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N-ACETIL-L-CYSTEINE AND 2-AMINO-2-THIAZOLINE N-ACETYL-L-CYSTEINATE AS A POSSIBLE CANCER CHEMOPREVENTIVE AGENTS IN MURINE MODELS

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The aim of this study was to investigate N-acetyl-cysteine (NAC) and its 2-amino-2-thiazoline salt (NACAT) as potential chemopreventive agents on experimentally induced lung tumours by urethane (U) in mice. Female BALB/c mice were used. U was given by intraperitoneal injections during 2 weeks (single dose – 10 mg/mouse, total – 50 mg/mouse). Mice were treated daily *per os* with NAC 1/10 LD_{50}, NACAT 1/10 or 1/100 LD_{50} starting 2 weeks prior U administration, then during U treatment and thereafter for 2 months. The duration of experiment was 4 months. The results showed that NAC (1000 mg/kg) reduced the lung tumour incidence to 30% that of controls, P # 0.05. Most effective of NACAT was 100 mg/kg dose; it reduced an average of lung adenomas per mouse by 26%, P # 0.05, but lower dose (10 mg/kg) was less effective. In order to achieve similar chemopreventive effect (~30%) on mice, it is necessary to use 0.38 mM/kg of NACAT or 6.13 mM/kg of NAC. It means that 16 times less of NACAT is required, if calculated by molar concentration. In general, NAC and NACAT have a moderate chemopreventive effect on lung tumorigenesis induced by urethane in mice.

Keywords: Cancer chemoprevention – N-acetyl-L-cysteine – 2-amino-2-thiazoline N-acetyl-L-cysteinate – pulmonary adenomas

INTRODUCTION

One of the goals in cancer chemoprevention research is to assess the efficacy of chemopreventive agents in inhibiting carcinogenesis, using various experimental cancer models on animals. The experimental and clinical data have shown that antioxidant N-acetyl-cysteine (NAC) is one of the most promising chemopreventive agent [1–4]. The therapeutic association of NAC with doxorubicin shows a remarkable synergic effect in inhibiting cancer metastasis formation [5]. The chemopreventive agent NAC acts through a variety of mechanisms, including its antioxidant and nucleophilic properties [4, 6–9].

The aim of this study was to investigate NAC and its 2-amino-2-thiazoline salt (NACAT) as potential chemopreventive agents on experimentally induced lung

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tumours by urethane (U) in mice. 2-Amino-2-thiazoline, an inducer of reverse transformation, inhibited carcinogenesis and prolonged the long-term survival of mice with dimethylhydrazine-induced colon tumours and abolished tumours in rectum [10].

MATERIALS AND METHODS

Agents

N-acetyl-L-cysteine was purchased from Aldrich. 2-Amino-2-thiazoline N-acetyl-L-cysteinate was synthesized according to the procedure [11] and commercial urethane (ethyl carbamate) was used ("pure", Kiev, Ukraine).

Animals

Female BALB/c mice (from Institute of Immunology, Vilnius) 6–7 weeks old were used in this study. This mouse strain has been shown to be sensitive to urethane tumorigenicity by developing in 4 months, a high incidence of lung tumours. The animals were kept at the laboratory conditions for at least 5 days prior to the test. Before the test, animals were randomized and assigned into treatment groups, each group consisted of 10 mice. Initial mice body weight was 17–19 g. During the experiment body weight was recorded weekly. Throughout the study mice were looked after in accordance with European Convention, the Guide for care and use of laboratory animals and Lithuanian laws [12–14]. Animals were kept in standard housing conditions in the Vivarium of the Institute of Biochemistry. Mice were given commercial pellets and water *ad libitum* throughout the acclimatization and experimental periods. Animals were sacrificed by cervical dislocation.

Selection of the dose levels and assay protocols

Acute toxicity test following OECD guideline [15] was determined for NACAT. The LD_{50} and MTD (maximum tolerated dose, oral) was determined prior to the beginning of the lung tumour bioassay.

Mice were injected intraperitoneally with U twice a week (single dose – 10 mg/mouse, total – 50 mg/mouse). Animals were treated daily *per os* with NAC 1/10 LD_{50} (1000 mg/kg), NACAT 1/10 LD_{50} (100 mg/kg) and 1/100 LD_{50} (10 mg/kg) starting 2 weeks prior to U administration, then during U treatment and thereafter for 2 months.

The experimental groups were as follows:

1. gr. U,
2. gr. NAC (1000 mg/kg) + U,
3. gr. NACAT (100 mg/kg)+ U,
4. gr. NACAT* (10 mg/kg) +U.

The duration of experiment was 4 months. Then mice were sacrificed, the lungs were removed and fixed in 10% formaldehyde solution. After 14 days of fixation lung tumours, which appeared as pearly white nodules on the surface of the lungs, were counted. Random samples of nodules were taken from the lungs for histopathological evaluation and confirmation of adenoma. Criteria of evaluation was percentage of mice with lung adenomas, mean number of tumours per tumour-bearing mouse, time taken to develop tumours. Data were analyzed statistically by Student's *t*-test.

RESULTS AND DISCUSSION

Acute oral administration of NACAT showed that LD_{50} is 1000 mg/kg and maximal tolerated dose (MTD) – 800 mg/kg. NACAT showed a moderate toxicity.

Table 1 indicates the results on NAC and NACAT compounds currently being tested in the animal model. Pulmonary tumours were found in every mouse which received U, U and NAC or U and NACAT. Number of tumour/mouse in our experiments on BALB/c mice was approximately the same as we found earlier in our experiments on this strain of mice [16, 17]. There was no significant difference in the size of adenomas in various groups (usually 1 mm in diameter) and time required to develop tumours. Pathology showed benign adenoma in the lungs in all groups. Apart from the number of tumours the lungs showed no essential difference between the groups. It is well known that multiciplity of lung adenomas directly related to the dose of U is an important quantitative dividend [18, 19]. Lung-tumour multiplicity was sulficiently high to evaluate the effect of chemopreventive agents.

When NAC (1000 mg/kg) was given *per os,* the number of tumours per animal was reduced by 30%, P # 0.05 (Fig. 1). According to the literature [4], administration of a diet supplemented with 0.2% NAC, protected Swiss albino mice from the induction of lung tumours by a single *i.p.* injection of U (1 g/kg body weight). Significant reductions of both incidence and multiciplicity, as compared to mice

Table 1
Effect of NAC and NACAT on lung carcinogenesis induced with urethane in mice BALB/c

Groups No.	Treatment	Number of animals			Lung tumours/ mouse ± SD
		initial	effective	% of mice with adenomas	
1.	U	10	9	100	11.4±1.9
2.	NAC + U	10	8	100	8.0±1.2
3.	NACAT + U	10	8	100	8.5±1.4
4.	NACAT* + U	10	10	100	9.8±2.5

AC – 1000 mg/kg, NACAT – 100 mg/kg, NACAT* – 10 mg/kg

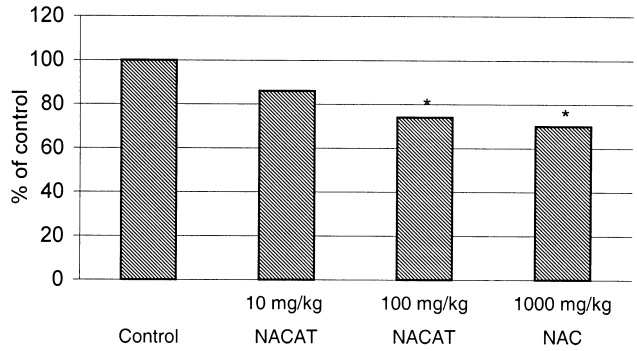

Fig. 1. Dose dependent chemopreventive effect of NAC and NACAT on lung tumours incidence (multiplicity)

treated with U only, were recorded in mice, starting 15 days before the U injection and continuing for 4 additional months. No significant change in tumour incidence or multiciplicity was observed when NAC was given only either during the 15 days preceding injection of the carcinogen, or one and two months later [20]. This suggests that, at variance with other anti-initiating antioxidants, NAC has no promoting effects in this animal model [4].

NACAT (dose 100 mg/kg) reduced the lung tumour incidence to 26% that of controls, P # 0.05, but lower dose of this compound – 10 mg/kg has less expressed chemopreventive effect, it reduced tumour incidence only by 14%. It was found that using pretreatment and treatment of structurally related compounds NAC and NACAT in 1/10 LD_{50} dose (1000 mg/kg and 100 mg/kg, accordingly), pulmonary adenoma formation in mice was reduced similary. Chemopreventive efficiency of NACAT was lower when it was used in lower dose – 1/100 LD_{50}. In order to achieve similar chemopreventive (~30%) effect on mice, it is necessary to use 0.38 mM/kg of NACAT or 6.13 mM/kg of NAC. It means that 16 times less of NACAT is required, if calculated by molar concentration.

The body weight-gain of mice during the experiment is presented in Fig. 2. As shown the initial body weight was the same in all groups. The administration of U did not cause decrease in body weight which gradually increased. As seen in Fig. 2, regardless of treatment regimen with NAC or NACAT the average body weight of mice during the course of treatment and later till the final of the study was not significantly different between groups. It was increasing till the end of experiment. In contrary, as it is known that the depression of weight-gain is frequently used as a non-specific indicator of toxicity in animal chemoprevention studies [21]. In our case it is shown that the examined compounds were not toxic for mice. No remarkable difference in behaviour of mice was noted between the groups during the experiment.

In conclusion, the results of our experiment demonstrate that NAC and NACAT possess moderate chemopreventive effect on urethane induced pulmonary tumours in mice BALB/c.

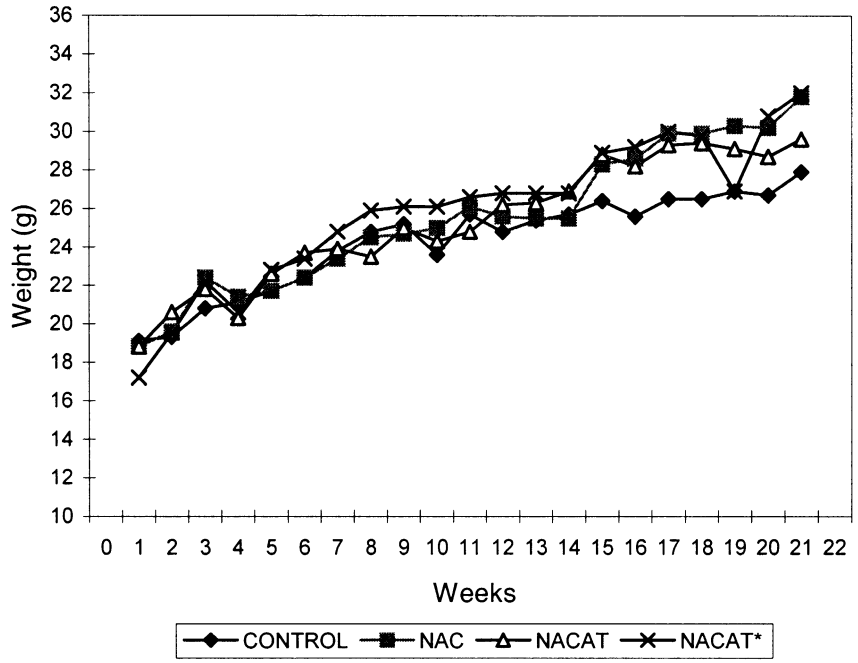

Fig. 2. The body weight-gain of mice during the experiment.
NAC – 1000 mg/kg, NACAT – 100 mg/kg, NACAT* – 10 mg/kg

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