HCT* increased phagocytic activation and iNOS expression in Salmonella pathogen.[28)]

The suppression of prostate cancer by *HCT* could be mediated through the regulation of multiple signaling pathways in PC-3 cell lines. In this study, extracts of *HCT* were examined by MTS assay and Western blot analysis. The cell viability of PC-3 cells was measured by MTS assay. Western blot analysis was performed to assess COX-2 expression and iNOS expression.

Methanol extracts of *HCT* significantly suppressed the cell viability in a dose-dependent manner. It significantly inhibited about 50% of the cell viability at the low concentration of 200㎍/㎖ with treatment for 48 hours. But when compared to the control group, the macromolecule extracts from the hot water extracts of *HCT* had no significant effect on the cell viability.

For medical use, not macromolecule extracts over 40,000 KDa but micromolecules below 3,000 KDa are needed in order to be adopted as an adjuvant for the treatment of prostate cancers.

In order for *HCT* to be beneficial to prevent and treat prostate cancer, both COX-2 and iNOS pathways should be regulated down by decreasing COX-2 and iNOS expressions. Increased expressions of COX-2 and iNOS result in poorer prognoses for patients.

Western blot analysis suggested that methanol extracts of *HCT* suppressed about 60% of COX-2 protein expression at the low density of 100㎍/㎖. However, abundant iNOS expression in this study suggested that *HCT* might have its possibility to promote a cancer-inducing effect. The possibility of chemoprevention of *HCT* against prostate cancer could not be expected with this result. This result was completely different from those results Tang et al. and Lai et al. reported, respectively, even though species of cancers and extraction solvents were different. It could be expected that various concentrations of *HCT* could bring out different

results from those in this study. Therefore, reexperiment with isolated and purified *HCT* is required such as at the density of 900~1000㎍/㎖ of more 5 times concentration than the low density of 200㎍/㎖ used in this study.

Also Western blot analysis resulted in that the hot water extracts of *HCT* reduced COX-2 and iNOS expressions in a dose dependent manner. At the density of 600㎍/㎖, the hot water extracts of *HCT* reduced about 80% of COX-2 expression and about 65% of iNOS expression, respectively. So, *HCT* could be considered to have the potentiality of chemoprevention for prostate cancers. The hot water extracts, macromolecules, could be adopted as foods through edibile processing.

However, because of the elevated iNOS expression, further studies associated with its safety would be necessary. In this study, it was not investigated which components of *HCT* could suppress the proliferation of PC-3 cells, and by which components of *HCT* and through which signaling pathways abundant iNOS expression was caused in spite of the suppressive effect of the cell proliferation. Therefore, further researches on biologically active components of *HCT* and the mechanism of *HCT* activity would be required. And then it would be expected that reexperiment not only with isolated and purified *HCT* but also with other extraction solvents such as ethanol might exhibit its potentiality of chemoprevention on prostate cancer.

Ⅴ. CONCLUSION

MTS assay and Western blot analysis were performed in this study to investigate argumentive suppressive effects of *HCT* extracts on PC-3 human prostate cancer cells. The followings are the results of the study.

MTS assay results:

1. Methanol extracts of *HCT,* micromolecules below 3,000 KDa, significantly suppressed the cell viability in a dose-dependant manner with incubation for 48 hours. Especially, it significantly inhibited about 50% of the cell survival rate at the low concentration of 200㎍/㎖ with the treatment for 48 hours.

2. Macromolecule extracts over 40,000 KDa from the hot water extracts of *HCT* had no significant effect on cell viability.

Western blot analysis results:

3. Methanol extracts of *HCT* suppressed about 60% of COX-2 protein expression at the low density of 100㎍/㎖. In contrast, abundant iNOS expression implicated the possibility to promote a cancer-inducing effect.

4. Hot water extracts of *HCT* reduced COX-2 and iNOS expressions in a dose dependent manner. At the density of 600㎍/㎖, the hot water extracts of *HCT* reduced about 80% of COX-2 expression and about 65% of iNOS expression, respectively.

This study is a basic research to investigate the suppression effects of *HCT,* a traditional medicinal herb, on PC-3 human prostate cancer cells. Hot water extracts, macromolecules, can be adopted as foods by edible processing. So, *HCT* could be considered to have the potentiality of chemoprevention for prostate cancers.

Methanol extracts of *HCT,* micromolecules below 3,000 KDa, could play a role on the prevention and treatment of prostate cancer cells, since certain constituents of *HCT* extracts significantly suppressed the cell viability of PC-3 at a low density of 200㎍/㎖.

However, because of elevated iNOS expression in methanol extracts, further studies related to its safety would be necessary. Therefore, further researches on biologically active components of *HCT* would be still necessary. Reexperiment with other various extraction solvents as well as with isolated and purified *HCT* would be needed so that *HCT* could identify its potentiality of chemoprevention on prostate cancer. Moreover, additional studies would be required to verify the mechanism of action of *HCT*.

Ⅵ. 국 문 초 록

어성초 추출물이 인체 전립선암 세포주 PC-3에 미치는 영향

지도교수 : 문 경 래
조선대학교 보건대학원
대체의학과 나 백 희

목적 : 어성초가 인체 전립선암 세포주 PC-3에 대해 암세포의 증식을 억제할 수 있는지 그리고 암 표적인자 COX-2와 iNOS의 발현을 억제할 수 있는지를 탐색하였다.

방법 : 어성초의 열수 추출물과 메탄올 추출물이 PC-3 세포주의 세포 생존율에 미치는 영향을 측정하기 위해 MTS assay를 수행하고, 암 표적인자 COX-2 와 iNOS의 발현에 미치는 영향은 Western Blot analysis를 수행하여 측정하였다.

결과 : MTS assay를 수행한 결과, 어성초의 메탄올 추출물은 인체 전립선암 세포를 농도 의존적으로 유의하게 억제하였다. 특히, 48시간 처리했을 때 200㎍/㎖의 낮은 농도에서 50% 정도 인체 전립선암 세포의 성장을 억제하였다. 그러나 어성초의 열수 추출물은 인체 전립선암 세포에 영향을 미치지 못했다.

Western blot analysis 결과, 어성초의 열수 추출물은 COX-2와 iNOS의 발현을 농도 의존적으로 억제하였다. 600㎍/㎖의 농도에서 COX-2의 발현은 80% 정도, iNOS의 발현은 65% 정도 억제하였다. 어성초의 메탄올 추출물은 100㎍/㎖의 저농도에서 COX-2의 발현을 60% 정도 억제하였다. 그러나 메탄올 추출물에서는 많은 양의 iNOS 단백질이 발현되었다.

결론 : 저분자 화합물인 어성초의 메탄올 추출물은 200㎍/㎖의 저농도에서 전립선암 세포의 증식을 50% 정도 억제하는 효과를 나타냈다. 그러나 이 실험에서 많은 양의 iNOS 발현은 암유발 효과 가능성을 시사하며 항암효과나 화학예방효과를 기대하기 힘들다. 따라서 어성초의 화학예방효과의 가능성을 확인하기 위해서는 좀 더 정밀한 정제와 분리과정, 그리고 여러 가지 추출방법을 통해 기전을 밝히는 추가적인 연구와 재실험이 요구된다.

키 워드: 어성초, 전립선암 세포주 PC-3, MTS, COX-2, iNOS

Ⅶ. REFERENCES

1) Torricelli P, Ricci P, Provenzano B, Lentini A, Tabolacci C.: "*Synergic effect of a -tocopherol and naringenin in transglutaminase-induced differentiation of human prostate cancer cells*", Amino Acids. 2010 Oct 28.

2) Jiang C, Lee HJ, Li GX, Guo J, Malewicz B, Zhao Y, Lee EO, Lee HJ, Lee JH, Kim MS, Kim SH, Lu J.: "*Potent antiandrogen and androgen receptor activities of an Angelica gigas-containing herbal formulation: identification of decursin as a novel and active compound with implications for prevention and treatment of prostate cancer*", Cancer Res. 2006 Jan 1;66(1):453-63.

3) Ministry of Health and Welfare, National cancer information center.

4) Landis SH, Murry T, Bolden S, Wingo PA.: "*Cancer statistics*", CA Cancer J. Clin. 1999, 49:8-21.

5) Kuo PL, Chen YH, Chen TC, Shen KH, Hsu YL.: "*CXCL5/ENA78 increased cell migration and epithelial-to-mesenchymal transition of hormone-independent prostate cancer by early growth response-1 /Snail signaling pathway*", J Cell Physiol. 2010 Oct 13.

6) Warrington RJ, Lewis KE.: "*Natural antibodies against nerve growth factor inhibit in vitro prostate cancer cell metastasis*", Cancer Immunol Immunother. 2010 Oct 26.

7) Kim CH, Park SJ, Lee SH.: "*A Targeted inhibition of DNA-Dependent Protein Kinase Sensitizes Breast Cancer Cells Following Ionizing Radiation*", J Pharmacol Exp Ther. 2002 Nov;303(2):753-9.

8) Surh YJ.: "*Cancer chemoprevention with dietary phytochemicals*", Nat Rev Cancer. 2003 Oct;3(10):768-80.

9) Zhang HN, Yu CX, Zhang PJ, Chen WW, Jiang AL, Kong F, Deng JT, Zhang JY, Young CY.: " *Curcumin downregulates homeobox gene NKX3.1 in prostate cancer cell LNCap*", Acta Pharmacol Sin. 2007 Mar;28(3):423-30.

10) RS Cotrans, V Kurma, T Collin, RL Robbins: "*Robbins Pathologic Basics of Disease*", 6th edition. W. B. Saunders Company. 1990, 1-29.

11) B Halliwell : "*Antioxidants in Human and Disease*", Annual Review of Nutrition. 1996, 16:33-50.

12) Abdulah R, Faried A, Kobayashi K, Yamazaki C, Suradji EW, Ito K, Suzuki K, Murakami M, Kuwano H, Koyama H.: "*Selenium enrichment of broccoli sprout extracts increases chemosensitivity and apoptosis of LNCaP prostate cancer cells*", BMC Cancer. 2009 Nov 30;9:414.

13) Herr I, Büchler MW.: "*Dietary constituents of broccoli and other cruciferous vegetables: Implications for prevention and therapy of cancer*", Cancer Treat Rev. 2010 Aug;36(5):377-83.

14) Bosland MC, McCormick DL, Melamed J, Walden PD, Zeleniuch-Jacquotte A, Lumey LH.: "*Chemoprevention strategies for prostate cancer*", Eur J Cancer Prev. 2002 Aug;11 Suppl 2:S18-27.

15) Chiang LC, Chang JS, Chen CC, NG LT, Lin CC.: "*Anti-Herpes simplex virus activity of Bidens pilosa and Houttuynia cordata*", Am J Chin Med. 2003;31(3):355-62.

16) Ji B, Zhao K.: "*Clinical application of Houttuynia cordata injection*", Chin Med Pharm J, 2003, 2:14-16.

17) Cho EJ, Yokozawa T, Rhyu DY, Kim SC, Shibahara N, Park JC.: "*Study on the inhibitory effects of Korean medicinal plants and their main compounds on the 1,1-diphenyl-2-picrylhydrazyl radical*", Phytomedicine. 2003;10(6-7):544-51.

18) Meng J, Dong XP, Zhou YS, Jiang ZH, Leung KS, Zhao ZZ.: "*Orthogonal experiment using SFE-CO2 in extractsion of essential oil from fresh Houttuynia cordata and analysis of essential oil by GC-MS*", Zhongguo Zhong Yao Za Zhi. 2007 Feb;32(3):215-7.

19) Duan X, Zhong D, Chen X.: "*Derivatization of beta-dicarbonyl compound with 2,4-dinitrophenylhydrazine to enhance mass spectrometric detection: application in quantitative analysis of houttuynin in human plasma*", J Mass Spectrom. 2008

Jun;43(6):814-24.

20) Xu X, Ye H, Wang W, Yu L, Chen G.: "*Determination of flavonoids in Houttuynia cordata Thunb. and Saururus chinensis (Lour.) Bail. by capillary electrophoresis with electrochemical detection*", Talanta. 2006 Jan 15;68(3):759-64.

21) Miyata M, Koyama T, Yazawa K.: "Water extracts of Houttuynia cordata Thunb. leaves exerts anti-obesity effects by inhibiting fatty acid and glycerol absorption", J Nutr Sci Vitaminol (Tokyo). 2010;56(2):150-6.

22) Lin TY, Liu YC, Jheng JR, Tsai HP, Jan JT, Wong WR, Horng JT.: "*Anti-enterovirus 71 activity screening of chinese herbs with anti-infection and inflammation activities*", Am J Chin Med. 2009;37(1):143-58.

23) Lau KM, Lee KM, Koon CM, Cheung CS, Lau CP, Ho HM, Lee MY, Au SW, Cheng CH, Lau CB, Tsui SK, Wan DC, Waye MM, Wong KB, Wong CK, Lam CW, Leung PC, Fung KP.: "*Immunomodulatory and anti-SARS activities of Houttuynia cordata*", J Ethnopharmacol. 2008 Jun 19;118(1):79-85.

24) Abou-Karam M, Shier WT.: "*Isolation and characterization of an antiviral flavonoid from Waldsteinia fragarioides*", J Nat Prod. 1992 Oct;55(10):1525-7.

25) Wu LL, Yang XB, Huang ZM, Liu HZ, Wu GX.: "*In vivo and in vitro antiviral activity of hyperoside extractsed from Abelmoschus manihot (L) medik*", Acta Pharmacol Sin. 2007 Mar;28(3):404-9.

26) Ren X, Sui X, Yin J.: "*The effect of Houttuynia cordata injection on pseudorabies herpesvirus (PrV) infection in vitro*", Pharm Biol. 2010 Oct 13.

27) Choi HJ, Song JH, Park KS, Kwon DH.: "*Inhibitory effects of quercetin 3-rhamnoside on influenza A virus replication*", Eur J Pharm Sci. 2009 Jun 28;37(3-4):329-33.

28) Kim GS, Kim DH, Lim JJ, Lee JJ, Han DY, Lee WM, Jung WC, Min WG, Won CG, Rhee MH, Lee HJ, Kim S.: "*Biological and antibacterial activities of the natural herb Houttuynia cordata water extracts against the intracellular bacterial pathogen salmonella within the RAW 264.7 macrophage*", Biol Pharm Bull. 2008 Nov;31(11):2012-7.

29) Shim SY, Seo YK, Park JR.: "*Down-regulation of FcepsilonRI expression by Houttuynia cordata Thunb extracts in human basophilic KU812F cells*", J Med Food. 2009 Apr;12(2):383-8.

30) Toda S.: "*Antioxidative effects of polyphenols in leaves of Houttuynia cordata on protein fragmentation by copper-hydrogen peroxide in vitro*", J Med Food. 2005 Summer;8(2):266-8.

31) Lee JS, Kim IS, Kim JH, Kim JS, Kim DH, Yun CY.: "*Suppressive effects of Houttuynia cordata Thunb (Saururaceae) extracts on Th2 immune response*", J Ethnopharmacol. 2008 Apr 17;117(1):34-40.

32) Park E, Kum S, Wang C, Park SY, Kim BS, Schuller-Levis G.: "*Anti-inflammatory activity of herbal medicines: inhibition of nitric oxide production and tumor necrosis factor-alpha secretion in an activated macrophage-like cell line*", Am J Chin Med. 2005;33(3):415-24.

33) Chen YY, Liu JF, Chen CM, Chao PY, Chang TJ.: "*A study of the antioxidative and antimutagenic effects of Houttuynia cordata Thunb. using an oxidized frying oil-fed model*", J Nutr Sci Vitaminol (Tokyo). 2003 Oct;49(5):327-33.

34) Tang YJ, Yang JS, Lin CF, Shyu WC, Tsuzuki M, Lu CC, Chen YF, Lai KC.: "*Houttuynia cordata Thunb extracts induces apoptosis through mitochondrial-dependent pathway in HT-29 human colon adenocarcinoma cells*", Oncol Rep. 2009 Nov;22(5):1051-6.

35) Lai KC, Chiu YJ, Tang YJ, Lin KL, Chiang JH, Jiang YL, Jen HF, Kuo YH, Agamaya S, Chung JG, Yang JS.: "*Houttuynia cordata Thunb extracts inhibits cell growth and induces apoptosis in human primary colorectal cancer cells*", Anticancer Res. 2010 Sep;30(9):3549-56.

36) Lisi S, Botta R, Lemmi M, Sellari-Franceschini S, Altea MA, Sisti E, Casini G, Nardi M, Marcocci C, Pinchera A, Marinò M.: "*Quercetin decreases proliferation of orbital fibroblases and their release of hyaluronic acid*", J Endocrinol Invest. 2010 Oct 27.

37) Chou SC, Su CR, Ku YC, Wu TS.: "*The Constituents and Their Bioactivities of*

Houttuynia cordata", Chem Pharm Bull (Tokyo). 2009 Nov;57(11):1227-30.

38) Shin S, Joo SS, Jeon JH, Park D, Jang MJ, Kim TO, Kim HK, Hwang BY, Kim KY, Kim YB.: "*Anti-inflammatory effects of a Houttuynia cordata supercritical extracts*", J Vet Sci. 2010 Sep;11(3):273-5.

39) Faried A, Kurnia D, Faried LS, Usman N, Miyazaki T, Kato H, Kuwano H.: "*Anticancer effects of gallic acid isolated from Indonesian herbal medicine, Phalerria macrocarpa (Scheff.) Boerl, on human cancer cell lines*", Int J Oncol. 2007 Mar;30(3):605-13.

40) Zhou JR, Yu L, Zhong Y, Blackburn GL.: "*Soy phytochemicals and tea bioactive components synergistically inhibit androgen-sensitive human prostate tumors in mice*", J Nutr. 2003 Feb;133(2):516-21.

41) Ko SH, Choi GJ, Lee JH, Han YA, Lim SJ, Kim SH.: "*Differential effects of selective cyclooxygenase-2 inhibitors in inhibiting proliferation and induction of apoptosis in oral squamous cell carcinoma*", Oncol Rep. 2008 Feb;19(2):425-33.

저 작 물 이 용 허 락 서

본인이 저작한 학위논문에 대하여 다음과 같은 방법 및 조건하에 대학교에 저작권을 위임할 것을 서약합니다.

1. 인터넷 및 온라인 서비스와 아카이빙을 위하여 저작물의 내용을 변경하지 않는 편집상 혹은 포맷상의 변경을 통한 복제를 허락함.
2. 저작물의 DB 구축과 인터넷을 포함한 정보통신망에 공개하여 논문 일부 또는 전부의 복제·배포 및 전송을 허락함.
3. 저작물에 대한 이용 기간은 3년으로 하고 계약 종료 2개월 이내에 별도의 의사표시가 없는 경우 기간을 계속 연장함.
4. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판 허락을 하였을 경우 1개월 이내에 소속 대학에 통보함.
5. 배포, 전송된 학위논문은 이용자가 다시 복제 및 전송할 수 없으며 이용자가 연구 목적이 아닌 상업적 용도로 사용하는 것을 금함.
6. 소속대학은 학위논문 위임 서약 이후 해당 저작물로 인한 타인의 권리 침해에 관하여 일체의 법적 책임을 지지 않을 것을 확인함.
7. 소속대학의 협약기관 및 한국교육학술정보원에 논문 제공을 허락함.

동의여부 : 동의(V) 조건부 동의( ) 반대( )

※ 조건부 동의 및 반대인 경우 사유 및 조건을 기재하여 주시기 바랍니다.

사유 :

조건 :

저작자 성명 : 나 백 희

주소 : 광주광역시 북구 연제동 광명아파트 201동 1401호

(연락처 : 011-9221-3356)

2010 년 12월 일

이름 : 나 백 희 (인)

조 선 대 학 교 총 장 귀하

저자명(한글)	나백희	(한자)	羅白熙
저자명(영문)	Na, Baek Hee		
학 과	대체의학과	학 번	20098621
전화번호	062-417-3356		
핸드폰	011-9221-3356		
E-mail 주소	102102na@hanmail.net		
E-mail 주소 공개 여부	공개 ( ) 미공개 (∨)		

논문명(국문)	어성초 추출물이 인체 전립선암 세포주 PC-3에 미치는 영향		
논문주제분야	총류( ), 철학( ), 종교( ), 사회과학( ), 순수과학( ), 기술과학(∨), 예술( ), 어학( ), 문학( ), 역사학( )		
학위구분	석사 (∨) 박사 ( )		
초록기술언어	영어, 한글	논문 쪽수	30 쪽

한글초록	MTS assay를 수행한 결과, 어성초의 메탄올 추출물은 인체 전립선암 세포를 농도의존적으로 유의하게 억제하였다. 특히, 48시간 처리했을 때 200㎍/㎖의 낮은 농도에서 50% 정도 인체 전립선암 세포의 성장을 억제하였다. 그러나 어성초의 열수 추출물은 인체 전립선암 세포에 영향을 미치지 못했다. Western Blot analysis 결과, 어성초의 열수 추출물은 COX-2와 iNOS의 발현을 농도의존적으로 억제하였다. 600㎍/㎖의 농도에서 COX-2의 발현은 80% 정도, iNOS의 발현은 65% 정도 억제하였다. 어성초의 메탄올 추출물은 100㎍/㎖의 저농도에서 COX-2의 발현을 60% 정도 억제하였다. 그러나 메탄올 추출물에서는 많은 양의 iNOS 단백질이 발현되었다.
주제어(국문)	어성초, 전립선암, 세포생존율, COX-2, iNOS
논문명(원문)	Effects of *Houttuynia cordata Tunb.* Extracts on PC-3 Human Prostate Cancer Cells
본문기술언어	영어
초록(원문)	Methanol extracts of *HCT* significantly suppressed the cell survival rate in a dose dependant manner. Especially, it significantly inhibited about 50% of the cell viability at the low density of 200㎍/㎖ with 48 hour treatment. Hot water extracts of *HCT* had no significant effect on cell viability. Methanol extracts of *HCT* suppressed about 60% of COX-2 protein expression at the low density of 100㎍/㎖. However, iNOS expression was elevated abundantly in methanol extracts. Hot water extracts of *HCT* reduced COX-2 expression and iNOS expression in a dose dependent manner.
주제어(원문)	*Houttuynia cordata Thunb.,* PC-3 human prostate cancer cells, COX-2, iNOS