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Preventive Effects of Quercetin against Benzo[a]pyrene-Induced DNA Damages and Pulmonary Precancerous Pathologic Changes in Mice

Nian-zu Jin[1], Yan-ping Zhu[1], Jian-wei Zhou[1], Li Mao[1], Ren-cheng Zhao[1], Tai-hui Fang[2] and Xin-ru Wang[1]

[1]Institute of Toxicology, Nanjing Medical University, 140 Hanzhong Road, Nanjing 210029, and
[2]School of Pharmacy, Nanjing University of Chinese Medicine, 282 Hanzhong Road, Nanjing 210029, China

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Abstract: The aim of this study was to investigate the preventive effects of quercetin against benzo[a]pyrene-induced blood lymphocyte DNA damages and pulmonary precancerous pathologic changes in mice, and to reveal the potential mechanism behind these effects. In this study, mice in quercetin-treated groups were given quercetin for 90 days. After one week of treatment, mice in the quercetin-treated groups and the positive control group received a single intraperitoneal dose of benzo[a]pyrene (100 mg/kg body weight). The results of single cell gel electrophoresis assay showed that the average lengths of the comet cell tail and DNA damage in the peripheral blood lymphocytes of mice induced by benzo[a]pyrene decreased significantly as a result of quercetin treatment dose-dependently. Light microscopic examination showed that the degrees of pulmonary precancerous pathologic changes in the quercetin-treated groups decreased significantly compared with those in the positive control group. Meanwhile, the cytochrome P4501A1-linked 7-ethoxyresorufin O-dealkylase activities in lung microsomes of mice decreased as the dose of quercetin increased. The results of this *in vivo* study revealed that quercetin had a significant preventive effect on benzo[a]pyrene-induced DNA damage, and had a potential chemopreventive effect on the carcinogenesis of lung cancer induced by benzo[a]pyrene. The mechanism of these effects of quercetin could be related to the inhibition of cytochrome P4501A1 activity.

The bioflavonoid quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a polyphenolic compound widely distributed in the plant kingdom and is concentrated in some fruits and vegetables (Michael *et al.* 1992; Alan *et al*. 1997; Schieber *et al*. 2001; Ross & Kasum 2002). It has been demonstrated to possess chemopreventive effects during carcinogenesis and exert cytostatic activity against a variety of tumour cells *in vitro* and *in vivo* (Caltagirone *et al.* 2000; Ranelletti *et al.* 2000; Pawlikowska *et al.* 2001; Sengupta *et al.* 2001; Kuo *et al.* 2002; Lin *et al.* 2002; Lee *et al.* 2002; Mouria *et al.* 2002; Chan *et al.* 2003; De *et al.* 2004; Nair *et al.* 2004; Nguyen *et al.* 2004; Lambert *et al.* 2005; Ramos *et al.* 2005; Zhang *et al.* 2005).

Previous studies have proved that the cytochrome P4501A1 (CYP1A1) enzyme bioactivates several procarcinogens, such as benzo[a]pyrene and other polycyclic aromatic hydrocarbons (Dogra *et al.* 1998; Hasler 1999; Omiecinski *et al.* 1999). In some *in vitro* studies, quercetin had been shown to inhibit CYP1A1, and thus inhibit the bioactivation of certain procarcinogens (Schwarz *et al.* 2003 & 2005). On the other hand, Le Marchand *et al.* (2000) investigated the possible relationship between the intake of flavonoid-intensive dietary and lung cancer risk using a population-based, case-control study in Hawaiians. They found an inverse association between the intake of quercetin and the risk of lung cancer. CYP1A1, which assists in the bioactivation of procarcinogens, may play a role in this association. Neuhouser (2004) reviewed data from four cohort studies and six case-control studies, which have examined associations of flavonoid intake with cancer risk. He found that there was consistent evidence from these studies that flavonoids, especially quercetin, may reduce the risk of lung cancer. These findings suggested that foods which are rich in quercetin may prevent lung cancer, and the decrease of procarcinogenic bioactivation by the inhibition of CYP1A1 should be explored as underlying mechanisms. There were few *in vivo* studies in this field of research, and the evidences found by *in vitro* studies and epidemiological studies should be proved and further clarified by experimental animal studies. We investigated the potential effects of quercetin on pulmonary precancerous pathologic changes and blood lymphocytes DNA damages in mice induced by benzo[a]pyrene, and detected changes in CYP1A1 activity in lung microsomes in this process. The aim of this study is to reveal the potential effects of quercetin on the early stage of carcinogenesis induced by benzo[a]pyrene, and to explore its mechanism.

Author for correspondence: Nian-zu Jin, Institute of Toxicology, Nanjing Medical University, 140 Hanzhong Road, Nanjing 210029, China (fax +86 25 86527613, e-mail jinnianzu2004@yahoo.com.cn).

Materials and Methods

Chemicals. Chemicals were obtained as follows: quercetin from Shanghai Second Reagent Inc (Shanghai, China); benzo[a]pyrene, 7-ethoxyresorufin, resorufin, β-nicotinamide adenine dinucleotide phosphate (NADP), D-glucose 6-phosphate disodium salt, glucose-6-phosphate dehydrogenase, bovine serum albumin and Triiton-

X100 from Sigma Chemical Co. (St. Louis, MO, USA); ethidium bromide and brilliant blue G from Fluka (Buchs, Switzerland); normal melting point agarose and low melting point agarose from Bio-Rad (Hercules, CA, USA); dimethyl sulfoxide (DMSO) and all other chemicals and reagents used were of highest commercially available quality.

Animals. ICR mice (clean animal, provided by the Experimental Animal Center of Jiangsu Province, China) weighing 17±1 g were housed in plastic cages. The animal facilities had an ambient temperature of 22±2°, a relative humidity of 55±5%, and a 12 hr light-dark cycle. The mice had free access to conventional laboratory feed and water. They were randomly divided into five treatment groups: high, middle, low doses of quercetin oral administration groups, the control group and the positive control group. Each group consisted of five male mice and five female mice. Animals in high, middle, low doses of quercetin oral administration groups were given quercetin (with 1% sodium carboxymethylcellulose as vehicle) by gavage at doses of 2.0, 1.0, 0.5 g/kg body weight respectively, once a day, for a duration of 90 days; animals in the control group and positive control group were dosed with the vehicle only. After one week of treatment, animals in all groups except the control group received a single intraperitoneal dose of benzo[a]pyrene (at the dose of 100 mg/kg body weight). After 90 day treatment of the animals, peripheral blood was drawn from the tail vein of mice for the SCGE assay, following which lung samples of the mice were taken out and prepared for pathomorphological examination and microsome isolation.

Single cell gel electrophoresis (SCGE) assay. The procedure of SCGE assay (or comet assay) was basically performed as described by Singh-*et al.* (1988). Briefly, a 5 µl murine blood sample mixed with 75 µl of 0.6% low melting point agarose was transferred to a microscope slide precoated with 1.0% normal melting point agarose and covered with a coverglass for 2 min. After allowing the low melting point agarose to solidify by putting the slide on a cooled metal plate for 2 min., the coverglass was carefully removed. Seventy µl of 0.6% low melting point agarose was placed on the slide as the top layer and covered with a coverglass again. When the top layer of agarose solidified, the coverglass was carefully removed and the slides submersed into alysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris-Hcl, pH 10; and 1% Triton X-100, 10% DMSO added freshly before use) at 4° for 24 hr to remove cellular proteins. Slides were then placed in a horizontal gel electrophoresis tank filled with fresh electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH 13) and left in the solution for 20 min. at 4° to allow the DNA to unwind and the alkali labile damage to occur. Electrophoresis was then carried out for 20 min. at 25 V, 300 mA. After electrophoresis, the slides were removed from the electrophoresis tank, neutralized with 3×5 min. washes with Tris-HCl (0.4 M, pH 7.5) and stained with 50 µl of ethidium bromide (0.05 mM). DNA migration which looks like a comet was viewed by a fluorescence microscope (Axioskop 2 plus, Zeiss). Images were captured by CCD camera.

The comet cell tail length (the distance from the center of comet head to the end of comet tail) viewed in the images were measured. In addition, according to the relative intensity of fluorescence in the tail, the percentage of DNA migration (the percentage of DNA within the comet tail in total DNA of the comet cell) were estimated, and used as the index of DNA damage degrees (Singh *et al.* 1988). There were five degrees for differentiating the level of DNA damage:

Degree 0: with no DNA damage, DNA migration<5%, comet tail was not in evidence.
Degree 1: with low DNA damage, DNA migration was in the range of 5%~20%, with mild comet tail.
Degree 2: with middle DNA damage, DNA migration was in the range of 20%~40%, with evident comet tail.
Degree 3: with high DNA damage, DNA migration was in the range of 40%~95%, with severe comet tail.
Degree 4: with complete DNA damage, DNA migration>95%, become snippet.

Pulmonary pathomorphological examination. Murine lung samples were secured by mild formalin, embedded in paraffin, sliced up to sections with 4 µm thickness and stained with haematoxylin+eosin for microscopic examination. Light microscopically, diagnostic standard for pulmonary pathomorphological examination was as follows:

1. Normal bronchi epithelium: normal bronchi epitheliums are cylindrical or cubical which lie on the surface of basal cell and cilium of epithelial cells stretch out to lumen.
2. Squamous epithelial metaplasia: squamous epithelial substitute with normal bronchi pseudostratified ciliated columnar epithelium (the latter may be a result of keratinization).
3. Atypical hyperplasia: bronchi epithelium constitute of some irregular cells, which vary in size and diversity in morphology. There is hyperchromatin and a large nucleus and nucleus/cytoplasm ratio increase. Mitosis can be observed.
4. Carcinoma *in situ*: normal bronchi epitheliums are completely displaced by severe atypical hyperplasia cells.
5. Squamous cell carcinoma: tumour cells display cord and/or sheet arrangement and marked atypia. "Keratin Pearls" and intercellular bridges may be seen.

Preparation of murine microsomes. Pieces of murine lung samples were homogenized with 4:1 (v/w) ice-cold 1.15% KCl using a glass

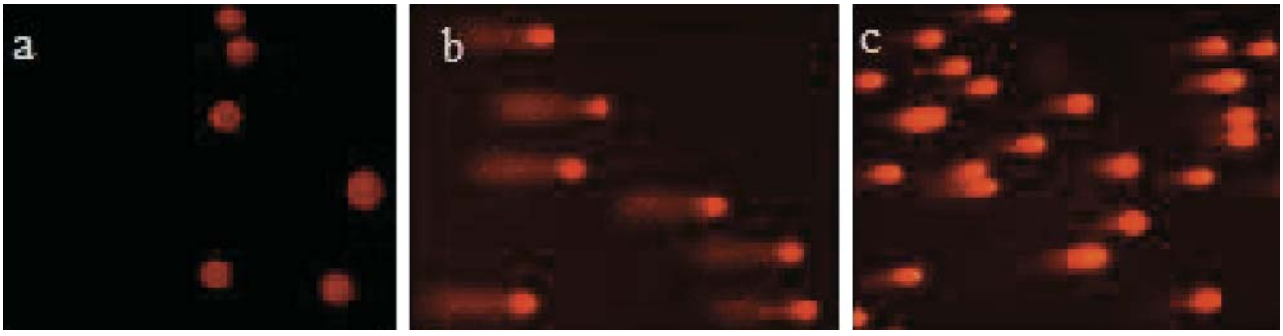

Fig. 1. Effect of quercetin on the DNA damage of blood lymphocytes in mice induced by benzo[a]pyrene. The morphologic observation of murine peripheral blood lymphocytes under fluorescence microscope: (a) The cells in the control group were undamaged, showing fluorescent circular nucleus; (b) The cells in the positive control group were damaged significantly and with severe comet tail; (c) The cells in the quercetin treatment (with a dose of 1.0 g/kg·day) group showed a substantial decrease of damage compared with that of the positive control group.

Table 1.

The frequency distribution of DNA damages with different degrees within the treatment groups in the SCGE assay. According to the relative intensity of fluorescence in the tail, the percentage of DNA migration were estimated, and used as the index of DNA damage degrees. A total of 600 lymphocytes were scored per treatment group by randomization for the evaluation of the DNA damage degrees.

		The frequency distribution and proportion (%)				
Sex	Treatment groups	Degree 0	Degree 1	Degree 2	Degree 3	Degree 4
Male	Control[b,c,d,e]	466 (77.7)	110 (18.4)	20 (3.3)	2 (0.3)	2 (0.3)
	Positive control[a,c,d,e]	123 (20.5)	99 (16.5)	124 (20.7)	75 (12.4)	180 (29.9)
	Low quercetin dose[a,b,d,e]	282 (47.0)	164 (27.2)	110 (18.3)	36 (6.0)	9 (1.5)
	Middle quercetin dose[a,b,c,e]	230 (38.3)	263 (43.8)	86 (14.3)	13 (2.2)	8 (1.4)
	High quercetin dose[a,b,c,d]	317 (52.8)	191 (31.8)	76 (12.6)	15 (2.6)	1 (0.2)
Female	Control[b,c,d,e]	494 (82.4)	98 (16.3)	6 (1.1)	1 (0.2)	0 (0)
	Positive control[a,c,d,e]	72 (12.0)	148 (24.7)	172 (28.7)	124 (20.8)	83 (13.8)
	Low quercetin dose[a,b,d,e]	129 (21.5)	142 (23.6)	147 (24.5)	111 (18.5)	72 (11.9)
	Middle quercetin dose[a,b,c]	376 (62.6)	168 (28.0)	43 (7.2)	12 (2.0)	1 (0.2)
	High quercetin dose[a,b,c]	397 (66.1)	160 (26.6)	41 (6.8)	3 (0.5)	0 (0)

[a] Significantly different from control group at $P<0.01$.
[b] Significantly different from positive control group at $P<0.01$.
[c] Significantly different from low quercetin dose group at $P<0.01$.
[d] Significantly different from middle quercetin dose group at $P<0.01$.
[e] Significantly different from high quercetin dose group at $P<0.01$.

homogenizer. The homogenate was centrifuged at 10,000×g for 20 min. and the resultant supernatant was centrifuged at 105,000×g for 60 min. The precipitate was re-suspended with ice-cold 0.25 M sucrose solution with a volume equivalent to the original weight of lung sample. The microsomal suspensions were stored at −70° before use. The protein contents were determined by the method used by Bradford (Darbre 1986)using bovine serum albumin as a standard.

Measurement of enzyme activity. 7-Ethoxyresorufin O-dealkylase (EROD) activity was used as a marker of CYP1A1 activity. The EROD activity was detected according to the procedure of Takahashi *et al.* (1995) with some modification. The NADPH generating system containing 4 mM NADP, 10 mM D-glucose 6-phosphate disodium salt, 4 units/ml glucose-6-phosphate dehydrogenase in Tris-$MgCl_2$ buffer (pH 7.4). The reaction system containing 10 µl NADPH generating system, 0.5 mg murine lung microsomes in 2 ml Tris-$MgCl_2$ buffer were pre-incubated at 37° for 30 min. The reaction was started with the addition of 10 µl 0.2 mM 7-ethoxyresorufin, followed by incubation at 20° for 10 min. The reaction was terminated with the addition of 0.5 ml methanol. The incubation mixtures were subsequently centrifuged at 13,000 r.p.m. for 10 min. The fluorescence intensity of the supernatant fraction was determined by a fluorescence spectrophotometry (RF-5000 Fluorescence Spectrophotometer, Shimadzu, λex: 560 nm; λem: 580 nm). All samples were run in duplicate. A resorufin standard curve was done beforehand for calculating the product contents of the enzymatic reaction. The linear regression coefficient was 0.9998 for the resorufin concentrations from 25 to 400 nM, showing an evident linear relationship between the resorufin concentration and the fluorescence intensity. The contents of resorufin in the supernatant fractions were analysed with the resorufin standard curve. EROD activities were expressed as picomoles of resorufin per mg protein/min.

Data analysis. In this study, statistical analysis software of SPSS for Windows version 10.0 was used. For pulmonary pathologic examinational evaluation, and for the frequency distribution of the different degrees of DNA damage between the experiment groups in SCGE assay, significance of the difference was determined by χ^2 test. For comet tail length measurement in SCGE assay, and for EROD activity measurement, data was expressed as mean±S.D., significance of difference between mean values was determined by ANOVA followed by LSD multiple comparison test. $P<0.05$ was considered significant.

Results

Profile of DNA damages in peripheral blood lymphocytes.

Fig. 1 shows the morphological observation of peripheral blood lymphocytes under fluorescence microscope. The cells in the control group were undamaged, showing a fluorescent circular nucleus. The cells in quercetin-treated

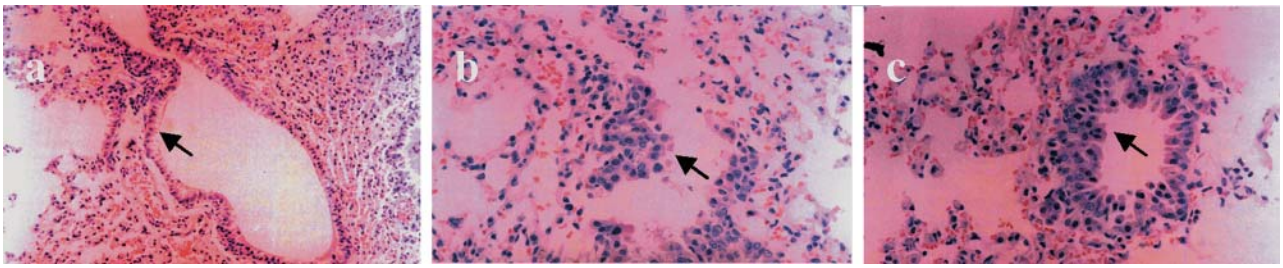

Fig. 2. Pulmonary pathomorphological changes of mice. By light microscopic examination (haematoxylin+eosin, magnification ×200): normal bronchi epithelium tissue (a), and pulmonary pathomorphological changes, such as squamous epithelial metaplasia (b) and atypical hyperplasia (c) were observed in the samples.

Table 2.

Effect of quercetin on the average comet cell tail length of peripheral blood lymphocytes in mice. The comet cell tail lengths (the distance from the center of comet head to the end of comet tail) viewed in the images were measured. A total of 200 lymphocytes were scored per treatment group by randomization for the measurement of the comet cell tail length.

	Comet cell tail length (μm)	
Treatment groups	Female	Male
Control	12.28±4.96	12.08±3.91
Positive control	83.88±29.34△	80.51±40.63△
Low quercetin dose	78.29±26.65*△	68.56±24.97**△
Middle quercetin dose	55.46±12.43**△	62.01±19.89**△
High quercetin dose	54.99±12.16**△	53.37±15.16**△

Values represented as mean±S.D.
* Significantly different from positive control group at $P<0.05$.
** Significantly different from positive control group at $P<0.01$.
△ Significantly different from control group at $P<0.01$.

Table 4.

The effects of quercetin on the activities of CYP1A1 in lung microsomes of mice. 7-Ethoxyresorufin O-dealkylase (EROD) activity was used as a marker of CYP1A1 activity. EROD activities were expressed as picomoles of resorufin per mg protein/min.

	EROD activities (pmol/min./mg protein)	
Treatment groups	Female	Male
Control	0.262±0.014	0.482±0.027
Positive control	0.810±0.038△	1.181±0.126△
Low quercetin dose	0.522±0.079*△	0.612±0.117*△
Middle quercetin dose	0.512±0.062*△	0.604±0.072*△
High quercetin dose	0.392±0.058*△▲	0.503±0.024△▲

Values represented as mean±S.D. n=5.
* Significantly different from positive control group at $P<0.01$.
△ Significantly different from control group at $P<0.01$.
▲ Significantly different from the quercetin treatment groups at $P<0.05$.

groups and the positive control group were damaged in varying degrees. A total of 600 lymphocytes were scored per experiment group by randomization for the evaluation of the extent of DNA damage. As shown in table 1, the results of statistical analysis revealed a substantial decrease of DNA damage in quercetin-treated groups compared with that of the positive control group, and there was a significant difference between different quercetin dose groups except in the female groups between the middle quercetin dose group and the high quercetin dose group. A total of 200 lymphocytes were scored per experimental group by randomization for the measurement of the comet cell tail length. As shown in table 2, the results of statistical analysis also revealed that the average length of the comet cell tail in the quercetin-treated groups decreased significantly compared with that of the positive control group.

Pulmonary pathomorphological examinational evaluation.

By light microscopic examination, normal bronchi epithelium tissue and pulmonary pathomorphological changes, such as squamous epithelial metaplasia and atypical hyperplasia, were observed in the samples (fig. 2). Table 3 shows the statistical data of observed pathomorphological changes in the treatment groups. Results revealed that squamous epithelial metaplasia and atypical hyperplasia were observed after benzo[a]pyrene treatment. Pulmonary pathologic changes in quercetin-treated groups were significantly weaker than those in the positive control group.

Table 3.

Pulmonary pathomorphological changes of mice. Pulmonary pathomorphological changes were observable in the samples of treatment groups (n=10) by light microscopic examination. Slides were read by two pathologists.

Treatment groups	Normal bronchi epithelium	Squamous epithelial metaplasia	Atypical hyperplasia
Control	10	0	0
Positive control	2	2	6
Low quercetin dose*	4	3	3
Middle quercetin dose*	4	4	2
High quercetin dose*	8	2	0

* Significantly different from positive control group at $P<0.05$.

The effects of quercetin on EROD activities in lung microsomes of mice.

Table 4 shows the effects of quercetin on EROD activities in lung microsomes of female mice and male mice respectively. The EROD activities in mice lung microsomes of benzo[a]pyrene-treated groups (positive control group and quercetin-treated groups) increased significantly compared with those of the control group. The EROD activities in lung microsomes of quercetin-treated groups decreased significantly compared with those of the positive control group, and the effect on the quercetin-treated group with a quercetin dose of 2.0 g/kg body weight was significantly stronger than those with a quercetin dose of 1.0 or 0.5 g/kg body weight. Besides these common changes, the EROD activities in the lung microsomes of male mice were significantly higher than those of female mice ($P<0.05$).

Discussion

The results of the SCGE assay revealed that DNA damage in the peripheral blood lymphocytes of mice due to benzo[a]pyrene treatment decreased significantly as a result of quercetin treatment at different doses, and dose-dependently. Also, the average lengths of the comet cell tail decreased gradually as the dose of quercetin increased. The results showed that quercetin had significant chemopreventive effects on benzo[a]pyrene-induced DNA damage in mice. Since DNA damage could lead to genetic disorders that occur at different stages of carcinogenesis, protection from such damages may in the long run help in preventing development of cancer.

It is considered that the carcinogenesis of lung cancer

may be due to the transformation of normal bronchi epithelium to the precancerous bronchi epithelium, and finally become the squamous cell carcinoma. In this study, we observed precancerous pathologic changes in varying degrees in the bronchi epithelium of mice in the benzo[a]pyrene-treated groups, and the degrees of precancerous pathologic changes in the quercetin-treated groups were decreased significantly compared with those in the positive control group, revealing the potential chemopreventive effect of quercetin on the carcinogenesis of lung cancer induced by benzo[a]pyrene.

The rationale for the chemopreventive effects of quercetin against DNA damage and precancerous changes in cells was brought forward by some researchers in their *in vitro* studies. Kang *et al.* (1999) concluded from their *in vitro* findings that quercetin suppresses benzo[a]pyrene-induced DNA damage in human Hep G2 cells by altering CYP1A1 gene expression. Schwarz *et al.* (2003 & 2005) found that quercetin inhibited CYP1A1 and CYP1A1-catalyzed 7, 8-diol-benzo[a]pyrene epoxidation which is the terminal reaction leading to the ultimate carcinogenic product, diolepoxide 2. The reaction was examined with three of the most common allelic variants of human CYP1A1, namely wild-type CYP1A1.1, CYP1A1.2, and CYP1A1.4. Quercetin potently inhibited diolepoxide 2 formation by all CYP1A1 types with IC50 values between 1.6 and 7.0 mM. The data from the above studies suggests that the protective effects of quercetin against benzo[a]pyrene-induced DNA damages and precancerous changes in cells are initiated upstream from CYP1A1. But the data reported by Lautraite *et al.* (2002) conflicts with the results of the above studies. They found that DNA damage was elevated in V79 h1A1-MZ cells expressing human CYP1A1 when treated with benzo[a]pyrene and this was inhibited by chrysin and apigenin, but not by quercetin. In our study, the EROD assay was conducted using lung microsomes of mice, since the induced CYP1A1 is concentrated in the lungs of animals, and the lungs of mice were the target tissues in this study. The results of our study revealed that the CYP1A1-linked EROD activities in lung microsomes of mice decreased as the dose of quercetin increased. The data from our *in vivo* study are in accord with the results of the *in vitro* study reported by Kang *et al.* (1999) and Schwarz *et al.* (2003). Moreover, the results in the previous study by Siess *et al.* (1990) revealed that quercetin inhibits the EROD activity of liver microsomes in man and rat. Considering the aforementioned evidence, we conclude that the mechanism of preventive effects of quercetin against pulmonary precancerous pathologic changes and DNA damage in the blood lymphocytes of mice due to the benzo[a]pyrene treatment could be related to the inhibition of CYP1A1 activity in microsomes of the lung or liver of mice. The rationale for the processes conducted in our study is as follows: The procarcinogen benzo[a]pyrene was bioactivated to ultimate carcinogen by CYP1A1 in the microsomes of lung or liver of mice, and the ultimate carcinogen covalently binds to DNA, causing the blood lymphocyte DNA damages and pulmonary precancerous pathologic changes. Quercetin can block the bioactivation of procarcinogen benzo[a]pyrene by inhibiting the CYP1A1 activity in microsomes of lung or liver of mice, and thus decrease the degree of blood lymphocytes DNA damage and pulmonary precancerous pathologic changes. For the DNA damages and the precancerous pathologic changes in cells that can lead to carcinogenesis, this is also the potential mechanism for the chemopreventive effects of quercetin on the early stage of carcinogenesis induced by benzo[a]pyrene and other procarcinogens.

References

Alan, C., E. J. L. Michael, S. M. Morag & B. Christine: Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *J. Agric. Food Chem.* 1997, **45**, 590–595.

Caltagirone, S., C. Rossi, A. Poggi, F. O. Ranelletti, P. G. Natali, M. Brunetti, F. B. Aiello & M. Piantelli: Flavonoids apigenin and quercetin inhibit melanoma growth and metastatic potential. *Int. J. Cancer* 2000, **87**, 595–600.

Chan, M. M., D. Fong, K. J. Soprano, W. F. Holmes & H. Heverling: Inhibition of growth and sensitization to cisplatin-mediated killing of ovarian cancer cells by polyphenolic chemopreventive agents. *J. Cell Physiol.* 2003, **194**, 63–70.

Darbre, A.: Analytical methods. In: *Practical protein chemistry: A handbook*. Ed.: A. Darbre. John Wiley and Sons, Inc. Press, New York, USA, 1986, pp. 227–335.

De, S., R. N. Chakraborty, S. Ghosh, A. Sengupta & S. Das: Comparative evaluation of cancer chemopreventive efficacy of alpha-tocopherol and quercetin in a murine model. *J. Exp. Clin. Cancer Res.* 2004, **23**, 251–258.

Dogra, S. C., M. L. Whitelaw & B. K. May: Transcriptional activation of cytochrome P450 genes by different classes of chemical inducers. *Clin. Exp. Pharmacol. Physiol.* 1998, **25**, 1–9.

Hasler, J.A.: Pharmacogenetics of cytochromes P450. *Mol. Aspects Med.* 1999, **20**, 12–137.

Kang, Z. C., S. J. Tsai & H. Lee: Quercetin inhibits benzo[a]pyrene - induced DNA adducts in human Hep G2 cells by altering cytochrome P-450 1A1 gene expression. *Nutr. Cancer* 1999, **35**, 175–179.

Kuo, H. M., H. J. Ho, P. D. Chao & J. G. Chung: Quercetin glucuronides inhibited 2-aminofluorene acetylation in human acute myeloid HL-60 leukemia cells. *Phytomedicine* 2002, **9**, 625–631.

Lambert, J. D., J. Hong, G. Y. Yang, J. Liao & C. S. Yang: Inhibition of carcinogenesis by polyphenols: evidence from laboratory investigations. *Amer. J. Clin. Nutr.* 2005, **81**, suppl. 1, 284S–291S.

Lautraite S., A. C. Musonda, J. Doehmer, G. O. Edwards & J. K. Chipman: Flavonoids inhibit genetic toxicity produced by carcinogens in cells expressing CYP1A2 and CYP1A1. *Mutagenesis* 2002, **17**, 45–53.

Lee, L. T., Y. T. Huang, J. J. Hwang, P. P. Lee, F. C. Ke, M. P. Nair, C. Kanadaswam & M. T. Lee: Blockade of the epidermal growth factor receptor tyrosine kinase activity by quercetin and luteolin leads to growth inhibition and apoptosis of pancreatic tumor cells. *Anticancer Res.* 2002, **22**, 1615–1627.

Le Marchand, L., S. P. Murphy, J. H. Hankin, L. R. Wilkens & L. N. Kolonel: Intake of flavenoids and lung cancer. *J. Natl. Cancer Inst.* 2000, **92**, 154–160.

Lin, S. Y., S. J. Tsai, L. H. Wang, M. F. Wu & H. Lee: Protection by quercetin against cooking oil fumes-induced DNA damage in human lung adenocarcinoma CL-3 cells: role of COX-2. *Nutr. Cancer* 2002, **44**, 95–101.

Michael, H. L. H., C. H. H. Peter & P. V. Dini: Optimization of quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *J. Agric. Food Chem.* 1992, **40**, 1591–1598.

Mouria, M., A. S. Gukovskaya, Y. Jung, P. Buechler, O. J. Hines, H. A. Reber & S. J. Pandol: Food-derived polyphenols inhibit pancreatic cancer growth through mitochondrial cytochrome C release and apoptosis. *Int. J. Cancer* 2002, **98**, 761–769.

Nair, H. K., K. V. Rao, R. Aalinkeel, S. Mahajan, R. Chawda & S. A. Schwartz: Inhibition of prostate cancer cell colony formation by the flavonoid quercetin correlates with modulation of specific regulatory genes. *Clin. Diagn. Lab. Immunol.* 2004, **11**, 63–69.

Neuhouser, M. L.: Dietary flavonoids and cancer risk: evidence from human population studies. *Nutr. Cancer* 2004, **50**, 1–7.

Nguyen, T. T., E. Tran, T. H. Nguyen, P. T. Do, T. H. Huynh & H. Huynh: The role of activated MEK-ERK pathway in quercetin-induced growth inhibition and apoptosis in A549 lung cancer cells. *Carcinogenesis* 2004, **25**, 647–659.

Omiecinski, C. J., R. P. Remmel & V. P. Hosagrahara: Concise Review of Cytochrome P450s and their roles in toxicology. *Toxicol. Sci.* 1999, **48**, 151–156.

Pawlikowska, P. B., G. J. Jakubowicz, J. Rzymowska & A. Gawron: The effect of quercetin on apoptosis and necrosis induction in human colon adenocarcinoma cell line LS180. *Folia. Histochem. Cytobiol.* 2001, **39**, 217–218.

Ramos, S., M. Alia, L. Bravo & L. Goya: Comparative Effects of Food-Derived Polyphenols on the Viability and Apoptosis of a Human Hepatoma Cell Line (HepG2). *J. Agric. Food Chem.* 2005, **53**, 1271–1280.

Ranelletti, F.O., N. Maggiano, F. G. Serra, R. Ricci, L. M. Larocca, P. Lanza, G. Scambia, A. Fattorossi, A. Capelli & M. Piantelli: Quercetin inhibits p21-RAS expression in human colon cancer cell lines and in primary colorectal tumors. *Int. J. Cancer* 2000, **85**, 438–445.

Ross, J. A. & C. M. Kasum: Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annu. Rev. Nutr.* 2002, **22** 19–34.

Schieber, A., P. Keller & R. Carle: Determination of phenolic acid and flavonoids of apple and pear by high performance liquid chromatography. *J. Chromotogr. A* 2001, **910**, 265–273.

Schwarz, D., P. Kisselev & I. Roots: St. John's wort extracts and some of their constituents potently inhibit ultimate carcinogen formation from benzo[a]pyrene-7,8-dihydrodiol by human CYP1A1. *Cancer Res.* 2003, **63**, 8062–8068.

Schwarz, D., P. Kisselev & I. Roots: CYP1A1 genotype-selective inhibition of benzo[a]pyrene activation by quercetin. *Eur. J. Cancer* 2005, **41**, 151–158.

Sengupta, A., S. Ghosh & S. Das: Modulation of DMBA induced genotoxicity in bone marrow by quercetin during skin carcinogenesis. *J. Exp. Clin. Cancer Res.* 2001, **20**, 131–134.

Siess, M. H., A. M. Le Bon, M. Rat & P. Suschetet: Inhibition of ethoxyresorufin deethylase activity by natural flavonoids in human and rat liver microsomes. *Food Addit. Contam.* 1990, **7**, suppl. 1, S178–S181.

Singh, N. P., M. T. McCoy, R. R. Tice & E. L. Schneider: A simple technique for quantification of low levels of DNA damage in individual cells. *Exp. Cell Res.* 1988, **175**, 184–191.

Takahashi, N., R. H. Dashwood, L. F. Bjeldanes, G. S. Bailey & D. E. Williams: Regulation of hepatic cytochrome P4501A by indole-3-carbinol: Transient induction with continuous feeding in rainbow trout. *Food Chem. Toxicol.* 1995, **33**, 111–120.

Zhang, X. M., J. Chen, Y. G. Xia & Q. Xu: Apoptosis of murine melanoma B16-BL6 cells induced by quercetin targeting mitochondria, inhibiting expression of PKC-alpha and translocating PKC-delta. *Cancer Chemother. Pharmacol.* 2005, **55**, 251–262.