

EFFECT OF A PLANT-ORIGINATED NATURAL COMPOUND, COD™ EXTRACT, ON C-MYC, HA-RAS AND P53 GENE EXPRESSION IN SHORT-TERM ANIMAL EXPERIMENTS

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Several plant-originated natural compounds were shown to exhibit chemopreventive effect in carcinogenesis. In our studies, we have investigated CoD™ extract, a complex plant extract made from several tropical plants with possible cancer preventive effect in animal experiments. c-myc, p53 and Ha-ras gene expressions were investigated 24, 48 and 72 h after CoD™ extract and dimethylbenz(a)-anthracene (DMBA) treatment in CBA/Ca(H-2^K) mice. CoD™ extract administered together with DMBA diminished c-myc, p53 and Ha-ras gene expressions both in the 24 and 48 h experiments, but not in the 72 h experiments. Further *in vivo* and human studies are needed to clarify the possible role of CoD™ extract in the prevention of tumour formation after carcinogenic exposures.

Keywords: CoD™ extract, plant-originated natural compounds, gene expression, “short-term” studies, anticarcinogenic effect

Several plant-originated natural compounds such as resveratrol, lycopene, epigallocatechine-3-gallate, quercetin and β -carotene were shown to exhibit chemopreventive effect in carcinogenesis (JOHNSON, 1998; FREMONT, 2000; FUJIKI et al., 2000; MANSON, 2003; PARK & SURH, 2003). CoD™ is a complex plant extract made from several tropical plants and is known to have a forceful oncostatic effect (DÁVID, 1997). In our studies, we have investigated the CoD™ extract to determine whether it shows cancer preventive effect in short-term experiments in animal model.

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1. Materials and methods

1.1. Treatments

CoDTM solution was prepared according to the manufacturer's instructions (DÁVID, 1997). Conventionally kept 6-week-old CBA/Ca(H-2^K) mice (10 animals/group, 5 males and 5 females) received CoDTM extract dissolved in bidistilled water i.p. 24 h prior to (CoDTM+DMBA) together with (DMBA×CoDTM) and 24 h after (DMBA+CoDTM) i.p. dimethyl-benz(a)-anthracene/DMBA administration. Controls received the same volume of corn oil, saline solution, and the same dose of CoDTM extract and DMBA. Details of treatments are given in Table 1. Each treatment condition was repeated in 3 different animals groups. One group of animals was autopsied 24 h, the 2nd 48 h and the 3rd group 72 h after drug administration.

Table 1. Protocol of i.p. treatment of CBA/Ca(H-2^K) mice

Animal group	Treatment
Saline control	0.1 ml physiologic saline solution
Oil control	0.1 ml sterile corn oil
CoD TM	0.167 g/kg body weight CoD TM extract dissolved in 0.1 ml bidistilled water
Dimethyl-benz-anthracene (DMBA) – <u>group A</u>	20 mg/kg body weight DMBA dissolved in 0.1 ml corn oil
CoD TM +DMBA – <u>group B</u>	0.167 g/kg body weight CoD TM extract dissolved in 0.1 ml bidistilled water 24 h later
CoD TM × DMBA – <u>group C</u>	0.167 g/kg body weight CoD TM extract dissolved in 0.1 ml bidistilled water +20 mg/kg body weight DMBA dissolved in 0.1 ml corn oil
DMBA+CoD TM – <u>group D</u>	20 mg/kg body weight DMBA dissolved in 0.1 ml corn oil 24 h later
	0.167 g/kg body weight CoD TM extract dissolved in 0.1 ml bidistilled water

1.2. Gene expression studies

One hundred mg tissue samples of lung, thymus, kidney, liver, spleen, paracoecal lymph nodes and bone marrow were removed from each animal and total cellular RNA was isolated by using TRIZOL Reagent (GIBCO, Grand Island, NY, USA). After RNA concentration and quality check at 260/280 nm, 5 µg of RNA was dot blotted onto Hybond N⁺ nitrocellulose membranes and hybridised with chemiluminescently-labelled (ECL Kit, Amersham, Little Chalfont, UK) c-myc, p53 (ATCC, Manassas, USA) and Ha-ras (courtesy of Prof. J. SZEBERÉNYI, University of Pécs, Hungary) DNA probes. Signals were detected on X-ray films and quantified by Quantiscan software (Biosoft, Cambridge, UK). Gene expressions were evaluated as percentages of β-actin. The arbitrary unit in the Figures is equivalent to 100% expression of β-actin.

2. Results and discussion

CoDTM extract administered simultaneously with the carcinogenic DMBA (DMBAxCoDTM group) decreased the expression of all the investigated genes (c-myc, Ha-ras and p53), especially the expression of Ha-ras in the 24-h experiments (Fig. 1). In the 48-h experiments, simultaneous administration of CoDTM with DMBA decreased the expression of all genes, especially that of the c-myc (Fig. 2). No effect of simultaneous CoDTM administration on the expression of c-myc, Ha-ras and p53 genes was seen in the 72-h experiment (data not shown). Neither pre-treatment with CoDTM extract, nor administration of CoDTM after the DMBA exposure was able to decrease the elevation of gene expressions caused by the carcinogenic treatment at any time point.

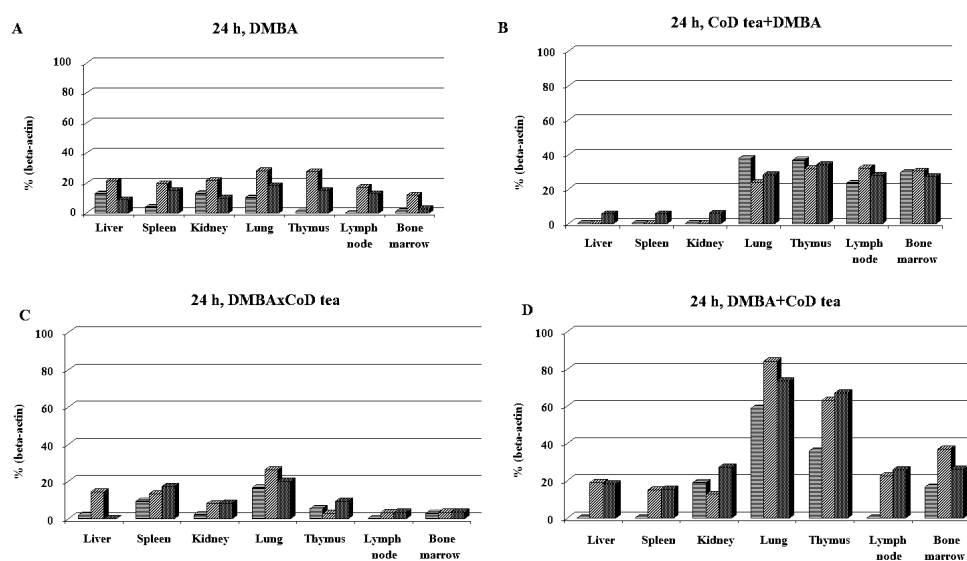


Fig. 1. c-myc, Ha-ras and p53 expression in different organs of CBA/Ca(H-2^K) mice 24 h after different treatment protocols. Gene expressions in the investigated organs are evaluated as percentages of beta-actin. The arbitrary unit of the vertical axis is equivalent to 100% expression of beta-actin. A: DMBA treatment; B: CoDTM treatment 24 h prior to DMBA treatment; C: Simultaneous CoDTM and DMBA treatment; D: CoDTM treatment 24 h after DMBA treatment.

□: c-myc; ▨: Ha-ras; ■: p53

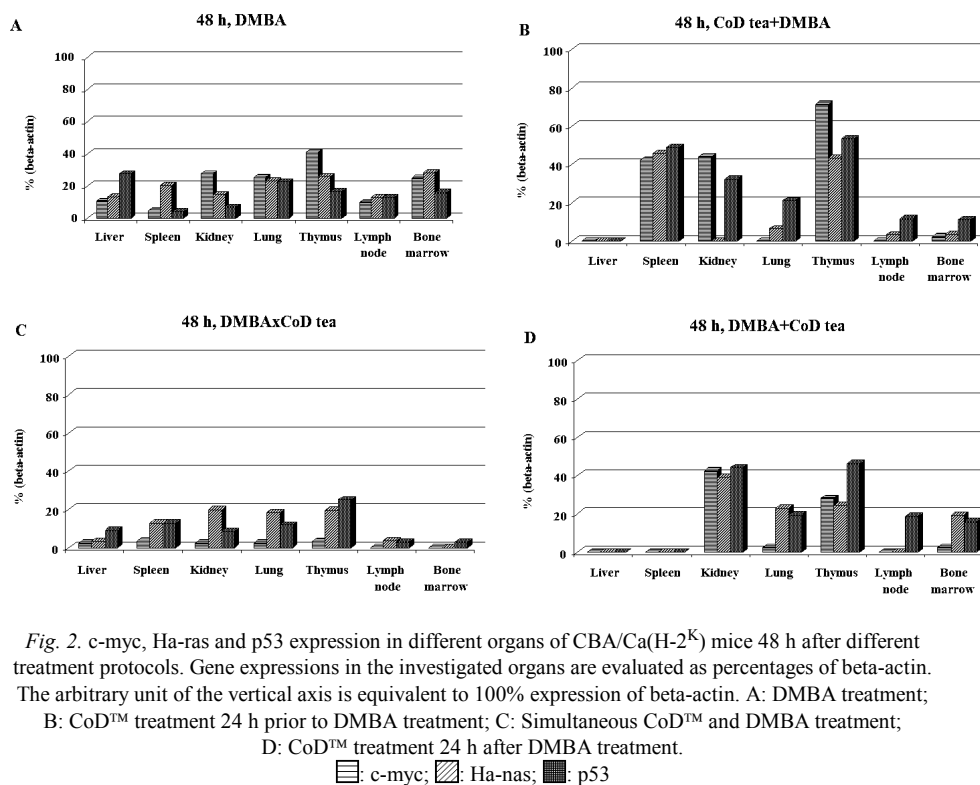


Fig. 2. c-myc, Ha-ras and p53 expression in different organs of CBA/Ca(H-2^K) mice 48 h after different treatment protocols. Gene expressions in the investigated organs are evaluated as percentages of beta-actin. The arbitrary unit of the vertical axis is equivalent to 100% expression of beta-actin. A: DMBA treatment; B: CoDTM treatment 24 h prior to DMBA treatment; C: Simultaneous CoDTM and DMBA treatment; D: CoDTM treatment 24 h after DMBA treatment.

CoDTM is a mixture, mainly composed of extracts from plants growing in the tropical part of South-America. The mixture is known to have forceful oncostatic and strong antioxidant and free radical-eliminating effects (DÁVID, 1997). This is in accordance with the generally accepted experiences suggesting that oncostatic compounds usually exhibit antioxidant or other free radical-eliminating effects (DÁVID, 1997; JOHNSON, 1998; MANSON, 2003). Furthermore, CoDTM exhibits strong lipoxigenase (enzymes which protect the plants against the damages caused by free oxygen radicals) activity, which is most active in acidic milieu (KOSÁRY et al., 2001; 2003). This strong lipoxigenase activity may also be responsible for the oncostatic properties of CoDTM (KOSÁRY et al., 2003).

The investigated genes, c-myc, Ha-ras and p53 are key genes in the process of developing malignant diseases. Determining the expressions of these genes gives us possibilities to investigate the early events of the carcinogenesis at three different points. p53 plays an important role in the apoptosis and repair of the cellular DNA after damages (NÉMETH et al., 2005). Besides these functions, p53 is a tumor suppressor gene, as well. Ha-ras exhibits a central role in the signal transduction during carcinogenesis, mainly through the mitogen-activated protein kinase pathway. c-myc is

absolutely necessary for proliferation and immortalisation, being part of the balance of genes regulating proliferation and/or apoptosis. Early changes/abnormalities indicating exposure and early biologic effect can be monitored by the expression level of these genes.

Our “short-term” animal model system, which was used for testing the in vivo effects of CoD™, is able to detect the effect of different carcinogenic and anticarcinogenic compounds (GYÖNGYI et al., 2001; 2002; NÉMETH et al., 2003; NÁDASI et al., 2005). The ability of CoD™ to decrease Ha-ras gene expression, as shown in our experiments, may be considered an anticarcinogenic property, since the activation of Ha-ras has been shown an early genetic change in the development of tumours (BOS, 1987; BALMAIN et al., 1988).

3. Conclusions

In summary, anticarcinogenic effect of CoD™ may result from its strong antioxidant, free radical-eliminating and lipoxigenase effects together with its ability to decrease Ha-ras expression induced by carcinogenic compounds. Further in vivo and human studies may clarify the possible role of CoD™ extract in the prevention of tumour formation after carcinogenic exposures.

Further “long-term” studies are needed to investigate the late effects of CoD™ extract on the expression of other onco- and suppressor genes. Individually designed DNA-chips may also be useful to identify new biomarker genes underlying the anticarcinogenic effect of CoD™ extract.

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