Modulation of apoptosis by cigarette smoke and cancer chemopreventive agents in the respiratory tract of rats

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Preclinical studies may elucidate the meaning of biomarkers applicable to epidemiologic studies and to clinical trials for cancer prevention. No study has explored so far the effect of cigarette smoke on apoptosis in vivo. We evaluated modulation of apoptosis in cells of the respiratory tract of smoke-exposed Sprague-Dawley rats both by morphological analysis and TUNEL method. In a first study, exposure of rats to mainstream cigarette smoke for either 18 or 100 consecutive days produced a significant and time-dependent increase in the proportion of apoptotic cells in the bronchial and bronchiolar epithelium. Oral N-acetylcysteine did not affect the background frequency of apoptosis but significantly and sharply decreased smoke-induced apoptosis. In a second study, exposure of rats to a mixture of sidestream and mainstream smoke for 28 consecutive days resulted in a >10-fold increase in the frequency of pulmonary alveolar macrophages undergoing apoptosis. Dietary administration of either 5,6-benzoflavone, 1,2-dithiole-3-thione or oltipraz did not affect the frequency of smoke-induced apoptosis, whereas phenethyl isothiocyanate produced a further significant enhancement. Again, N-acetylcysteine and its combination with oltipraz significantly decreased smoke-induced apoptosis. In both studies exposure to smoke resulted in a sharp increase of cells positive for proliferating cell nuclear antigen (PCNA), which was unaffected by the examined chemopreventive agents. These findings highlight the concept that modulation of apoptosis has diversified meanings. Different meanings (as explained in the following lines). First, the apoptotic process is triggered as a defense system against genotoxic agents, such as the components of cigarette smoke. The further induction produced by phenethyl isothiocyanate, favoring removal of damaged cells, represents an example of a detoxification mechanism. Inhibition of smoke-induced apoptosis by N-acetylcysteine should be interpreted as an epiphenomenon of antigenotoxic mechanisms, as shown in parallel studies evaluating modulation of DNA alterations in the respiratory tract of the same animals. Thus, it is important to discriminate between whether the opposite modulation of apoptosis is per se a protective mechanism or the beneficial outcome of other mechanisms inhibiting genotoxicity.

Introduction

One of the major goals of preclinical chemoprevention studies is to validate biomarkers which can be applied to phase II clinical trials, and to provide mechanistic insights with regard to the investigated endpoints (1).

Within the variety of protective mechanisms which can be exploited for preventative purposes (2,3), apoptosis represents a physiological process by which cells are removed when their DNA is damaged by noxious agents. At the same time, this process can be stimulated by certain chemopreventive agents, including for instance retinoids, sulindac sulfone, tamoxifen, butyric acid, genistein and apigenin (2,4). These agents stimulate the programmed death of cells prior to their further evolution towards cancer or other mutation-related chronic degenerative diseases (5).

However, from a conceptual point of view, the meaning attributable to modulation of this biomarker is rather ambiguous, and interpretation of the effects of chemopreventive agents can be double-edged for several reasons. First of all, since apoptosis is stimulated both by genotoxic agents and by certain chemopreventive agents, one cannot rule out the doubt that upregulation by a putative chemopreventive agent is not due to some adverse effect. In addition, in the case when a chemopreventive agent inhibits some mechanisms preceding apoptosis, the result may be an apparent down-regulation of this biomarker. This is not a mechanism per se but rather the consequence of other protective mechanisms intervening upstream in the chain of events which ultimately trigger apoptosis (3). Thus, inhibition of DNA damage may simultaneously decrease apoptosis. Still, there is the possibility that the proportion of apoptotic cells may appear to be unchanged after treatment with a chemopreventive agent because a potential stimulation of this process is counter-balanced by inhibition of genotoxic damage.

With the goal of giving further experimental support to the above concepts, we assessed by two different methods the frequency of apoptosis in the bronchial and bronchiolar epithelium and in the pulmonary alveolar macrophages (PAM) of rats exposed to cigarette smoke (CS), either mainstream or environmental (ECS), in the framework of two separate studies which also evaluated a variety of other biomarkers. In parallel, due to the close interconnection of the molecular pathways leading to cell apoptosis and proliferation (6), we determined the positivity for proliferating cell nuclear antigen (PCNA) in the same cells and, in one study, also in the lingual epithelium. Overall, five chemopreventive agents were administered orally, including 1,2-dithiole-3-thione (1,2-D3T) and its substituted analogue 5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-

Abbreviations: 1,2-D3T, 1,2-dithiole-3-thione; 5,6-BF, 5,6-benzoflavone; BAL, bronchoalveolar lavage; CS, cigarette smoke; ECS, environmental cigarette smoke; NAC, N-acetylcysteine; OPZ, oltipraz or 5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione; PAM, pulmonary alveolar macrophages; PCNA, proliferating cell nuclear antigen; PEITC, phenethyl isothiocyanate; TUNEL, TdT-mediated dUTP nick end labeling.

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Materials and methods

the idea that certain agents endowed with chemopreventive effects on apoptosis. Morphological analysis and TUNEL method was also tested. The findings of the present study, in conjunction with the results obtained by evaluating other end-points in the same animals, show the powerful induction of apoptosis in CS and strengthen the idea that certain agents endowed with chemopreventive properties have diversified effects on apoptosis.

Thione or oltipraz (OPZ), phenethyl isothiocyanate (PEITC), 5,6-benzoflavone (5,6-BF) and N-acetyl-L-cysteine (NAC). A combined treatment with NAC and OPZ was also tested. The statistical analysis: significantly different from either sham-exposed rats \((P < 0.01, {P}^{<} 0.001)\) or ECS-exposed rats \((P < 0.05, {P}^{<} 0.01, {P}^{<} 0.001)\).

Table I. Frequency of apoptotic cells and of proliferating cells (PCNA-positive) in the respiratory tract of rats, as related to exposure to mainstream cigarette smoke (CS) and/or treatment with NAC.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Apoptotic cells in the bronchial/bronchiolar epithelium (%) (means ± SD)</th>
<th>PCNA-positive cells (%) (means ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morphological analysis</td>
<td>TUNEL method</td>
</tr>
<tr>
<td>Sham-exposed</td>
<td>1.8 ± 0.8</td>
<td>4.4 ± 1.7</td>
</tr>
<tr>
<td>NAC</td>
<td>1.6 ± 0.6</td>
<td>4.8 ± 1.9</td>
</tr>
<tr>
<td>CS-exposed (18 days)</td>
<td>3.2 ± 1.1b</td>
<td>8.6 ± 1.1b</td>
</tr>
<tr>
<td>CS-exposed (18 days) + NAC</td>
<td>2.6 ± 1.1</td>
<td>5.4 ± 2.3d</td>
</tr>
<tr>
<td>CS-exposed (100 days)</td>
<td>17.2 ± 5.9c</td>
<td>36.4 ± 9.2c</td>
</tr>
<tr>
<td>CS-exposed (100 days) + NAC</td>
<td>9.8 ± 1.9d</td>
<td>18.2 ± 4.3c</td>
</tr>
</tbody>
</table>

NT, Not tested. Statistical analysis: significant increase as compared with sham-exposed rats \((P < 0.05, {P}^{<} 0.01, {P}^{<} 0.001)\), or significant decrease by NAC as compared with CS-exposed rats \((P < 0.05, {P}^{<} 0.01)\).

Table II. Induction of apoptosis and proliferation (PCNA-positive cells) in PAM of rats as related to exposure to environmental cigarette smoke (ECS) and treatment with chemopreventive agents.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Apoptotic cells (%) (means ± SD)</th>
<th>PCNA-positive cells (%) (means ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morphological analysis</td>
<td>TUNEL method</td>
</tr>
<tr>
<td>Sham-exposed</td>
<td>0.5 ± 0.8</td>
<td>1.2 ± 1.2</td>
</tr>
<tr>
<td>ECS-exposed</td>
<td>6.5 ± 1.4b</td>
<td>12.9 ± 5.0f</td>
</tr>
<tr>
<td>ECS + 1.2-D3T</td>
<td>7.0 ± 1.9b</td>
<td>14.2 ± 6.6b</td>
</tr>
<tr>
<td>ECS + OPZ</td>
<td>6.8 ± 1.9b</td>
<td>12.8 ± 4.0f</td>
</tr>
<tr>
<td>ECS + NAC</td>
<td>4.0 ± 1.7ab</td>
<td>8.8 ± 3.5c</td>
</tr>
<tr>
<td>ECS + NAC + OPZ</td>
<td>3.8 ± 1.9cd</td>
<td>7.2 ± 5.2c</td>
</tr>
<tr>
<td>ECS + PEITC</td>
<td>9.1 ± 3.1bc</td>
<td>19.9 ± 4.9d</td>
</tr>
<tr>
<td>ECS + 5.6-BF</td>
<td>6.8 ± 2.3b</td>
<td>15.8 ± 5.2b</td>
</tr>
</tbody>
</table>

Statistical analysis: significantly different from sham-exposed rats \((P < 0.01, {P}^{<} 0.001)\) or ECS-exposed rats \((P < 0.05, {P}^{<} 0.01, {P}^{<} 0.001)\).

Materials and methods

Animals

Adult male Sprague–Dawley rats were used in both studies. In the first study (study A), 36 rats, aged 6 weeks and weighing 240–260 g at the start of the experiment, were purchased from Morini (S.Polo d’Enza, Italy) and maintained on MII standard rodent chow (Morini). In the second study (study B), 64 rats, aged 8 weeks and weighing 290–310 g at the start of the experiment, were purchased from Harlan Italy (Correzzana, Milan) and maintained on Teklad IRM Rat/Mouse diet (Harlan, Italy). All animals were given drinking water *ad libitum*.

The rats were housed in a climatized environment at a temperature of 22 ± 1°C, relative humidity of 50 ± 2%, ventilation accounting for 15 air renewal cycles/h, and with a 12 light-dark cycle. Prior to treatment, rats were acclimatized for 7–14 days. Animal care and treatments were in accordance with Italian and institutional guidelines.

Exposure to cigarette smoke

Groups of rats, each composed either of 6 (study A) or 8 (study B) animals, were exposed whole-body to CS. The general outline of the two studies is reported in Tables I and II, respectively.

In study A, mainstream CS was obtained, as previously described (7), by using commercially available filter-tipped cigarettes, having a declared content of 14 mg tar and 0.9 mg nicotine. Briefly, each one of the groups of rats undergoing this treatment was placed in a 20 l sealed glass chamber, that was subsequently filled by means of a 50 ml syringe with the mainstream CS generated by one cigarette. After 10 min the chamber was opened and, once the air was completely renewed (1–2 min), it was filled again with fresh CS a further eight times, accounting for exposure to the smoke generated by 9 cigarettes/day for either 18 consecutive days (total of 162 cigarettes/group) or 100 consecutive days (900 cigarettes/group). Under these conditions, the total suspended particulate (TSP) in the exposure chamber, where the animals were exposed each day for 90 min, was on an average 700 mg/m³.

In study B, a mixture of sidestream smoke (50%) and mainstream smoke (11%), mimicking an exposure to ECS, was produced by using a smoking machine (model TE-10, Pfeuge Enterprises, Davis, CA), where each smoldering cigarette was puffed for 2 s, once every min for a total of 8 puffs, at a flow rate of 10.5 l/min to produce a standard puff of 35 cm³ (8). Kentucky 2R1 reference cigarettes (Tobacco Research Institute, University of Kentucky, Lexington, KY), having a declared content of 44.6 mg tar and 2.45 mg nicotine each, were used. Before use, the cigarettes were kept for 48 h in a standardized atmosphere humidified with a mixture of 70% glycerol and 30% water. The smoking machine was set to burn five cigarettes at one time, 6 h a day divided into two 3 h rounds with a 3 h interval. TSP in the four exposure chambers, where the position of cages was rotated daily, was on an average 83 mg/m³. Exposure to smoke lasted for 28 consecutive days, during which 3360 cigarettes were burned.

In both studies sham-exposed rats were kept in filtered air for corresponding periods of time.

Treatment with chemopreventive agents

In study A, NAC was given to groups of rats, either sham-exposed or exposed to mainstream CS for 18 or 100 days. NAC was given with drinking water in the form of a water soluble commercial preparation containing 200 mg of the drug (Flunucil, Zambon, Vicenza, Italy), at a concentration accounting for a calculated daily intake of 2 g/kg body weight. The daily administration of NAC started 2 days before the first exposure to CS, and lasted until the end of the experiment (100 days in sham-exposed rats and either 18 or 100 days in CS-exposed rats).

In study B, the chemopreventive agents were only given to ECS-exposed rats, starting 3 days before the first exposure to CS, and continuing until the end of exposure to ECS (28 days). ECS, either alone or in combination with OPZ, was given as in study A, except that the calculated daily intake was 1 g/kg body weight. The other agents, including 5.6-BF (Sigma Chemical, St Louis, MO) and OPZ, 1,2-D3T and PEITC (Division of Cancer Prevention Repository, National Cancer Institute, Rockville, MD), were incorporated in Teklad Diet (Harlan Teklad, Madison, WI) at concentrations of either 400 (OPZ and 1,2-D3T) or 500 mg/kg diet (PEITC and 5.6-BF). The diets were kindly supplied by Dr C.J. Grubbs from the University of Alabama at Birmingham.

Collection of biological samples

At the time of the rats were starved for 24 h, anesthetized with ethyl ether and killed by cervical dislocation. In both studies bronchoalveolar lavage (BAL) was performed by lavaging the lungs of each rat with two 10 ml aliquots of cold (4°C) 0.15 M NaCl solution infused via a cannula inserted into the trachea. BAL cells were washed twice with phosphate buffered saline (pH 7.4) and then spun onto slides in a cytocentrifuge and fixed with absolute methanol for the assessment of PCNA and apoptosis. In study A samples of tongue and lung were also collected, fixed in buffered formaline and embedded
in paraffin. For the assessment of PCNA and apoptosis in the bronchial/bronchiolar and lingual epithelium, 5 μm sections were cut and placed onto slides treated with poly-l-lysine (Poly-Prep™ Slides, Sigma Diagnostics, St Louis, MO).

Detection of apoptosis

Apoptotic cells were identified both by morphological analysis and by TdT-mediated dUTP nick end labeling (TUNEL) method. For morphological analysis, the slides were stained according to Feulgen reaction. Briefly, the slides were treated to mild acid hydrolysis for 1 h in 5 N HCl. After several rinses in distilled water, the slides were immersed in filtered Schiff’s reagent (Sigma Chemical, St Louis, MO) for 30 min, washed in distilled water and left in running tap water for 5 min in order to intensify the staining of the Schiff’s reagent. Finally, the slides were blotted dry and mounted in Eukitt (BDH Chemicals, Poole, UK). Apoptotic cells have condensed, darkly stained nuclei, which often are smaller in size and occasionally may be fragmented.

For the TUNEL method we used the In Situ Cell Death Detection kit (Boehringer Mannheim GmbH, Mannheim, Germany), following the manufacturer’s instructions. TUNEL quantifies apoptotic cell death at the single cell level and preferentially labels apoptosis, thereby discriminating it from necrosis and from primary DNA strand breaks (9).

With both methods the slides were scored at a magnification of ×400, and 1000 cells per rat were examined. The results are expressed as percent of apoptotic cells.

PCNA immunohistochemistry

PCNA immunoreactivity was detected by using the NCL-PCNA kit (Novocastra Laboratories, Newcastle upon Tyne, UK), following the manufacturer’s instructions. This detection kit is based on an anti-PCNA monoclonal antibody (clone PC10) and employs avidin/biotinylated horseradish peroxidase complex technology (ABC technique). Slides were scored at a magnification of ×400, and 1000 cells per rat were examined. The results are expressed as percent of PCNA-positive cells.

Statistical analysis

The data were expressed as means ± SD of the results obtained within each group of either six (study A) or eight rats (study B). Inhibition of a biomarker by chemopreventive agents was calculated as [100 – (C – A/B – A)×100], where A, B and C are the mean values recorded in sham-exposed rats, smoke-exposed rats without chemopreventive agents and smoke-exposed rats with each chemopreventive agent, respectively. The effects of exposure to mainstream CS (study A) or ECS (study B) and treatments with chemopreventive agents, regarding body weight gain, percentage of apoptotic cells, and percentage of PCNA-positive cells, were evaluated by Student’s t-test for unpaired data.

Results

Cell apoptosis and proliferation in the respiratory tract of rats exposed to mainstream cigarette smoke and/or treated with oral NAC

The overall body weight gain during the 100 days of this study was +96.2% in sham-exposed rats, +72.8% in NAC-treated rats, +54.2% in CS-exposed rats and +62.6% in CS-exposed rats treated with NAC. Irrespective of treatment with NAC, exposure to CS resulted in a significant decrease of body weight gain as compared with sham-exposed rats (P < 0.01).

The results of this study are summarized in Table I. Exposure to mainstream CS resulted in a significant and time-related stimulation of apoptosis in the bronchial/bronchiolar epithelium. Irrespective of the method used, apoptotic cells approximately doubled after 18 days and increased almost 10-fold after 100 days of treatment. Figure 1A shows an example of the appearance of apoptotic cells, as detected by TUNEL, in the bronchiolar epithelium of a CS-exposed rat. The daily administration of NAC with drinking water did not affect the background frequency of apoptosis, but significantly decreased apoptosis in smoke-exposed rats. The frequency of apoptosis was approximately half that of smoke-exposed rats in the absence of a chemopreventive agent. In fact, according to the formula indicated in Materials and methods, which takes into account the ‘spontaneous’ background level of apoptosis, inhibition by NAC was 42.9% (morphological analysis) and 76.2% (TUNEL method) after 18 days, and 48.1% (morphological analysis) and 56.9% (TUNEL method) after 100 days of exposure to CS.

The background frequency of PCNA-positive cells was very high in the lingual epithelium and was not affected by exposure to CS for 100 days, irrespective of NAC administration. In contrast, exposure to smoke produced a marked increase of PCNA-positive cells both in the bronchial/bronchiolar epithelium and in PAM. Stimulation of cell proliferation was already at plateau after 18 days of exposure to smoke, and was not further enhanced after 100 days. NAC administration failed to affect this parameter.

Apoptosis of pulmonary alveolar macrophages of rats exposed to environmental cigarette smoke and treated with chemopreventive agents

The overall body weight gain during the 28 days of this study was +23.4% in sham-exposed rats and +12.8% in ECS-exposed rats treated with NAC.
exposed rats ($P < 0.01$). The body weight gain was not further changed in ECS-exposed rats receiving OPZ (+11.7%), NAC (+7.7%), NAC plus OPZ (+9.3%), PEITC (+7.3%) and 5,6-BF (+10.0%), whereas no body weight gain at all was observed in ECS-exposed rats receiving 1,2-D3T ($P < 0.001$ as compared with both sham-exposed rats and ECS-exposed rats).

Figure 1B shows the distinctive appearance of two PAM processes with the TUNEL, one of which undergoing apoptosis (brown-stained nucleus) and the other one non-apoptotic (blue-stained nucleus). As summarized in Table II, exposure to ECS determined a >10-fold increase in the proportion of PAM undergoing apoptosis, as shown by morphological analysis (13.0-fold) and TUNEL (10.8-fold). The dietary administration of either 1,2-D3T, OPZ or 5,6-BF did not affect ECS-induced apoptosis. In contrast, PEITC produced a further significant increase of apoptotic activity, whereas treatment with NAC significantly inhibited this process. Even more effective was the NAC/OPZ combination, in spite of the fact that OPZ alone totally failed to modulate apoptosis.

In parallel, exposure to ECS resulted in a significant increase (3.9-fold) in the proportion of PCNA-positive PAM. Treatment with chemopreventive agents did not further affect induction of PCNA by ECS (Table II).

Discussion

The main goal of the present study was not to explore the intricate mechanisms regulating the apoptotic process but rather to evaluate the practical meaning of this biomarker, since apoptosis is often investigated in molecular epidemiology studies and in cancer chemoprevention clinical trials.

A variety of diseases, such as AIDS, neurodegenerative disorders, myelodysplastic syndromes, ischemic injury and toxin-induced liver disease, have been associated with excess apoptosis (10). In contrast, certain autoimmune disorders, viral diseases other than AIDS, and cancer have been associated with inhibition of apoptosis, since cell accumulation can result from either increased proliferation and/or failure of cells to undergo apoptosis (10).

The preclinical studies described in this paper evaluated apoptosis in the respiratory tract of rats, both in target cells, such as cells of the bronchial and bronchiolar epithelium, and in surrogate cells such as PAM. PAM have extensively been used as sentinel cells suitable for the evaluation of apoptosis induced by a variety of inhalable toxic agents (11). PAM are sweeping cells equipped with the metabolic machinery to activate and detoxify phagocytosed carcinogens (12). They also represent ideal surrogate cells in molecular epidemiology studies and cancer chemoprevention clinical trials, because these cells can be recovered by bronchoalveolar lavage, which is a semi-invasive technique, and, at variance with blood cells, PAM are selectively affected by inhalable agents (12).

The proportion of cells undergoing apoptosis in the respiratory tract of both sham-exposed and smoke-exposed rats was higher when assessed by TUNEL than by morphological analysis. This finding could be ascribed either to the fact that this method often provides labeling of morphologically normal nuclei (9) and/or to the possibility that the TUNEL method gives some false positive results (9,13).

The present study supports the view that interpretation of apoptosis modulation is not simple and straightforward, unless the information is completed with the parallel assessment of other biomarkers. In spite of the importance of this type of exposure, very few data are available in the literature on modulation of apoptosis following exposure to CS. For instance, apoptosis was induced in cultured human monocytes exposed to CS (14), gastric mucosa cells of CS-exposed rodents (15), and lung tissues of newborn rats exposed to sidestream smoke during pregnancy (16). The striking induction of apoptosis that we detected in the bronchial and bronchiolar epithelium of rats exposed to mainstream CS and in PAM of rats exposed to ECS is likely to represent a defense mechanism of the respiratory tract against toxic and genotoxic components of cigarette smoke. In any case, this mechanism was counterbalanced by a sharp increase in cell proliferation, as assessed by evaluating the proportion of PCNA-positive cells in the bronchial epithelium and PAM. Conversely, exposure to CS did not further increase the proportion of PCNA-positive cells in the lingual epithelium, which was remarkably high even in sham-exposed rats, presumably because this epithelium is one of the body tissues with the highest proliferation rate (17,18).

It has been shown that exposure to mainstream CS in rats resulted in the formation of $^{32}$P postlabeled DNA adducts in the tracheal epithelium (19), and exposure to ECS resulted in the alteration of multiple biomarkers in the respiratory tract, including formation of DNA adducts in the lung, tracheal epithelium and bronchoalveolar lavage cells, oxidative DNA damage in lung cells and cytogenetic damage in PAM (20).

The investigated chemopreventive agents, none of which apparently affected the evaluated proliferation marker, had differential effects on smoke-induced cell apoptosis. In fact, NAC significantly inhibited apoptosis both in the bronchial/bronchiolar epithelium of rats exposed to mainstream CS and in PAM of ECS-exposed rats. The latter effect was even more evident when NAC was combined with OPZ, although the difference between the NAC and NAC + OPZ groups was not statistically significant. This trend is in agreement with the additive or more than additive effects produced by combination of these two drugs on other biomarkers in the respiratory tract of ECS-exposed rats (20). In contrast, PEITC further enhanced the frequency of apoptotic PAM in ECS-exposed rats, and 1,2-D3T, OPZ and 5,6-BF were apparently without effect in this experimental system. All tested agents, with the exception of OPZ alone, inhibited the smoke-related induction of genotoxic effects in the respiratory tract of the same animals (19,20). Thus, it appears that modulation of apoptosis can be variously oriented depending on the mechanisms involved.

Upregulation of apoptosis by PEITC provides an example of the protective mechanism by which certain chemopreventive agents affect the survival of damaged cells (2–4), based on the hypothesis that the apoptotic process preferentially eliminates preneoplastic cells (21). PEITC has recently been shown to induce apoptosis in cultured mammalian cells (22–24). Mechanistic studies have shown that apoptosis induction by PEITC occurs through a p53-dependent pathway (22), and is independent or upstream the activation of caspase/interleukin-1 $β$-converting enzyme proteases, mediated by c-Jun N-terminal kinase activation (23). A further study, however, showed that certain concentrations of PEITC can also activate the caspase pathway (24). Although we did not test PEITC per se, in the absence of CS, it is thus likely that CS and PEITC may have both contributed to enhance the induction of apoptosis in rat PAM.
Downregulation of smoke-related apoptosis by NAC is likely to depend on the nucleophilic and antioxidant properties of this thiol (25), which can attenuate induction of genotoxic damage and ultimately of apoptosis by smoke components. Therefore, NAC provides an example of a chemopreventive agent which inhibits cell apoptosis because it works upstream in the chain of events signaling this process (3). To our knowledge, no previous study has addressed the issue of modulation of smoke-related apoptosis by NAC in vivo. In cultured human monocytes, NAC has been found to prevent CS-induced mitochondrial depolarization (deltapsim disruption) and apoptosis (26). It is noteworthy that NAC had even been found to inhibit PEITC-induced apoptosis, suggesting that the death signaling by PEITC is triggered by oxidative stress (23). More than 300 references on modulation of apoptosis by NAC, most of which deal with in vitro test systems, were available from MEDLINE (November 2000). As many as 106 studies, 103 in vitro and 3 in vivo, cover the effect of NAC on apoptosis induced by oxidative mechanisms, which play a key role in CS carcinogenesis and other CS-related pathological conditions. Of these 106, 94 (88.6%) showed the ability of NAC to inhibit apoptosis, 10 (9.4%) showed no effect and only two (1.8%) showed an enhancement of apoptosis by NAC. Interestingly, in one of these two studies NAC induced apoptosis in several transformed cell lines and transformed primary cultures but not in normal cells (27), which clearly highlights a protective effect of NAC in cancer cells. The three in vivo studies demonstrated the ability of NAC to inhibit apoptosis mediated by oxidative mechanisms under situations which may play a role in different pathological conditions. In fact, NAC downregulated the apoptotic process induced by contralateral tectal lesion in the eye retinal ganglion of chicken embryos (28), by experimental diabetes in pancreatic β-cells of C57BL/KJ-db/db mice (29), and by balloon-cather injury in carotid artery of rabbits (30).

The protective effect of NAC towards smoke-induced apoptosis in PAM and in the bronchial/bronchiolar epithelium may be relevant not only as a defense mechanism in smoke-related lung carcinogenesis, but also in the pulmonary inflammatory response, as well as in other pulmonary diseases. In fact, as shown by studies in PAM of mice exposed to bleomycin, the apoptotic process detected in these cells may contribute to the inflammatory burden present in the injured lung (11). Moreover, clinical studies provided evidence that apoptosis of bronchiolar and alveolar epithelial cells is associated with fibrosing lung disease (31), and in particular apoptosis of type II pneumocytes occurs in diffuse alveolar damage (32).

Failure of 1.2-D3T, OPZ, and 5,6-BF to affect ECS-induced apoptosis in PAM may be interpreted as a lack of influence of these agents on the investigated end-point. However, one cannot rule out that a potential stimulation of apoptosis may be masked by the simultaneous inhibition of genotoxic damage by certain agents.

As a general assumption, it is difficult to discriminate whether modulation of a given end-point is actually a specific mechanism or rather the epiphenomenon of other mechanisms. In cancer therapy, activation of apoptosis is a beneficial property shared by a number of chemotherapeutic drugs (10). The situation is different in the case of normal cells exposed to carcinogens, which can be protected by chemopreventive agents through a network of interconnected mechanisms. In conclusion, modulation of apoptosis should be interpreted with caution in both animal models and human studies since cancer chemopreventive agents can apparently have contrasting effects on regulation of this biomarker.

Acknowledgements

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References


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