Abstract. Background: Afobazole, a new 2-mercaptobenzimidazole derivative, exhibited antimutagenic activity in chromosome aberration tests and antioxidant properties. The aim of this study was to demonstrate the potential chemopreventive effect of afobazole on the level of early biological effects by analysing changes in oncogene and tumor suppressor gene expression. Materials and Methods: Single intraperitoneal (i.p.) treatment with 7,12-dimethylbenz[a]anthracene (DMBA) combined with afobazole was administered to CBA/Ca (sensitive H-2K haplotype) female mice. The expression of Ha-ras and p53 was determined in the vital organs (liver, spleen, lung, kidney, thymus, lymph nodes and bone marrow) 24, 48 and 72 hours later. Results: Coadministration of afobazole and DMBA resulted in a decrease of DMBA-induced overexpression of Ha-ras and p53. Reduction of the DMBA-induced gene expression was most striking when afobazole was given in parallel with DMBA. Discussion: Our results strengthen the previous assumption, which was based on in vitro results, that afobazole has a chemopreventive effect in vivo.

The incidence of head and neck carcinomas is tending to increase in almost all European countries, including Hungary. In the European Union 60,000 patients are registered with head and neck tumors every year (1). Despite successful surgical, radio- and chemotherapy, the survival in 50% of the patients is less than 5 years (2). This can be explained by tumor recurrence, development of distant metastases and therapy induced tumors. Due to the limited therapeutic possibilities, continuing improvements in primary prevention are necessary.

Molecular and predictive epidemiology play an important role in cancer prevention. With the recognition of early biomarkers such as changes in oncogene and tumor suppressor gene expression, tumor development can be monitored and the efficiency of therapy (e.g. "minimal residual disease") and primary preventive intervention can be followed (3).

A test system has already been developed in our institute to assess the potential chemopreventive effect of bioactive compounds by analysing the changes in the expression of an oncogene and a tumor suppressor gene. A number of chemopreventive drugs have been investigated in this test-system such as chalcone analogues as intermediary compounds of the flavonoid biosynthetic pathway (4-6).

Plotnikov et al. have reported that a compound, bemitil (2-mercaptobenzimidazole), which belongs to a new group of pharmaceuticals, the actoprotectors, showed a strong antimutagenic effect (7). In in vivo investigations bemitil reduced the 7,12-dimethylbenz[a]anthracene (DMBA)-induced overexpression of oncogenes and tumor suppressor genes in a short-term test (8). Pharmacologically active 2-mercaptobenzimidazole (bemitil, tomerzole, afobazole) derivatives are able to reduce the mutagenic effects of chemical prooxidants by inhibition of free radical oxidation-induced endogenous mutagen formation. A chromosome...
CBA/Ca (sensitive H-2 K haplotype) mice were used. The animals were obtained from Sigma-Aldrich, Buchs, France and from Moscow, Russia. Corn oil and DMBA were purchased from the Institute of Pharmacology, Russian Academy of Medical Sciences, provided by Professor Sergey Seredenin (State Zakusov Research Institute).

Afobazole (chemical structure Figure 1) was kindly provided by Professor Sergey Seredenin (State Zakusov Research Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow, Russia). Corn oil and DMBA were purchased from Sigma-Aldrich, Buchs, France.

Materials and Methods

Chemicals. Afobazole (chemical structure Figure 1) was kindly provided by Professor Sergey Seredenin (State Zakusov Research Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow, Russia). Corn oil and DMBA were purchased from Sigma-Aldrich, Buchs, France.

Six groups of six conventionally kept, 6-8 week-old female CBA/Ca (sensitive H-2K haplotype) mice were used. The animals weighed 20-25g. Four groups were treated intraperitoneally (i.p.) with a single 40 mg/kg bodyweight (bwkg) dose of DMBA dissolved in corn oil (8 mg/ml). Three of the four groups were additionally treated i.p. with a 50 mg/bwkg dose of afobazole dissolved in distilled water (10 mg/ml). The first group of animals was administered afobazole 24 hours after, the second group parallel with, and the third group 24 hours prior to the DMBA treatment. The fifth group was administered corn oil (i.p. 1 ml/bwkg) and the sixth group was treated with afobazole (i.p. 50 mg/bwkg) in a 10 mg/ml corn oil solution. The animals were autopsied 24, 48 and 72 hours after the final treatment. The thymus, spleen, liver, kidney, lung, bone marrow and lymph nodes were removed. The total RNA was isolated according to the acid guanidinium thiocyanate-phenol-chloroform method (Trizol, Invitrogen, Paisley, Scotland, UK). The RNA-quality was checked by denaturing gel-electrophoresis and absorption measurement was performed at 260/280 nm. Ten µg of total RNA was dot-blotted onto Hybond N+ nitrocellulose membrane (Amersham, Little Chalfont, England, UK) and hybridized with chemiluminescently labelled p53 and Ha-ras specific probes (ECL kit, Amersham). Hybridization and detection were performed according to the manufacturer’s instructions. The signals were detected on X-ray film and dots were evaluated by using Quantiscan software (Biosoft, Cambridge, UK). The results were expressed as the percentage of β-actin (constitutively expressed endogenous control).

Results

DMBA increased the expression of Ha-ras and p53 in the liver, spleen, lung, lymph nodes and bone marrow (Figure 2). Afobazole itself had little effect on the expression of either gene. Coadministration of afobazole and DMBA resulted in a decrease of DMBA-induced overexpression of both Ha-ras and p53. Reduction of the DMBA-induced gene expression was most striking when afobazole was given in parallel with DMBA, the expression of both genes in all the investigated tissues was reduced. Administration of afobazole 24 hours after the DMBA treatment reduced the DMBA-induced expression of Ha-ras and p53 in the liver, spleen, lung, thymus and lymph nodes. Administration of afobazole 24 hours prior to the DMBA treatment reduced the DMBA-induced gene expression in the liver, spleen, lung and lymph nodes, but had no effect on the level in the kidney, thymus or the bone marrow. A decrease in the reduction of DMBA-induced gene expression levels was observed after 48 hours in four tissues: the liver, spleen, lung and lymph nodes. A slight increase of the expression of both genes was also observed 72 hours after the treatment. This could be explained as a time-dependent effect of afobazole.

Discussion

Since oncogenes and tumor suppressor genes play a crucial role in carcinogenesis (11, 12), analysis of oncogene and tumor suppressor gene expressions are appropriate for the early detection of carcinogen exposure (13-14).

Both of the genes which were investigated in this study play an important role in carcinogenesis, Ha-ras in the initiation of carcinogenesis and p53 in the response to DNA damage (12, 15, 16). Overexpression of these genes can represent the early biological effect of temporary or continuous carcinogen exposure, and can be considered as early approximate predictive biomarkers (17-21). Afobazole has previously shown a tendency to decrease reactive oxygen species (ROS) accumulation and induce an increase in catalase (antioxidative) activity in rats (22). The antimutagenic activity determined by its antioxidant properties was dependent on the dose and treatment schedule (23).

In the present study the effect of DMBA on the expression of Ha-ras oncogene was in agreement with previous results (4, 5). The effect of afobazole on the DMBA-induced expression of the Ha-ras and p53 genes was time dependent. The most effective suppressor effect
Figure 2. continued
was observed 48 hour after afobazole treatment. This result was similar to the effect of E-2-(4'-methoxybenzylidene)-1-benzosuberone investigated earlier using our test system (5). In contrast, a slight increase of expression of the two genes was evident again 72 hours after administration indicating a decrease of the chemopreventive effect of afobazole. The chemopreventive effect was also depended on the administration schedule. The most striking reduction of DMBA-induced overexpressions was seen with parallel administration of afobazole and DMBA when the overexpression of both Ha-ras and p53 was reduced in all tissues.

Our findings suggest that afobazole might have an influence on the metabolic activation of DMBA, responsible for the mutagenic activity of the compound. These observations further strengthen our previous assumption, based on in vitro results, that afobazole has an in vivo chemopreventive effect.

References