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Modulation of apoptosis by cancer chemopreventive agents

Francesco D'Agostini [a,*], Alberto Izzotti [a], Roumen M. Balansky [a,b], Carlo Bennicelli [a], Silvio De Flora [a]

[a] *Department of Health Sciences, University of Genoa, via A. Pastore 1, I-16132 Genoa, Italy*
[b] *National Centre of Oncology, Sofia 1756, Bulgaria*

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Abstract

A review of almost 2000 studies showed that the large majority of 39 putative cancer chemopreventive agents induced "spontaneous" apoptosis. Inhibition of the programmed cell death triggered by a variety of stimuli was consistently reported only with ascorbic acid, α-tocopherol, and *N*-acetylcysteine (NAC). We performed experimental studies in rodents exposed to cigarette smoke, either mainstream (MCS) or environmental (ECS), and UV-A/B-containing light. The nonsteroidal anti-inflammatory drug sulindac did not affect the apoptotic process in the skin of light-exposed mice and in the lungs of ECS-exposed mice. Likewise, 5,6-benzoflavone, indole-3-carbinol, 1,2-dithiole-3-thione and oltipraz failed to modulate apoptosis in the respiratory tract of ECS-exposed rats. Phenethyl isothiocyanate further enhanced the frequency of apoptosis in pulmonary alveolar macrophages and bronchial epithelial cells, and upregulated several genes in the lung of ECS-exposed rats. Both individually and in combination with oltipraz, NAC inhibited apoptosis in the respiratory tract of rats exposed either to MCS or ECS. Moreover, NAC attenuated the ECS-related overexpression of proapoptotic genes and normalized the levels of proapoptotic proteins in rat lung. The transplacental administration of NAC to mice considerably attenuated gene overexpression in the liver of fetuses exposed to ECS throughout pregnancy. Inhibition of apoptosis by chemopreventive agents reflects their ability to counteract certain upstream signals, such as genotoxic damage, redox imbalances, and other forms of cellular stress that trigger apoptosis. On the other hand, enhancement of apoptosis is a double-edged sword, since it represents a protective mechanism in carcinogenesis but may contribute to the pathogenesis of other degenerative diseases. We suggest that stimulation of apoptosis by so many chemopreventive agents, as reported in the literature, may often reflect the occurrence of toxic effects at high doses.

Keywords: Apoptosis; Cancer chemopreventive agents; *N*-Acetylcysteine; Phenethyl isothiocyanate

Abbreviations: 5,6-BF, 5,6-benzoflavone; BITC, benzyl isothiocyanate; DFMO, difluoromethylornithine; 1,2-D3T, 1,2-dithiole-3-thione; ECS, environmental cigarette smoke; EGCG, epigallocatechin gallate; GSH, reduced glutathione; 4-HPR, *N*-(4-hydroxyphenyl) retinamide; I3C, indole-3-carbinol; MCS, mainstream cigarette smoke; NAC, *N*-acetyl-L-cysteine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; OPZ, oltipraz or 5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione; PAM, pulmonary alveolar macrophages; PCA, principal component analysis; PEITC, phenethyl isothiocyanate; SUL, sulindac; TUNEL, TdT-mediated dUTP nick end labelling

* Corresponding author. Tel.: +39 010 353 8487; fax: +39 010 353 8504.
E-mail address: fda@unige.it (F. D'Agostini).

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1. Introduction

Apoptosis is the most common and well-defined form of programmed cell death. It is a physiological cell suicide that is essential for the maintenance of homeostasis in embryonic, fetal and adult tissues [1,2]. The apoptotic process, which is characterized by cell shrinkage, chromatin condensation, internucleosomal DNA fragmentation and formation of apoptotic bodies, is triggered by two major pathways. The extrinsic pathway is induced by activation of death receptors on the cell surface, whereas the intrinsic pathway involves an increase of mitochondrial permeability and cytochrome *c* release [2,3]. Several protease families are implicated in apoptosis, the most prominent being caspases, a ubiquitous family of cysteine proteases, including both upstream initiators and downstream effectors [4]. This cascade leads to proteolytic cleavage of a variety of cytoplasmic and nuclear proteins, thereby favoring the prevalence of proapoptotic activities on antiapoptotic activities.

Both induction and inhibition of apoptosis can be related to either physiological stimuli, exposure to stress-inducing agents, or pathological conditions. Among pharmacological agents, many cancer chemotherapeutic drugs are known to activate apoptotic mechanisms of tumor cell death, suggesting this may be a mechanism for current cancer treatment [3,5]. Moreover, apoptosis has been proposed as a novel target for cancer chemoprevention [6], whose rationale is to remove cells undergoing neoplastic transformation, in situations where other defense mechanisms fail to block the carcinogenesis process upstream.

A huge literature is available on the modulation of apoptosis by putative cancer chemopreventive agents, either dietary principles, vitamins, or pharmacological agents. In order to have a comprehensive view of the available data, we made an ad hoc literature survey regarding 39 selected chemopreventive agents belonging to a variety of chemical or functional families. Table 1 summarizes the results of almost 2000 studies cited in MEDLINE from 1 January 1989 to 31 October 2004, using the name of the chemopreventive agent and the term "apoptosis" as key words. This survey excluded review articles and only included original research articles in which the abstract clearly reported the effect of the chemopreventive agent on apoptosis. Most studies used in vitro cellular systems, mainly employing cultured tumor cells. A minority of studies used animal models, and few of them involved trials in humans. The results reported in Table 1 are divided into three categories depending on the effect of the listed chemopreventive agents on apoptosis, including: (a) induction of "spontaneous" apoptosis by the chemopreventive agent, in the absence of any apoptosis inducer; (b) inhibition by the chemopreventive agent of apoptosis induced by either biological, physical or chemical agents; and (c) none of the above two situations. Other possible effects, such as a further increase by the chemopreventive agent of apoptosis induced by various agents, will be commented below. Due to the large number and complexity of the available data, Table 1 does not specify the test system and the inducer of apoptosis used.

As a general comment, it can be observed in Table 1 that the large majority of the reviewed chemopreventive agents induced "spontaneous" apoptosis, in the absence of other stimuli in most studies. This finding is impressive, and it is rather surprising that so many chemopreventive agents, belonging to different families and having distinctive mechanisms of action, share this property. We suspect that in many in vitro studies, induction of apoptosis may depend on toxicity of the compounds tested at high doses. A genuine protective mechanism should be supported by a selective induction of apoptosis in malignant or premalignant cells [6]. In only a limited number of studies was it shown that certain chemopreventive agents selective induce apoptosis in cancer cells but not in the corresponding noncancer cells. For instance, indole-3-carbinole induced apoptosis in tumorigenic but not in nontumorigenic breast epithelial cells [7]; 4-HPR stimulated apoptosis in malignant human leukaemia cells but not in normal lymphocytes [8]; ascorbic acid increased arsenite-induced apoptosis in NB4 malignant cells but not in normal cells [9]; in nude mice carrying colon cancer xenografts, Vitamin E succinate induced apoptosis in cancer cells but not in normal cells [10]; 1-selenomethionine selectively induced apoptosis in cancer but not in primary cells of the human prostate [11]; NAC enhanced the frequency of apoptotic cells in several transformed cell lines and transformed primary cultures but not in normal cells [12]; EGCG induced apoptosis in a variety of tumor cell lines but was less effective in normal cells [13].