Eur. J. Oncol., vol. 17, n. 1, pp. 11-21, 2012

Kinase inhibitors reduce 7,12-dimethylbenz[a]anthracene-induced onco-suppressor gene expression in short-term experiments

Inibitori delle chinasi riducono l'espressione genica dell'onco-soppressore indotto da 7,12- dimethylbenz[a]anthracene in esperimenti a breve termine

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Summary

Aims. **Elevated expression of oncogenes and tumour suppressor genes after treatment with 7,12- dimethylbenz[a]anthracene (DMBA) as early markers of carcinogenesis has been previously observed. The potential antineoplastic and chemopreventive properties of four protein tyrosine kinase (PTK) inhibitor molecules were studied.** ***Materials and methods.*** **In order to determine whether a promising antineoplastic activity would extend to anticarcinogenic properties, the effects of these molecules on the 7,12 DMBA-induced expression of *Ha-ras* oncogene and *p53* tumour suppressor genes in the liver, lungs, bone marrow and kidney of CBA/Ca inbred mice was investigated. The experimental molecules were administered (I) 24 hours prior to, (II) simultane-**

Riassunto

Finalità. **È stato precedentemente osservato che il trattamento con 7,12- dimethylbenz[a] anthracene (DMBA) induce l'espressione di oncogeni e di geni onco-soppressori, che costituiscono indicatori precoci di carcinogenesi. Il presente studio valuta le potenziali proprietà antineoplastiche e chemiopreventive di quattro inibitori delle proteine tirosin-chinasi (PTK).** ***Materiali e metodi.*** **Per determinare se una potenziale attività antineoplastica di queste molecole sia associata a proprietà anticarcinogeniche, è stato valutato il loro effetto sull'espressione dei geni onco-soppressori *Ha-ras* e *p53*, indotta dal DMBA, nel fegato, polmoni, midollo osseo e rene di topi di ceppo CBA/Ca. Le molecole in esame sono state somministrate (I) 24 ore prima, (II) durante, o (III) 24**

Received/Pervenuto 24.11.2011 - Accepted/Accettato 25.1.2012
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ously with, or (III) 24 hours after DMBA exposure. *Results.* PTK inhibitor (1*Z*)-1-(3,4-Dihydroxybenzylidene)-3,4-dihydro[1,4]oxazino[3,4-b]quinazolon-6(1H)-on and N-(3-bromophenyl)-6,7-dimethoxy quinazolin-4-amine reduced the expression of *Ha-ras* and *p53* genes in all of the examined organs in the different treated groups. Another two PTK inhibitor, 2-Benzyl-1-(4-hydroxyphenyl)-3-me-thyl-2,3-dihydroimidazo[5,1-b]quinazolin-9(1H)-one and 2-[(2E)-2-(3,4-dihydroxybenzylidene)hydrazino]-N-(3-nitrophenyl)-2-oxoacetamide reduced both *Ha-ras* and *p53* gene expression induced by DMBA in all of the examined organs (except for the kidneys, where only *Ha-ras* expression decreased) in all of the experimental settings. *Conclusions.* Our *in vivo* short-term experimental results provide some evidence, that PTK inhibitors exhibit antineoplastic and chemopreventive effects by reversing the overexpression of the early markers of carcinogenesis. This early molecular biomarker model indicates the first steps of malignant transformation at subcellular level and possible anti-tumour effects, as well. Eur. J. Oncol., 17 (1), 11-21, 2012

Key words: **DMBA, gene expression, Ha-ras, p53, tyrosine-kinase inhibitor**

ore dopo esposizione a DMBA. *Risultati.* Gli inibitori di PTK (1*Z*)-1-(3,4-Dihydroxybenzylidene)-3,4-dihydro[1,4]oxazino[3,4-b]quinazolon-6(1H)-uno e N-(3-bromophenyl)-6,7-dimethoxy quinazolin-4-amina hanno ridotto l'espressione dei geni *Ha-ras* e *p53* in tutti gli organi esaminati e per tutti gli schemi di somministrazione. Altri due inbitori di PTK, 2-Benzyl-1-(4-hydroxyphenyl)-3-methyl-2,3-dihydroimidazo[5,1-b]quinazolin-9(1H)-uno e 2-[(2E)-2-(3,4-dihydroxybenzylidene)hydrazino]-N-(3-nitrophenyl)-2-oxoacetamide, hanno ridotto l'espressione dei geni *Ha-ras* e *p53* indotta da DMBA in tutti gli organi esaminati (eccetto il rene, dove solo l'espressione di *Ha-ras* è diminuita). *Conclusioni.* I nostri risultati, concernenti la somministrazione a breve termine *in vivo*, forniscono una qualche evidenza circa un effetto antineoplastico e chemiopreventivo degli inibitori delle PTK prevenendo la sovra-espressione di indicatori precoci di carcinogenesi. Questo modello di bioindicatori molecolari precoci è indice dei primi stadi di trasformazione maligna a livello subcellulare, nonchè possibili effetti antitumorali. Eur. J. Oncol., 17 (1), 11-21, 2012

Parole chiave: **DMBA, espressione genica, Ha-ras, p53, inibitori delle tirosin-chinasi**

Introduction

Kanner *et al.* in 1991 proposed that p120 is a substrate of both nonreceptor- and ligand-activated transmembrane receptor tyrosine kinases and of serine/threonine kinases (Ser/ThrK) and is a component of both mitogen-stimulated and tyrosine kinase oncogene-induced signaling pathways (1). This theory was proven and the "age of protein kinases" had begun. Approximately 400 Ser/ThrK and 90 protein tyrosine kinases (PTK) and some dual specific protein kinases are estimated to exist in the human genome (2). Reducing secondary signaling pathway activity with PTK inhibitors (PTKi) is expected to be effective in antitumour therapy (3). PTKs play a fundamental rôle in signal transduction pathways, regulating a number of cellular functions such as cell growth, differentiation and cell death. Compounds with PTKi activity from a range of plant secondary metabolites were highlighted by Hollósy and Kéri. Structure activity analysis and optimization with chemical modification followed by *in vitro* testing resulted in the development of several anticancer drug candidates (4).

With the discovery of oncogenes and tumour suppressor genes, and an understanding of their role in the development of malignant diseases, new opportunities for therapy have risen. Cancer is a manifestation of deregulated signaling pathways that mediate cell growth and programmed cell death. Protein kinases are essential elements in these signaling pathways (2).

Potential antineoplastic and chemopreventive properties of four PTKi molecules are detailed in this paper. (1*Z*)-1-(3,4-dihydroxybenzylidene)-3,4-dihydro[1,4]oxazino[3,4-b]quinazolon-6(1H)-one

(compound 1), N-(3-bromophenyl)-6,7-dimethoxy quinazolin-4-amine (compound 2), 2-benzyl-1-(4-hydroxyphenyl)-3-methyl-2,3-dihydroimidazo[5,1-b]quinazolin-9(1H)-one (compound 3) and 2-[(2E)-2-(3,4-dihydroxybenzylidene)hydrazino]-N-(3-nitrophenyl)-2-oxoacetamide (compound 4) (fig. 1) are promising anticarcinogenic compounds, according to earlier *in vitro* studies (5-8).

Elevated expression of Ha-ras and *p53* key onco/suppressor genes after treatment with the chemical carcinogen 7,12- dimethylbenz[a] anthracene (DMBA) has been previously observed (9). Our research group has developed an animal model for the investigation of alterations in onco/suppressor gene expression due to external carcinogenic agents, and this model had already been used to evaluate the carcinogenic effects of cytostatic drugs in humans, an analysis of the effects of ethylene oxide exposure seemed to offer further information on the usefulness of gene expression as a biomarker (10). DMBA is used in "short-term" *in vivo* experiments to determine possible chemopreventive effects of potential anticarcinogen compounds, which, if effective, should abrogate DMBA-induced expression (11). In a recent study the inhibitory effect of agents were investigated on DMBA-induced molecular epidemiological biomarker *Ha-ras* onco- and *p53* tumour suppressor genes. A number of chemopreventive drugs have already been investigated in this test system such as chalcone analogues as intermediary compounds of the flavonoid biosynthetic pathway or 2-Mercaptoimidazole derivative Afobazole (12-14). Furthermore, natural compounds may also be evaluated in the *in vivo* model, since our research group investigated the potential chemopreventive effects of Uncaria and Tabebuia extracts (15).

Materials and methods

Animals and treatments

Six- to eight-week-old (20±4 g) conventionally maintained, CBA/Ca inbred H-2^K haplotype mice (6 females in each group) were used for this experiment. The experiment was approved by the Animal Experiment Committee of University of Pécs. The animals were acclimatized to laboratory conditions, and were given food and water *ad libitum*. Two

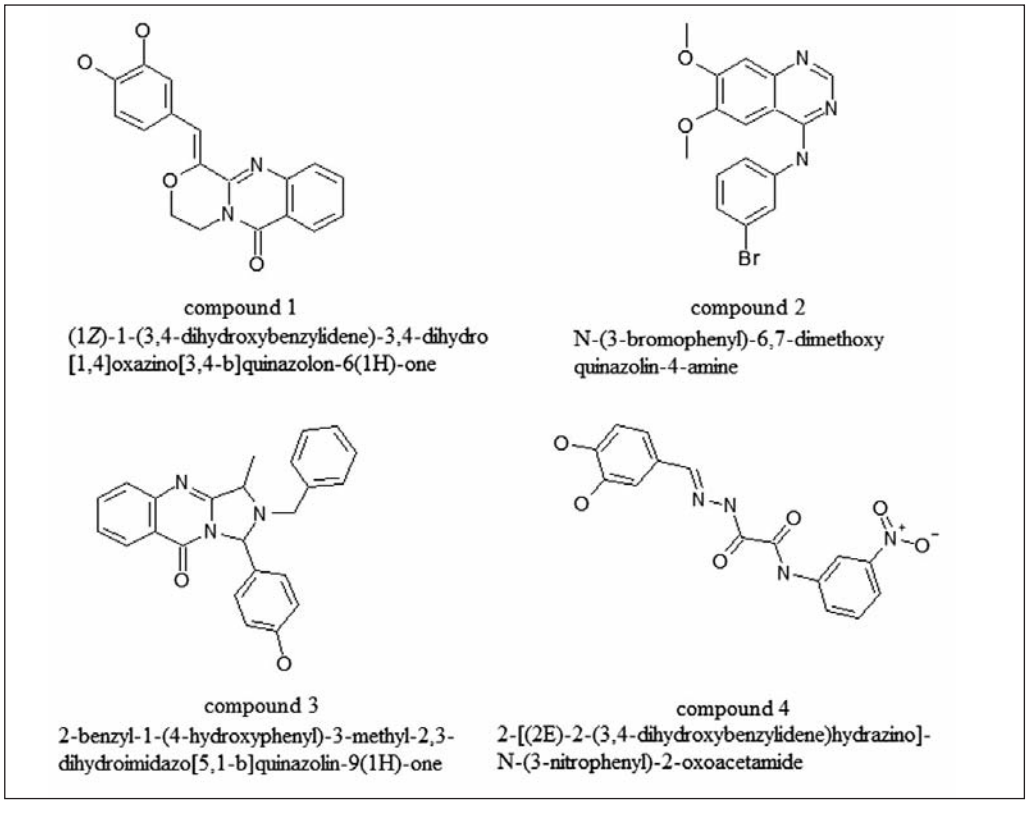

Fig. 1. Structure of experimental compounds.

control (one negative and one positive) sets were established to compare the experimental results. We had all the requisite licenses to perform the experiment. Four experimental sets were created for each of the four experimental agents (fig. 2). The control and experimental mice were autopsied twenty-four hours after the last DMBA or experimental agent treatment. Compound 1, 2, 3 and 4 were supplied by Vichem Chemie Ltd., Budapest, Hungary.

First set: Negative control.

One group of animals, which served as negative control, were intraperitoneally treated *(i.p.)* with a single 10 mg/kg body weight dose of dimethylsulfoxide (DMSO) (Sigma Aldrich Budapest, Hungary).

Second set: Positive control.

One group of mice were *i.p.* with a single 20 mg/kg body weight dose of DMBA (Sigma Aldrich Budapest, Hungary) dissolved in DMSO (Sigma Aldrich Budapest, Hungary) which served as positive control.

Third set: Simultaneous treatment.

Four groups of mice were simultaneously treated with experimental agents and DMBA dissolved in DMSO (methods and quantities as in the first two sets). Each of the four groups of mice were *i.p.* treated with a single 10 mg/kg body weight dose of compound 1 or 2 or 3 or 4.

Fourth set: Pretreatment with experimental agent.

The effect of DMBA on mice pretreated with the experimental agents was investigated in four groups of mice. Each of the groups was *i.p.* treated with a single dose of compound 1 or 2 or 3 or 4 (as described for the third set). After 24 hours, all four groups were treated with a single 20 mg/kg body weight dose of DMBA. Twenty-four hours later mice were autopsied.

Fifth set: Post treatment with experimental agent.

The effect of the experimental agents was examined on mice pretreated with DMBA. Four groups of mice were pretreated with DMBA, then after 24 hours were treated with experimental agents as above and 24 hours later were autopsied.

Sixth set: Treatment with experimental agents alone.

Each of the four groups of mice were *i.p.* treated with a single 10 mg/kg body weight dose of compound 1 or 2 or 3 or 4; 24 hours later mice were autopsied.

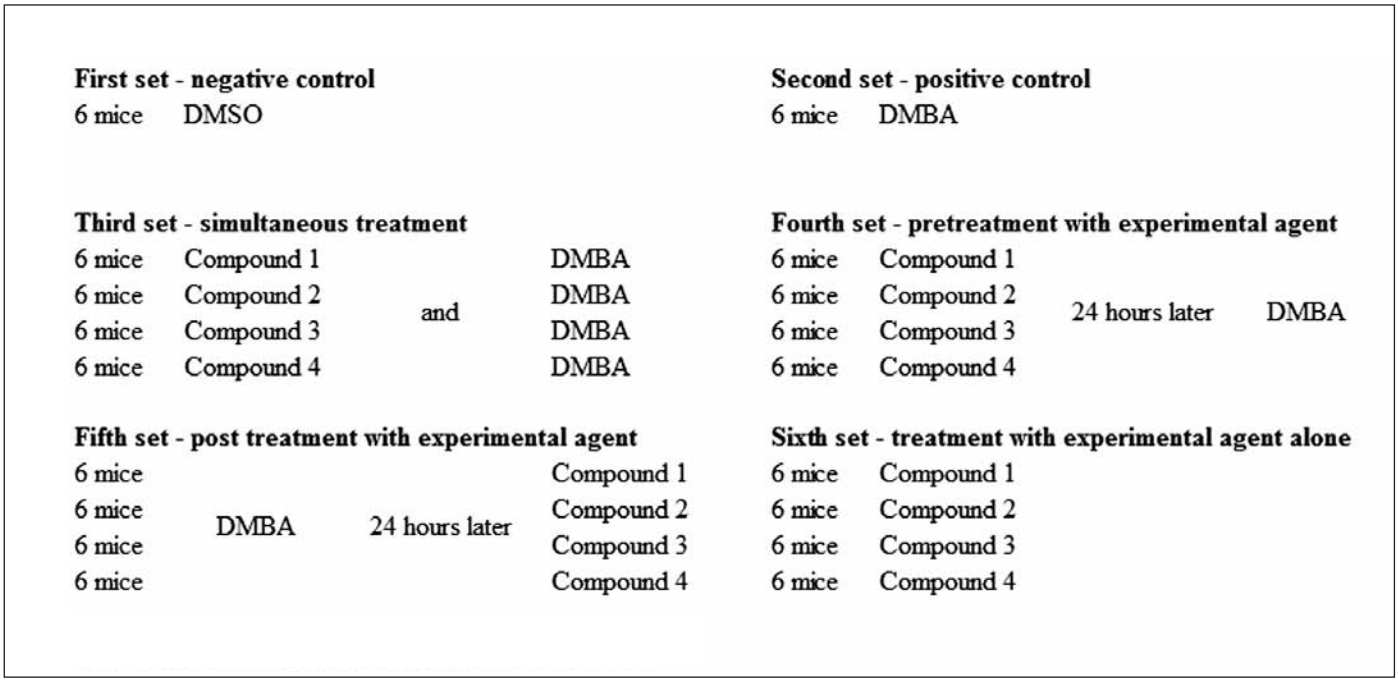

Fig. 2. Experimental plan administration and doses as in Materials and Methods

Gene expression investigations

The liver, the lungs, the bone marrow and the kidneys of the animals were removed and 100 mg samples of each tissue from the respective groups pooled. After homogenization of the organs, total cellular RNA was isolated using TRIZOL reagent (Invitrogen, Paisley, UK). The RNA quality was assessed by denaturing gel-electrophoresis, and absorption measurement at 260/280 nm (A260/A280 was >1.8). After necessary dilution, 10 μg RNA was dot-blotted onto Hybond N^+ nitrocellulose membrane (ECL kit, Amersham, Little Chalfont, UK) and hybridized with chemiluminescent specific probes for *p53* and *Ha-ras* genes (Professor J. Szeberényi, University of Pécs, Hungary). Isolation of RNA, hybridization and detection were performed according to the manufacturers' instructions. Chemiluminescent signals were detected on X-ray films, the films were scanned and evaluated by computer software Quantiscan (Biosoft, Cambridge, UK). The membranes rehybridized with constitutively expressed *β-actin* gene served as basis for comparison of gene expression. The results indicate the gene expression as a proportion of *β-actin* expression. Results are presented in figs. 3-6, each column represents the mean of the results for each set. Statistical analysis was carried out with *t-test* implemented in SPSS statistics. Bars over the columns represent 2x standard deviation. The significance of the ratios was computed from the t-scores, and a threshold of $p<0.05$ was applied to define differentially expressed genes.

Results

DMBA, a pluripotent chemical carcinogen, increased the expression of *Ha-ras* and *p53* genes in a remarkable manner, in our "short-term" animal experimental model. DMBA, given alone, elevated the expression of the examined key onco/suppressor genes in every organ in nearly every experimental set. DMSO, which served as negative control and was used as a solvent for DMBA and the experimental agents, applied alone had very little effect on the examined genes in each of the examined organs (figs 3-6). Generally, administration of the experimental molecules 24 hours prior to, simultaneously with, and 24 hours after DMBA exposure characteristically reduced DMBA-induced overexpression of the examined genes.

The liver

Gene expression patterns of the liver are presented in fig. 3.

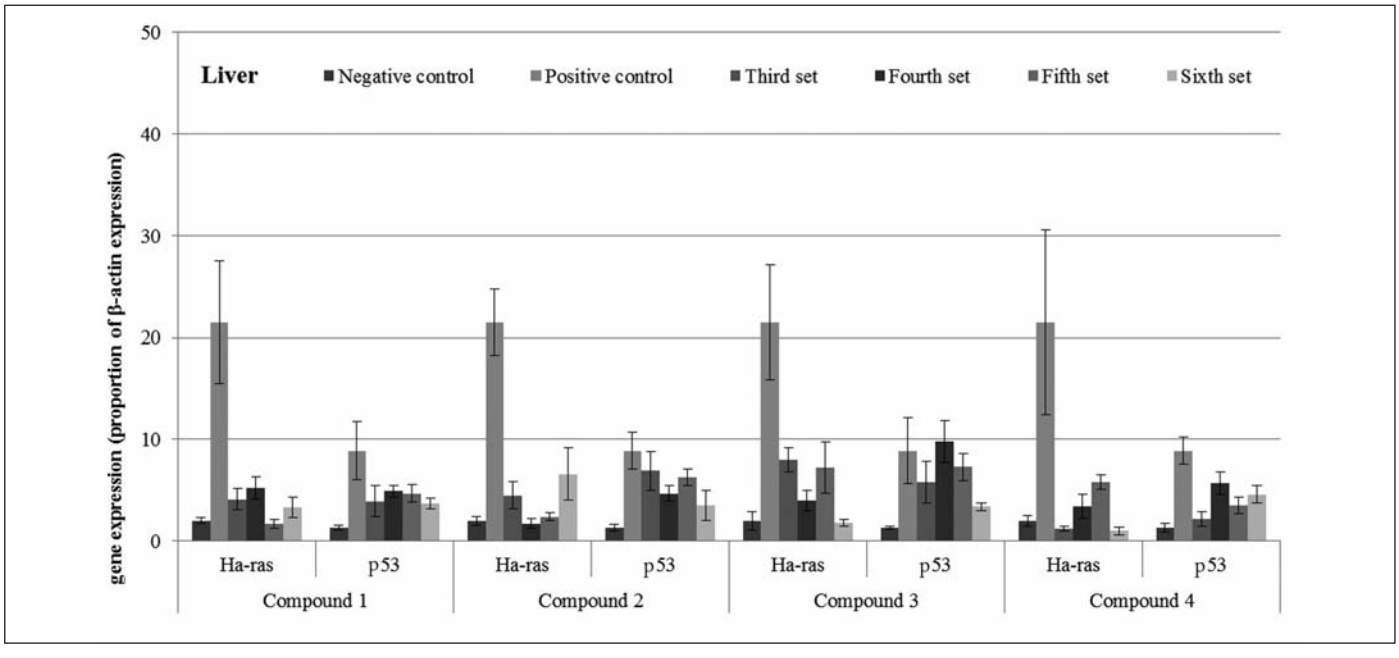

Fig. 3. *Ha- ras* and *p53* gene expression pattern from liver of CBA/Ca mice at 24 hours after treatment and their combinations

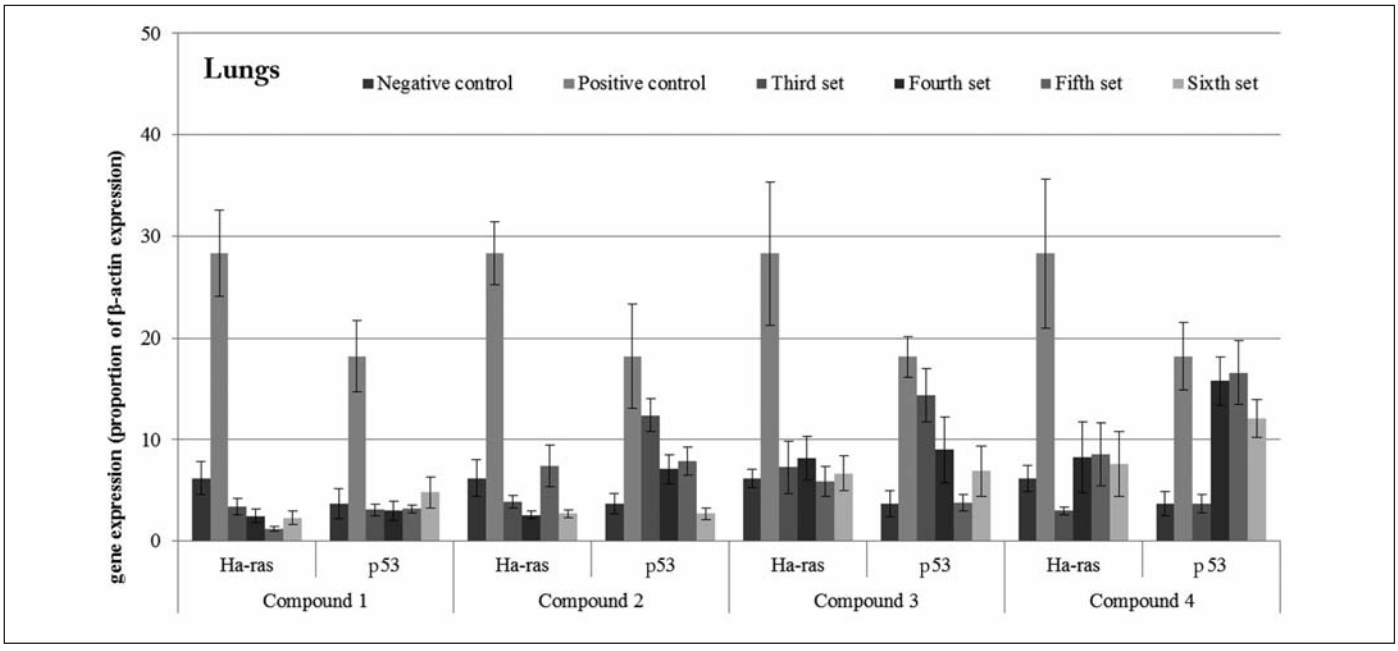

Fig. 4. *Ha- ras* and *p53* gene expression pattern from the lungs of CBA/Ca mice at 24 hours after treatment and their combinations

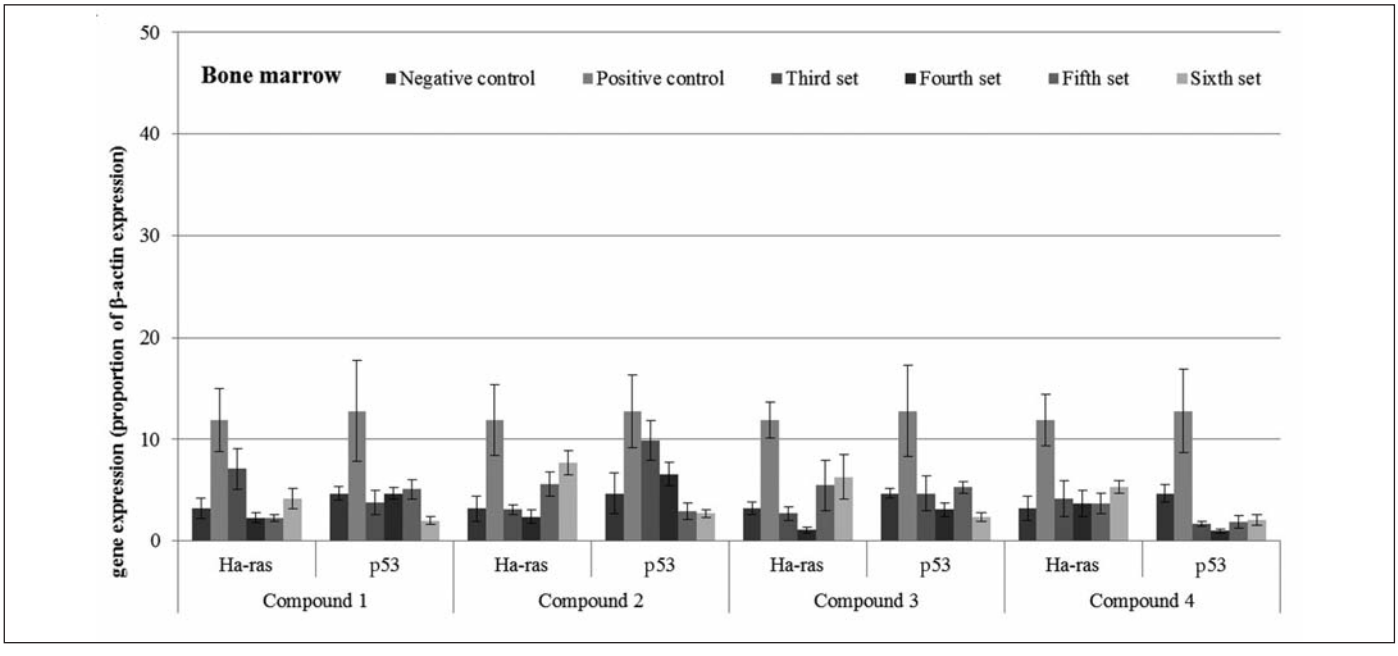

Fig. 5. *Ha- ras* and *p53* gene expression pattern from bone marrow of CBA/Ca mice at 24 hours after treatment and their combinations

All of the four compounds reduced DMBA-induced overexpression of *Ha-ras* in the liver. Compound 1 reduced *Ha-ras* expression in the fifth set, administered after DMBA exposure. Pre-treatment with compound 2 and 3 (fourth set) significantly ($p<0.05$) reduced expression of *Ha-ras* to the level of the negative control. Simultaneous DMBA and compound 4 administration decreased expression of *Ha-ras* gene (third set). The *p53* gene expression of the liver was reduced by compound 1 and 4 in the third, fourth and fifth set. In case of compound 1 no significance was found. Compound 4, while

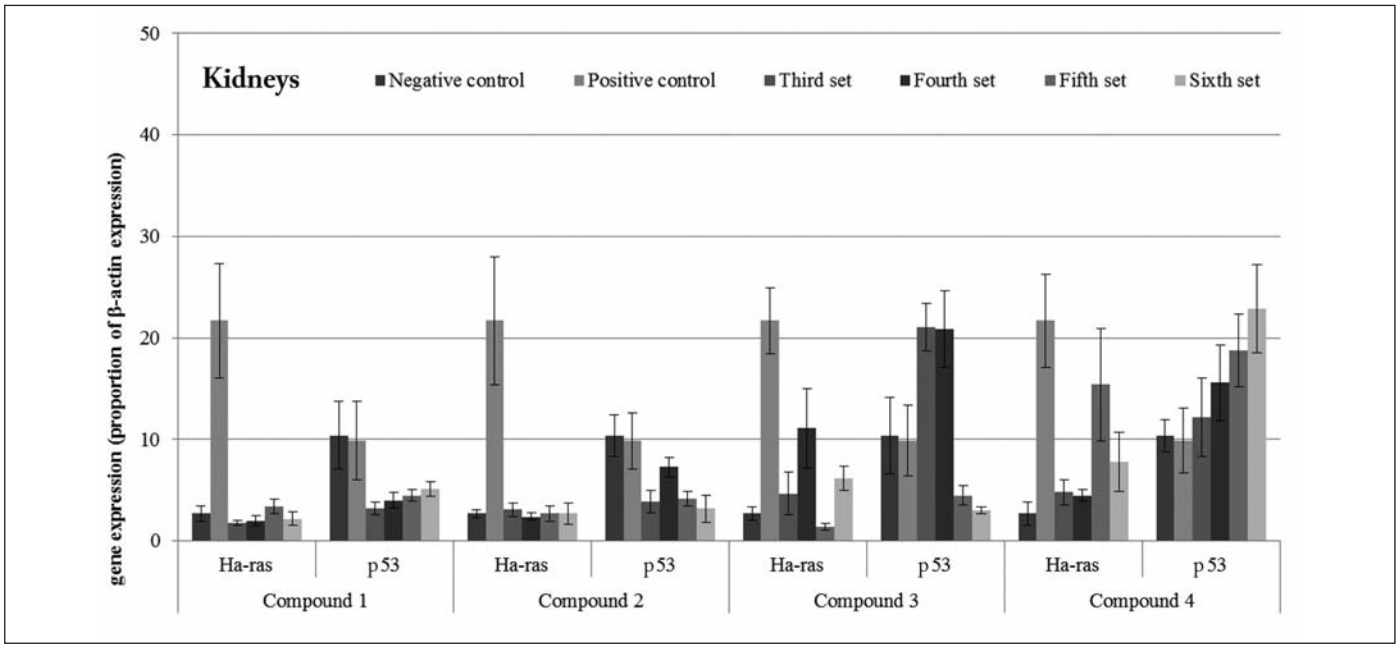

Fig. 6. *Ha- ras* and *p53* gene expression pattern from the kidneys of CBA/Ca mice at 24 hours after treatment and their combinations

simultaneously administered with DMBA, reduced *p53* mRNA expression significantly ($p<0.05$) to the level of the negative control.

The lungs

Gene expression patterns of the lungs are presented in fig. 4.

All four tyrosine kinase inhibitors reduced DMBA-caused *Ha-ras* gene expression to the level of the negative control. Comparing to the positive control, the decreasing is significant ($p<0.05$). Compound 2 was an effective inhibitor in the fourth set. Simultaneously administered with DMBA (third set) compound 4 significantly ($p<0.05$) reduced *Ha-ras* expression to the level of the negative control. DMBA-elevated *p53* mRNA level was significantly reduced ($p<0.05$) by compound 1 in the third, fourth and fifth set. Compound 2 and 3 treatment reduced elevated *p53* expression level (third, fourth, fifth set), the reduction is significant ($p<0.05$) in the fourth and fifth set.

Bone marrow

Gene expression patterns of the bone marrow are presented in fig. 5.

The DMBA-elevated *Ha-ras* mRNA expression was reduced by the experimental compounds.

Compound 2, 3 and 4 significantly reduced *Ha-ras* expression ($p<0.05$) in the third, fifth and sixth set. Compound 1 significantly ($p<0.05$) reduced *Ha-ras* gene expression when the animals were pre or post treated with it (fourth and fifth set). All of the four compounds reduced the expression of *p53* gene in all the three experimental sets (third, fourth and fifth). Compound 1 and compound 3 decreased expression of *p53* gene to the level of the negative control. Compound 4 significantly ($p<0.05$) reduced *p53* expression in all of the three treatment sets (third, fourth and fifth). The downregulation by compound 2 was not significant in the third set, but in the pre and post-treatment sets (fourth and fifth) *p53* mRNA level decreased in the bone marrow.

The kidneys

Gene expression patterns of the kidneys are presented in fig. 6.

All four compounds reduced *Ha-ras* gene expression comparing to the positive control.

Compound 1, 2 and 3 significantly reduced *Ha-ras* expression in the third, fourth and fifth set ($p<0.05$). Compund 1 and compound 2 reduced *Ha-ras* mRNA expression to the level of the negative control. Comparing to the third and fourth sets, compound 3 showed greater *Ha-ras* reduction in the fifth set.

The expression of *p53* tumour suppressor gene was found to be higher in the kidneys than in other examined organs. DMBA treatment (positive control) did not increased *p53* expression in the kidneys. Compound 1 and compound 2 reduced *Ha-ras* and *p53* gene expression in all of the experimental settings where they were administered. The reduction is significant ($p<0.05$), except for *p53* in the fourth set of compound 2.

Compounds 3 and 4 reduced *Ha-ras* expression significantly ($p<0.05$) in the third, fourth and fifth set. Compound 3 administered before and after DMBA exposure increased *p53* gene expression in the kidneys (third and fourth set). Compound 4 administered with DMBA and alone increased *p53* gene expression in the kidneys.

Discussion

Generally, the applied molecular epidemiological key onco/suppressor gene test system represented the biological effects of all of the compounds used in this experiment. DMSO and solvents containing DMSO (in applied concentration) used often in cell culture experiments practically do not interfere with biological functions in a measureable manner. As we use the expression of *Ha-ras* onco- and *p53* tumoursuppressor genes as an early biomarker of chemical carcinogenesis, the increase of their expression shows the carcinogen exposure of the tissues. A potential chemopreventive agent might inhibit the effect of the carcinogen thus decreasing the expression of the biomarkers compared to the positive control. DMBA – a pluripotent and complete carcinogen (16) – increased the expression of the examined genes in the examined organs. In our previous experiments we demonstrated, that significant overexpression of *Ha-ras* and *p53* may be detected 24 hours after administration of DMBA in the liver, lungs and kidneys of female CBA/Ca (sensitive H-2k haplotype) mice. Our previous results strongly correlate with recent experiment (17).

However DMBA did not exert remarkable effect of *p53* gene expression in the kidneys, due to lipophilic distribution (18) and also the need for its metabolic activation (19). DMBA is a carcinogen foremost in the lungs (CYP1A1 highly expressed), liver (CYP1A2 highly expressed) and in other organs which highly express activating enzymes (17, 19, 20). Elevated *p53* expression in lung carcinomas (21) and phase I metabolizing enzyme activity induction caused by ubiquitous chemical carcinogens (*e.g.* DMBA) (22) correlates with the results of this paper.

Compounds 1, 2 and 3 given alone in all of the experimental settings, in all of the examined organs exerted less expression increasing effect, than the DMBA treated group, indicating chemopreventive effect of the examined quinazolin type molecules.

Tyrosine Kinases play a crucial rôle in both mitogen-stimulated and oncogene-induced signaling pathways (1). Epidermal Growth Factor (EGF) and Platelet-Derived Growth Factor (PDGF) are important components of these cell proliferation-stimulation secondary signal transduction pathways (1) and can be blocked by tyrosine kinase inhibitors (23).

Compound 1 and compound 2 reduced both DMBA increased *Ha-ras* and *p53* gene expressions in all of the examined organs in all of the experimental settings due to PTKi effect. The inhibitory effect of certain PTKi agents lasts for at least 48 hours (24) on PTK secondary signal transduction pathway. This underpins our results in the applied time period, 24 hours prior to, simultaneously with and 24 hours after DMBA exposure.

Moreover TK inhibitory effect (IC_{50}=70-100 μM) of compound 1 on SW-620 human colon carcinoma cells has been shown (5). Liu *et al.* demonstrated that PTK inhibition (caused by genistein) inhibited basal and also PDGF induced rat hepatic stellate cell proliferation for 48 hours by suppressing tyrosine phosphorylation (24). A reduction in *Ha-ras* expression corresponds to inhibition of Ras Extracellular signal-Regulated Kinase (ERK) pathway (which directs signals to the immediate-early genes *e.g. c-fos*, *c-jun* up-regulating transcriptional factors, enhancing cell proliferation), thus exerting a chemopreventive effect (25). Moreover Liu *et al.* found with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay-based flow cytometry that PTK inhibition reduced the protein level of cyclin D1, a cell cycle-related protein (24). This reduced the activation of the ERK pathway and also the inducibility of cyclin D1 (26), leading to lower cell proliferation. Cheng *et al.* corroborated these

findings, highlighting that oridonin induced G2M arrest and apoptosis in L929 cells, which is regulated by promoting ERK-p53 apoptotic pathway and suppressing PTK-mediated survival pathway (27).

Therefore abrogation of DMBA-induced *Ha-ras* oncogene expression caused by all four PTKi experimental agents in all experimental settings in the liver corresponds to the literature data earlier mentioned (1, 23-25). This is also the case for *p53* suppressor gene expression (26, 27).

The same is true for compound 3 and 4, except for the kidneys, where only *Ha-ras* gene expression moderately decreased. This may be explained by the accumulation of the lipophilic agents in the kidneys and the mutual significance of *p53* overexpression in malignant diseases: early *p53* overexpression is partially a consequence of *Ha-ras* (and of *c-myc* as well) overexpression caused by carcinogen exposure (28). *p53* overexpression is a crucial component in the initiation of EGFR TK pathway, resulting in reduction of cell proliferation *via* G0G1 cell cycle arrest and induction of apoptosis, with dramatic clinical relevance *e.g.* in patients with non-small cell lung cancer (NSCLC) (29).

Compound 4 treated group – alone and in co-administration with DMBA as well – elevated *p53* gene expression in the kidneys in each setting. This result differs from expected based upon previous *in vitro* experiments (5-8). Since gene expression patterns of compound 1, 2 and 3 treatment confirms the *in vitro* experiments, further investigations of the effect of compound 4 on the kidneys are needed. Since *p53* has a Janus-faced function it is pro-apoptotic and also transactivates genes whose products act in an anti-apoptotic manner (30). Our test system is able to indicate other apoptotic pathways, in order to determine the events behind compound 4 elevating *p53* expression, other apoptotic genes should also be investigated in future experiments. Activation of receptor tyrosine kinase signaling, including EGFR, has been implicated in the development of malignant disease *e.g.* high-grade gliomas and hemangioblastomas (31). Moreover, Chang *et al.* revealed in patients with primary lung cancers (with corresponding lymph node metastases), that *p53* and EGFR mutations usually precede lymph node metastasis (but heterogeneity of EGFR expression should be considered in therapeutic aspects) (32). Thus, the complex nature of EGFR biology allows potential opportunities for EGFR inhibitors in a number of areas of cancer therapy, including proliferative, angiogenic, invasive and metastatic aspects (3). For example, the alkaloid staurosporine acts as a potent inhibitor of protein kinase C and PDGF receptor proteins. Molecules blocking growth factor signaling at several points by inhibiting protein kinase C, phosphoinositide specific phospholipase C and inositol(1,4,5) trisphosphate induced Ca^{2+} release (33) supports *in vivo* antitumour mechanisms and enhance beneficial health effects of chemopreventive agents. Inhibition of the mentioned signalling pathways may explain the effects of these experimental agents and corresponds with a great concordance to the results of the *in vivo* experiment detailed in this paper. In our previous studies with the *in vivo* model we found that the reducing effect of the investigated compound also depends on the administration schedule (12). A chemopreventive agent might interact in several ways with the multistage carcinogenesis (34) for example by modifying transmembrane transport, modulating metabolism, blocking reactive species, inhibiting cell replication, maintaining DNA structure, modulating DNA metabolism and repair, and controlling gene expression (35). For example a blocking agent inhibiting the activation of a carcinogen to its ultimate carcinogenic form is more potent given prior to or with the carcinogen itself. On the other hand a blocking agent that modifies metabolising enzyme systems acts later (36). To evaluate whether the examined compounds have another effect besides kinase inhibition, or have a time-dependent effect on DMBA carcinogenesis, we established three experimental sets depending on the order of the administered DMBA and experimental compounds. In recent experiment some tendencies may be found that might help further investigations about potential chemopreventive mechanism of investigated compounds.

Conclusion

Our *in vivo* short-term experimental results provide some evidence for the supposed antineoplastic and chemopreventive effect of the four examined

compounds. In this study the applied early molecular epidemiological biomarker model indicates the first steps of malignant transformation by cell level and possible anti-tumour effects, as well. Our findings suggest that compounds 1, 2 and 3 have a preventive effect on the early steps of DMBA induced carcinogenesis. In case of compound 4 promising results were shown in the liver, lungs and bone marrow, but in the kidneys only *Ha-ras* expressions confirms *in vitro* results, further investigation is needed. These observations further strengthen the previous assumption, based on *in vitro* results, that the four compounds have an *in vivo* chemopreventive effect.

Future experiments

The effect of gene expression on protein synthesis could occur via microRNAs (miRNAs) modification. MiRNAs are 21-24 nucleotide (nt) long, endogenous non-coding RNA molecules that are involved in the post-transcriptional regulation of gene expression in multicellular eukaryotic organisms. Certain miRNAs are able to regulate transcription factors *e.g.* Hepatic Nuclear Factor (HNF) (37). Our research group is currently working on several miRNA experiments, and we studied that miR-21 – corresponding to the literature (38) – has an effect on *p53* and other anti-apoptotic gene expressions. We are planning to expand recent experiments concerning miRNA measurements.

Acknowledgements

The authors express their special thanks to Zsuzsanna Bayer and Mónika Herczeg for valuable technical assistance.

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