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In vivo synergistic cytogenetic effects of aminophylline on lymphocyte cultures from patients with lung cancer undergoing chemotherapy

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ABSTRACT

Background: The anti-cancer and cytogenetic effects of aminophylline (AM) have been demonstrated in several clinical trials. The aim of the present study was to investigate the in vivo cytogenetic effects of AM in newly diagnosed patients with small cell (SCLC) and non-small cell lung cancer (NSCLC), receiving chemotherapy for the first time.
Methods: Sister chromatid exchanges (SCEs) and proliferation rate index (PRI) were evaluated in peripheral blood lymphocyte cultures from six patients with SCLC and six patients with NSCLC after the in vitro addition of AM and after the in vivo administration of AM in patients receiving chemotherapy.
Results: The in vitro addition of AM significantly increased SCEs only in SCLC patients ($p < 0.001$). The in vivo administration of AM after chemotherapy increased SCEs in both cancer types (SCLC: $p < 0.001$, NSCLC: $p = 0.003$) and this increase was synergistic, the rates of SCEs in the presence of AM were higher than the expected SCE values if the increases above background for chemotherapy and AM were independent and additive (SCLC: $p < 0.001$, NSCLC: $p = 0.008$). Although in both groups of patients cell division delays were observed after the combined chemotherapy plus in vivo AM treatment, the correlation between the magnitude of the SCE response and the PRI depression was not statistically significant ($p > 0.05$).
Conclusions: These observations suggest that AM enhances the results of concurrently administered chemotherapy by synergistically increasing its cytogenetic effects in patients with lung cancer.

1. Introduction

Lung cancer represents the leading cause of cancer and cancer-related mortality worldwide [1,2]. Chemotherapy is the cornerstone of treatment for small cell lung cancer (SCLC) and for the majority of cases of non-small cell lung cancer (NSCLC), since only 25% of patients are candidates for surgical treatment at the time of the diagnosis [3,4]. Despite some gradual improvement, treatment of lung cancer remains largely unsatisfactory with very low 5-year survival rates [4,5].

The target of the anti-tumor agents is DNA damage and subsequent cancer cell death. Sister Chromatid Exchanges (SCEs), i.e. exchanges among DNA parts of two sister chromatids during the S phase of the cell cycle before the final separation of chromatids in two chromosomes, have been frequently used as a sensitive indicator of DNA damage and subsequent repair [6]. SCEs assay reflects DNA instability or deficiency in DNA repair mechanisms [7] and it is considered as a sensitive cytogenetic index for the detection of carcinogens and for the evaluation of chemotherapy [8,9]. SCEs have been used for the investigation of the cytogenetic effects [7,10–12] and the evaluation of the protective role of various agents [6,13]. Delay in cell division as assessed by the Proliferation Rate Index (PRI) is a valuable indicator of cytostaticity [7] and has been used for the assessment of the cytostatic action of various therapeutic agents [6,14].

Given the disappointing results and the toxicity of the currently used anti-tumor agents, several other drugs have been investigated regarding their possible contribution in lung cancer treatment [6,15]. Aminophylline is a complex of the bronchodilator theophylline, structurally classified as a methylxanthine, and ethylenediamide which improves solubility [16]. AM is a nonselective adenosine receptor antagonist and a phosphodiesterase inhibitor that elevates intracellular levels of cyclic AMP. High intracellular levels of cAMP are considered capable of arresting growth, inducing apoptosis and attenuating cancer cell migration [15,17–21]. In that setting methylxanthines have been investigated as anti-cancer drugs in small-cell lung carcinoma [22] and

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Table 1
SCE values of lymphocyte cultures from SCLC patients at the four different phases of the study.

Patients	SCEs ± SE				
	Phase 1	Phase 2	Phase 3	Phase 4	
1	6.57 ± 0.86	9.28 ± 0.55	42.02 ± 3.35	55.07 ± 6.74	(44,73)
2	6.23 ± 0.31	8.36 ± 0.48	53.34 ± 2.21	64.04 ± 3.36	(55,47)
3	5.58 ± 1.28	7.87 ± 0.44	30.19 ± 1.73	37.87 ± 1.61	(32,48)
4	9.48 ± 0.4	12.02 ± 0.59	32.62 ± 2.77	46.89 ± 2.49	(35,16)
5	8.48 ± 0.77	10.55 ± 2.4	30.28 ± 1.14	40.55 ± 1.78	(32,35)
6	13.26 ± 0.61	14.4 ± 0.83	44.07 ± 2.66	51.5 ± 2.35	(45,21)
Mean value	8.26 ± 2.85	10.41 ± 2.46	38.75 ± 9.32	49.32 ± 9.67	

Phase 1: control before chemotherapy, phase 2: before chemotherapy and after the in vitro addition of aminophylline, phase 3: 20 h after chemotherapy and phase 4: 20 h after chemotherapy and 20 min after in vivo administration of aminophylline.
Numbers in parentheses are the expected values if the increases above background for chemotherapy and AM were independent and additive. The rates of SCEs in the presence of AM (phase 4) are of much higher significance ($p < 0.001$, by paired t-test) than those in the absence of AM (phase 3) over the respective controls.

non-small-cell lung carcinoma lines [23]. The aim of the present study was to investigate the in vivo cytogenetic and cytostatic effects of AM after administration in SCLC and NSCLC patients who were concurrently receiving chemotherapeutic drugs for the first time. In addition we also tried to evaluate the possible synergistic effect of AM with the chemotherapeutic agents.

2. Materials and methods

2.1. Patients

Twelve patients with recent diagnosis of inoperable lung cancer were included in the study just prior to receiving their first chemotherapy at the Pulmonary Department, Aristotle University of Thessaloniki, "G. Papanikolaou" Hospital. Six patients (mean age ± sd: 58.33 ± 2.98 years) suffered from SCLC and were treated with Carboplatin (Paraplatin) 400 mg/m^2 plus Ifosfamide (Holoxan) 2 g/m^2 plus Etoposid (Vepesid) 60 mg/m^2. In addition six patients (mean age ± sd: 59.66 ± 2.43 years) suffered from NSCLC of any histological type and were treated with Carboplatin (Paraplatin) 400 mg/m^2 plus Mitomycin-C (Mitomycin-C) 8 mg/m^2 plus Vintesine (Gesidin) 3 mg/m^2. All patients gave their informed consent and the study was approved by the Hospital's ethical committee.

Lymphocyte cultures were prepared from separate whole venous blood samples from each patient at four phases: phase 1: control before chemotherapy, phase 2: before chemotherapy plus in vitro addition of AM (50 μg/ml of culture, dose which corresponds to serum levels attained during chemotherapy), phase 3: 20 h after chemotherapy and phase 4: 20 h after chemotherapy and 20 min after intravenous administration of AM solution (3.3 mg/kg body weight, 250 mg AM diluted in 250 ml of normal saline). The time point of 20 h after chemotherapy was selected based on the fact that SCEs are increased during the first hours after chemotherapy while they are expected to decrease 20 h after [22]. We therefore administered AM 20 h after chemotherapy, in order to detect the possible synergistic effect of AM with the chemotherapeutic agents as an increase of the already low frequencies of SCEs [24].

2.2. In vitro SCE assay

Peripheral lymphocytes cultures were set up in universal containers by adding 11 drops of whole blood to 5 ml of chromosome medium B (Biochrom KG, Berlin, Germany). Bromodeoxyuridine (BrdU) at a final concentration of 5 μg/ml, was added at the beginning of the culture period. Cultures were then incubated at 37 °C for 72 h and were maintained in the dark to prevent or minimize BrdU photolysis. After 70 h, 0.5 μg/ml of Colchicine was added for 2 h and cultures were harvested at the end of the incubation period. Chromosome preparations were stained by a modified Fluorescence plus Giemsa technique [25]. Bromodeoxyuridine and Colchicine were obtained from Sigma (Sigma–Aldrich, St. Louis, MO, USA) and were dissolved in distilled water. Cells on the first (both chromatids staining dark), second (one chromatid of each chromosome staining dark) and third and subsequent mitotic divisions (a portion of chromosomes with both chromatids staining light) were counted.

Thirty suitably spread second division metaphases from each culture were scored on coded slides for SCEs and mean SCE values were evaluated. In order to establish the proliferation rate indices (PRIs), at least 100 cells were counted and the following formula was used: PRI = $(M_1 + 2M_2 + 3M_3+)/100$ where M_1 is the percentage of cells in the first division, M_2 in the second and M_3+ in the third and subsequent divisions.

2.3. Statistical analysis

Biostatistical analysis was performed by using SPSS for Windows, release 17.0.1 (Standard version, SPSS Inc.). The normality of distribution was assessed by Shapiro–Wilk test. Paired t-test was used for the comparison of SCE frequencies between different phases. The chi-square test was used for the cell kinetic comparisons (PRI). For the assessment of correlations between the SCEs and PRI frequencies, Pearson test (r correlation coefficient) was applied. The expected values of SCEs in the case that the influences of AM were additive was calculated as follows: expected value = phase2 − phase1 + phase3. The synergistic action of AM was calculated as follows: phase 4 − expected value.

3. Results

3.1. SCLC patients

SCE frequency and PRI values of lymphocyte cultures from SCLC patients at the four phases of the study are shown in Tables 1 and 2 respectively. The mean value of SCE after the in vitro addition of AM (phase 2) was statistically significantly higher than controls (phase 1) ($p < 0.001$, paired t-test). The mean value of SCE after the in vivo addition of AM (phase 4) was statistically significantly higher than SCE after chemotherapy alone (phase 3) ($p < 0.001$, paired t-test). SCE values at phase 4 were higher than the expected SCE values if the increases above background for chemotherapy and AM were independent and additive ($p < 0.001$, paired t-test, Table 1).

In patients 1,2,3,4 and 6 receiving the combined chemotherapy plus AM treatment (phase 4) a statistically significant ($p < 0.001$, by χ^2 test) change of the PRI rate was observed compared to control (phase 1) and to chemotherapy alone (phase 3). In Table 2, we observed a PRI increase in four patients and PRI decrease in two patients compared to the expected values if the differences in PRIs for chemotherapy and AM were independent and additive.

3.2. NSCLC patients

SCE frequency and PRI values of lymphocyte cultures from NSCLC patients at the four phases of the study are shown in

Table 2
PRI values of lymphocyte cultures from SCLC patients at the four different phases of the study.

Patients	PRI				
	Phase 1	Phase 2	Phase 3	Phase 4	
1	2.12	1.95	1.58	1.43	(1,41)
2	2.36	1.88	2.02	2.1	(1,54)
3	2.11	1.73	2.42	2.26	(2,04)
4	1.21	1.34	1.23	1.34	(1,36)
5	1.69	2.03	1.49	1.44	(1,83)
6	1.42	1.53	1.48	2.03	(1,59)
Mean value	1.81	1.74	1.7	1.76	

Phase 1: control before chemotherapy, phase 2: before chemotherapy and after the in vitro addition of aminophylline, phase 3: 20 h after chemotherapy and phase 4: 20 h after chemotherapy and 20 min after in vivo administration of aminophylline.
Numbers in parenthesis are the expected values if the differences in PRIs for chemotherapy and AM were independent and additive.