# Induction of cytochromes P450 in small intestine by chemopreventive compounds

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## Abstract

**OBJECTIVES**: Since flavonoids and other natural compounds exert beneficial effects on human health, their consumption rapidly increases. However, they can modulate the activity of xenobiotic-metabolizing enzymes involved in activation and detoxification of food and environmental carcinogens. Thus, their potential negative effects should be examined.

**METHODS**: The induction effects of selected chemopreventive compounds, administered per orally by gastric gavages to rats, on cytochrome P450 (CYP) 1A and 2B were determined in liver and small intestine using Western blotting analysis and specific metabolic activity assays.

**RESULTS**: Comparing CYPs expression along small intestine, the highest induction was observed in the proximal part near pylorus with rapid decrease towards the distal part. In response to chemopreventive compounds, the marked induction of CYP1A and CYP2B in liver was observed after diallyl sulphide and flavone treatment. In small intestine, β-naphthoflavone, diallyl sulphide and curcumin induced CYP1A1 and CYP2B1. In both tissues, resveratrol did not significantly affect CYPs expression. The results of Western blotting detection of CYPs correlate well with their specific enzymatic activities.

**CONCLUSIONS**: Presented data indicate ambiguous impact of chemopreventive compounds on cytochromes P450, main xenobiotic-metabolizing enzymes. Thus, the question of safety and unlimited consumption of these compounds arises.

**Abbreviations**

| | |
|---|---|
| CYP | - cytochrome P450 |
| EROD | - 7-ethoxyresorufin-O-deethylase |
| PROD | - 7-pentoxyresorufin-O-depenthylase |
| SDS | - sodium dodecyl sulphate |

ORIGINAL ARTICLE

## INTRODUCTION

Cancer is worldwide a life-threatening disease with one of the highest mortalities. Development of cancer is a complex process, resulting in uncontrolled cell growth and division. It has been estimated that dietary factors and smoking are associated with 35% of all human cancers (Doll and Peto, 1981). One of the approaches to reduce cancer risk is the prevention consisting in a healthy lifestyle and a natural diet. Moreover, increasing attention is being paid to the possibility of applying a wide range of natural chemopreventive compounds (e.g. vitamins, flavonoids, organosulphur compounds, dithiolthiones, glucosinolates, phytoestrogens) as food supplements. These compounds, which are considered to be safe because of their plant origin, are freely available in health food stores. Since these phytochemicals exert a great variety of beneficial effects (e.g. antiallergic, antiinflammatory, antiviral, antiproliferative, anticarcinogenic) (Hodek *et al*., 2002) on human health, they can be consumed in large quantities for long periods of time without any limitation. On the other hand, it has been reported that some flavonoids have prooxidant effects and/or mutagenic, e.g. quercetin, (Rietjens *et al*., 2005). They may interfere with essential biochemical pathways, such as indole-3-carbinol from cruciferous plants, which is able to alter metabolism of sex hormones (Higdon *et al*., 2007). Severe flavonoid-drug interactions causing an overdose or the loss of the drug therapeutic effects have been reported (Fuhr *et al*., 1998, Dresser *et al*., 2002).

The negative properties of these dietary supplements may come mainly from: (i) their own toxicity, (ii) metabolic conversion into cytotoxic or mutagenic agents, (iii) induction of carcinogen activating enzymes, and (iv) effect on human microflora.

Chemopreventive agents are xenobiotics and, therefore, they can undergo interactions with enzymes metabolizing foreign compounds. Among these enzymes, cytochromes P450 (CYPs) play the most prominent role. CYPs are monooxygenases involved in metabolism of xenobiotics (e.g. drugs, carcinogens) (Kotrbová *et al*., 2006) and endogenous substrates (e.g. steroids). Chemopreventive agents might inhibit or stimulate the activity of several CYPs, and/or induce an expression of certain CYPs (Hodek *et al*., 2006). Thus, the chemopreventive compounds show a double-edged sword activity on CYPs. In this respect, a detailed study on CYP-chemopreventive compound interactions (inhibition and induction of CYPs) is of great importance in order to elucidate the role of chemopreventive compounds in food and environment carcinogen activation.

The objective of the present study is to investigate the effects of chemopreventive compounds on CYP1A and 2B subfamily, namely on their induction and metabolic activities in small intestine and liver of rat model organism.

## MATERIAL AND METHODS

*Chemicals*

β-Naphthoflavone, flavone, morin, diallyl sulphide, resveratrol, curcumin, and anti-chicken IgG alkaline phosphatase conjugate were purchased from Sigma Chemical Co., USA. All other chemicals were purchased from standard commercial sources and were of the highest quality available.

*Animal treatment and microsome isolation.*

All studies with rats were carried out in accordance with the guidelines of the Ethical Committee of the Faculty of Science. Male Wistar rats (150 g) obtained from AnLab, Czech Republic, were housed in groups of 4 in wire cages at 22 °C with a 12h light/dark period and an *ad libitum* diet (ST-1 diet from Velaz, Czech Republic) and water access. The tested compounds (60 mg/kg body weight) were administered *p.o.* by gastric gavages, dissolved in sunflower oil (1 ml), daily for 5 consecutive days. The control group was treated with 1 ml of sunflower oil only. The treated rats were fasted overnight and twenty-four hours after the last treatment, they were sacrificed. Microsomal fractions were prepared from the colon and whole liver, immediately after sacrificing the rats, according to van der Hoeven and Coon (1974). Tissues from 4 rats were combined for each microsomal preparation. In brief, the colon was removed circa 2 cm under the stomach in a total length of approximately 20 cm. Tissues were perfused with homogenization buffer (50 mmol/l Tris, 150 mmol/l KCl, pH 7.5, 4 °C), excised, weighed and homogenized in a Potter-Elvehjem homogenizer. Standard differential centrifugation was used to isolate microsomes. Microsomal pellets were resuspended and washed with buffer (100 mM $Na_4P_2O_7$.10 $H_2O$, 1 mM EDTA, pH 7.2, 4 °C) at 100 000×g for 90 min. Final pellets were resuspended in buffer (150 mM KCl, 50 mM Tris, 1 mM EDTA, pH 7.4, 4 °C) containing 20% glycerol. Microsomes were stored at –80 °C before use.

*Determination of protein concentration and enzyme assays.*

Protein concentration in microsomes was measured according to Smith *et al*. (1985) using bicinchoninic acid with bovine serum albumin as the standard. The CYP1A and CYP2B protein amounts were determined by Western blotting on Immobilon-P membrane (Millipore, Bedford, MA) using specific chicken anti-CYP1A1 antibody. Since the content of cytochrome P450s is lower in small intestine, the samples used for SDS-electrophoresis and following Western blotting analysis were twice as concentrated as the ones from liver. 7-Ethoxyresorufin-O-deethylase (EROD) and 7-pentoxyresorufin-O-depenthylase (PROD) activity assays were determined according to the method described by Burke and Mayer (1974). Formation of the resorufin was continuously measured for 10 minutes by

monitoring its fluorescence (excitation and emission wavelengths of 530 and 585 nm, respectively).

## RESULTS

Our research is focused on the effect of chemopreventive compounds on CYPs. In the present study, we report the impact of selected chemopreventive compounds on CYPs expression and activity in small intestine and liver in a rat model organism.

*CYPs expression along small intestine*

As the small intestine is an organ highly exposed to chemopreventive compounds, after *p.o.* administration by gastric gavages, we focused on the CYPs induction in this tissue. First, it was necessary to find out where the highest content of CYPs is expressed in small intestine. The tissue was dissected into proximal (pylorus) and distal (cecum) parts, and microsomal fraction from each part was then prepared. The content of CYP1A1/2 and CYP2B1/2 was determined using Western blotting with specific antibodies. Immunoblots showing the CYPs expression in proximal and distal parts of small intestine are presented in Figure 1. The same induction pattern, significantly lower induction in the distal part than in the proximal part, was observed for the expression of CYP1A1 and CYP2B1 in small intestine after diallyl sulphide and morin treatment of rats. Thus, for further experiments, the microsomal fractions were prepared from the proximal part of small intestine.

*CYPs expression in small intestine and liver*

For comparison, the CYPs expression was also monitored in the other biotransformation tissue, in the liver. As representatives of 4 categories of chemopreventive compounds, flavonoids, stilbenes, curcumins, and organosuplhur compounds, we chose flavone, resveratrol, curcumin, and diallyl sulphide, respectively. In Figure 2, the results of Western blot detection of particular CYPs are shown. Although CYPs in extrahepatic tissues are several times less abundant than in liver, we succeeded in detecting both CYP1A1 and CYP2B1 in small intestine. Comparing the induction effects on CYP1A1 in liver and small intestine, β-naphthoflavone and diallyl sulphide were strong inducers. Flavone, however, induced CYP1A1 only in the liver. In case of CYP2B1, diallyl sulphide induced this CYP in both tissues, while β-naphthoflavone was effective only in small intestine. In addition, CYP2B1 in small intestine was significantly induced also by curcumin.

*Effects on xenobiotic-metabolizing enzyme activities*

While Western blotting is able to detect the CYPs expression at the protein level, it is essential to correlate the presence of the particular CYP with its specific metabolic activity. In order to confirm the expression of metabolically active CYPs in small intestine and liver, the marker metabolic activities of the respective CYPs were measured. When comparing the data presented in Figure 3, the CYPs induction observed using immunodetection analysis are in accordance with determined CYP1A and CYP2B activity assays, EROD and PROD, respectively.

## DISCUSSION

Cancer chemoprevention is one of the key facets of recent biomedical research. Since flavonoids and other natural compounds exert beneficial effects on human health, they are frequently used as potential chemopreventive substances. As a component of human diet rich in fruits, vegetables, herbs, they could reduce the cancer risk, especially of the colorectal cancer. These compounds have the potential to modulate the activity of cytochromes P450, mainly CYP1A subfamily, involved in the activation of food and environmental carcinogens. Thus, the inhibition of carcinogen activating enzymes is considered to be one of the major health

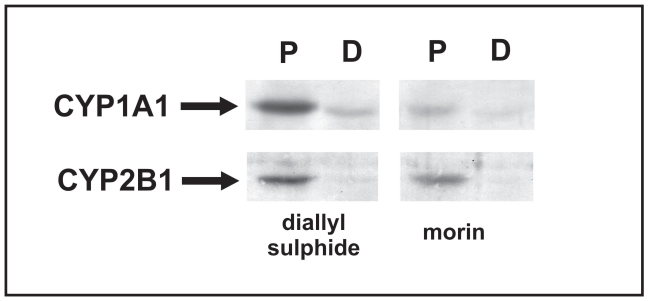


**Figure 1.** Immunoblot analysis of CYP1A1/2 and CYP2B1/2 expression in proximal (**P**) and distal (**D**) parts of rat small intestine after diallyl sulphide and morin treatment. Electrophoresed microsomal proteins (25 μg) were transferred to Immobilon-P membrane and probed with anti CYP1A or anti-CYP2B antibody.

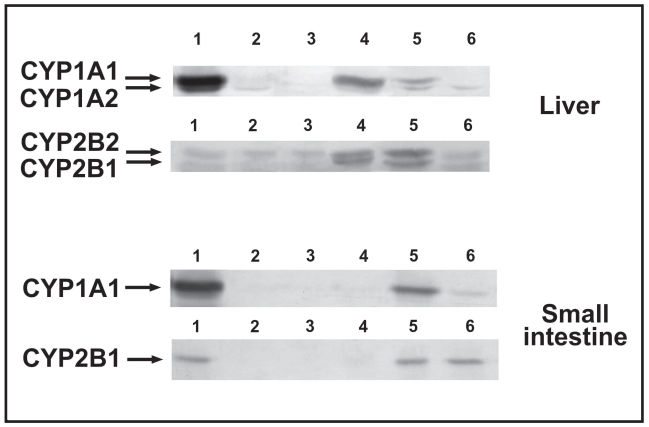


**Figure 2.** Immunoblot analysis in liver and intestinal microsomes from rats treated with chemopreventive compounds. Electrophoresed microsomal proteins (liver 12.5 μg, intestinal 25 μg) were transferred to Immobilon-P membrane and probed with anti CYP1A or anti-CYP2B antibody. β-Naphthoflavone (**1**), control (**2**), resveratrol (**3**), flavone (**4**), diallyl sulphide (**5**), curcumin (**6**).

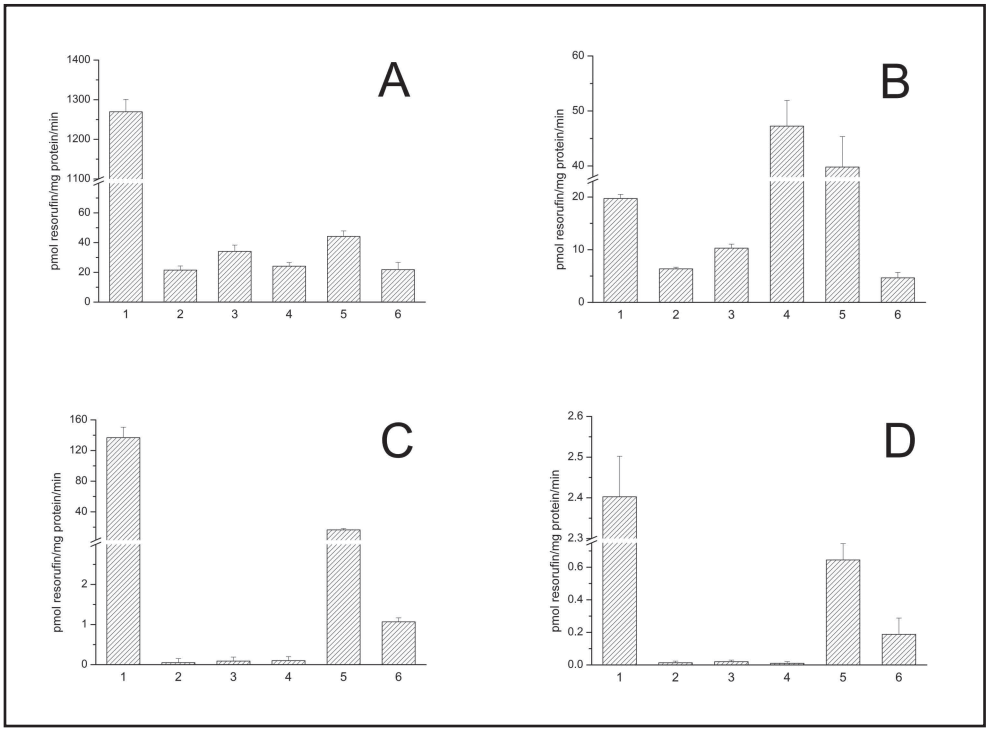


**Figure 3.** Effect of tested compounds on CYP1A and CYP2B marker activities in microsomal samples. EROD (panels **A**, **C**) and PROD (panels **B**, **D**) activities were determined in microsomes of liver (panels **A**, **B**) and small intestine (panels **C**, **D**) from rats treated with β-naphthoflavone (**1**), control (**2**), resveratrol (**3**), flavone (**4**), diallyl sulphide (**5**), or curcumin (**6**). Bars represent the means ± SD of 3 determinations.

promoting effects of chemopreventive compounds. On the other hand, the induction of e.g. CYPs by chemopreventive compounds might significantly increase the activation of ingested carcinogens in the human body. Most studies on xenobiotic-metabolizing enzymes have been carried out with liver enzymes. However, it is the gastrointestinal tract which is the first barrier met by the exogenous compounds of food or orally delivered drugs. Moreover, colorectal carcinoma is one of the leading causes of human death. Therefore, the present study is focused on the effects of chemopreventive agents on CYPs in two crucial organs, small intestine and liver of rat as a model organism.

To mimic the human chemopreventive agent intake, the tested compounds were administered *p.o.* by gastric gavages to male rats. An important asset of this study is the finding that the highest induction after diallyl sulphide and morin treatment was in the proximal part of small intestine near pylorus with rapid decrease towards the distal part. A similar induction pattern was observed also after β-naphthoflavone administration in a study by Zhang *et al.* (1997). The proximal part of small intestine is either more equipped with xenobiotic response proteins or the efficient absorption of foreign compounds in blood significantly reduces the exposure of the distal part to xenobiotics.

Another outcome of this work arises from the comparison of CYPs induction in liver and intestine revealing marked differences in response to tested chemopreventive compounds between these two organs. While β-naphthoflavone and diallyl sulphide induced respective CYPs in a similar way in both organs, flavone was an effective inducer of CYPs only in liver, and curcumin caused expression of CYPs solely in small intestine. Surprisingly, diallyl sulphide was able to induce all detectable CYPs (1A1, 1A2, 2B1, 2B2). β-Naphthoflavone, a known inducer of CYP1A in liver, strongly stimulated expression of CYP1A1 in small intestine, in addition to diallyl sulphide treatment. However, no CYP was detected in this organ in untreated rats and after flavone treatment. On the other hand, flavone was an efficient inducer of CYP1A1 in liver which could be explained by its easy pass from gut into the blood stream and then to liver. Moreover, diallyl sulphide as well as flavone induced CYP2B1/2 in liver. The activity of allyl sulphides on CYP2B1 in liver, lungs, and jejunum were recently reported (Lii *et al.*, 2006). Effects of flavone on both CYPs and their activities correlate well with findings in the study of Canivenc-Lavier *et al.* (1996). Curcumin increased CYPs expression in small intestine, while non-significant effects were detected in liver. A similar effect of curcumin was detected in MCF-7 cells (mammary epithelial cell line), where curcumin increased EROD activity and mRNA protein level (Ciolino *et al.* 1998). Contrary to all other tested chemopreventive compounds, resveratrol did not affect

CYPs expression apparently in both tissues. Hence, resveratrol seems to be a safe chemopreventive compound in respect of the potential cancer risk associated with CYPs induction.

Results of Western blot CYPs detection correlate well with marker enzymatic activities EROD and PROD of corresponding enzymes, except for diallyl sulphide and flavone EROD activities in liver. It could be explained by a contribution of another CYP to the EROD activity in the case of diallyl sulphide. In other words, the immunodetection of CYPs protein amounts clearly follows the marker enzymatic activities of respective CYPs. Even though, the samples were prepared from small intestine, rich in digestive enzymes, CYPs were nevertheless active.

In conclusion, the present study, focused on chemopreventive compounds administered *per orally* to rats, shows that the inducing ability of chemopreventive compounds is both, compound and tissue-specific. Presented data indicate ambiguous activity of chemopreventive compounds on CYPs, main xenobiotic-metabolizing enzymes, thus the question of safety and unlimited consumption arises.

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