Topical applications of caffeine or (−)-epigallocatechin gallate (EGCG) inhibit carcinogenesis and selectively increase apoptosis in UVB-induced skin tumors in mice

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SKH-1 hairless mice were irradiated with ultraviolet B (UVB) twice weekly for 20 weeks. These tumor-free mice, which had a high risk of developing skin tumors during the next several months, were then treated topically with caffeine (6.2 μmol) or (−)-epigallocatechin gallate (EGCG; 6.5 μmol) once a day 5 days a week for 18 weeks in the absence of further treatment with UVB. Topical applications of caffeine to these mice decreased the number of nonmalignant and malignant skin tumors per mouse by 44% and 72%, respectively. Topical applications of EGCG decreased the number of nonmalignant and malignant tumors per mouse by 55% and 66%, respectively. Immunohistochemical analysis showed that topical applications of caffeine or EGCG increased apoptosis as measured by the number of caspase 3-positive cells in nonmalignant skin tumors by 87% or 72%, respectively, and in squamous cell carcinomas by 92% or 56%, respectively, but there was no effect on apoptosis in nontumor areas of the epidermis. Topical applications of caffeine or EGCG had a small inhibitory effect on proliferation in nonmalignant tumors as measured by BrdUrd labeling (16–22%), and there was also a similar, but nonsignificant, inhibitory effect on proliferation in malignant tumors. The results suggest a need for further studies to determine whether topical applications of caffeine or EGCG can inhibit sunlight-induced skin cancer in humans.

tea constituents | programmed cell death | caspase 3

Skin cancer is a major cancer in the United States, and its incidence is expected to increase substantially because of increased recreational exposure to sunlight and depletion of the ozone layer (1, 2). The identification and use of protective agents should have an important impact on the formation of these cancers. Although the use of sunscreens is an approach that has decreased the risk of skin cancers (3, 4), there is also a need to identify additional approaches for skin cancer prevention in individuals previously exposed to high-dose levels of sunlight (high-risk individuals). Treatment of SKH-1 hairless mice with ultraviolet B (UVB) (30 mJ/cm^2) twice a week for 20 weeks resulted in mice without tumors but with epidermal hyperplasia and a high risk of developing skin tumors during the next several months in the absence of further UVB treatment (initiated high-risk mice) (5). This animal model resembles humans who are heavily exposed to sunlight early in life and then have reduced exposure later in life. We have used UVB-pretreated high-risk mice for evaluating the effects of potential chemopreventive agents on skin tumor formation in the absence of further exposure to UVB. Oral administration of green tea, black tea, or caffeine to UVB-pretreated high-risk mice inhibited tumorigenesis, but the decaffeinated teas had little or no activity, and reconstitution of the decaffeinated teas with caffeine restored biological activity (5). These observations indicate that caffeine is a major cancer chemopreventive constituent in tea. Potential antitumor mechanisms include inhibition of tumor growth and increased apoptosis. In accord with this concept, oral administration of black tea to UVB-pretreated mice with tumors inhibited the growth of these tumors by inhibiting proliferation and stimulating apoptosis in the tumors (6). In the present study, we evaluated the potential inhibitory effects of topical applications of the tea constituents caffeine and (−)-epigallocatechin gallate (EGCG) on tumorigenesis in UVB-pretreated high-risk mice, and we have also evaluated the effects of these treatments on apoptosis and proliferation in the tumors and nontumor areas of the skin as measured by the percent of caspase 3 (active form)-positive cells and BrdUrd labeling.

Materials and Methods

Chemicals and Animals. Acetone (HPLC grade) and 10% phosphate-buffered formalin were obtained from Fisher Scientific, caffeine (>99% pure) was obtained from Sigma, and EGCG (>98% pure) was obtained from Thomas J. Lipton (Englewood Cliffs, NJ).

Female SKH-1 hairless mice (6–7 weeks old) were purchased from the Charles River Breeding Laboratories, and the animals were kept in our animal facility for at least 1 week before use. Mice were given water and Purina Laboratory Chow 5001 diet from Ralston-Purina (St. Louis) ad libitum, and they were kept on a 12-h light/12-h dark cycle.

Treatment of Mice with UVB. The UV lamps used (FS72T12-UVB-HO; National Biological, Twinsburg, OH) emitted UVB (280–320 nm; 75–80% of total energy) and UVA (320–375 nm; 20–25% of total energy). There was little or no radiation below 280 nm or above 375 nm. The dose of UVB was quantified with a UVB Spectra 305 dosimeter (Daevlin, Byran, OH). The radiation was further calibrated with a model IL-1700 research radiometer/photometer (International Light, Newburyport, MA).

Mice were irradiated with UVB (30 mJ/cm^2) twice a week for 20 weeks, and UVB treatment was stopped. Three weeks later, these tumor-free high-risk mice were randomized and divided into three groups (30 mice per group). Each group was treated topically on the back with 100 μl acetone, caffeine (1.2 mg, 6.2 μmol) in 100 μl acetone, or EGCG (3.0 mg, 6.5 μmol) in 100 μl acetone once a day 5 days a week for 18 weeks. The mice were killed at 24 h after the last application of caffeine or EGCG. Tumors on the treated areas of the mice were counted and

Abbreviations: EGCG, (−)-epigallocatechin gallate; UVB, ultraviolet B.

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characterized by histological examination. The daily dose of caffeine or EGCG applied topically was about 40% of that ingested daily by mice treated with 0.6% green tea (6 mg tea solids per ml) as their sole source of drinking fluid.

Measurement of Tumor Size and Preparation of Skin Sections. Tumor volume was determined by measuring the three-dimensional size (height, length, and width) of each mass. The average of the three measurements was used as the diameter. The radius (r) was determined, and the volume was calculated by: volume $= 4\pi r^3/3$.

For histopathology examination and immunohistochemical analysis, the animals were killed and the dorsal skins were taken to include each of the grossly observed masses in the treated areas of the mice. The skins were stapled flat to a plastic sheet and placed in 10% phosphate-buffered formalin at 4°C for 24 h. The skin samples were then dehydrated in ascending concentrations of ethanol (80%, 95%, and 100%), cleared in xylene, and embedded in Paraplast (Oxford Labware, St. Louis). Four-micrometer serial sections of skin were made, deparaffinized, rehydrated with water, and used for regular hematoxylin-eosin staining or immunohistochemical staining. The counting and characterization of all tumors was done blinded with respect to treatment group as described (5, 7). All immunohistochemical determinations were made with 400-fold magnification with a light microscope. These examinations were also done blinded with respect to treatment group.

Caspase 3 Immunostaining. Affinity-purified polyclonal rabbit antibody that reacts with the mouse p20 subunit of caspase 3 but does not react with the precursor form was purchased from R&D Systems. Skin sections used for the measurement of caspase 3 were stained by the horseradish peroxidase-conjugated-avidin method with some modification. Endogenous peroxidase was blocked by incubating the tissue sections in 3% hydrogen peroxide in methanol for 30 min at room temperature. Sections were then treated with 0.01 M sodium citrate buffer (pH 6.0) in a microwave oven at high temperature for 10 min. The sections were incubated with a protein block (normal goat serum) for 10 min, followed by avidin D for 15 min and biotin blocking solution for 15 min (Avidin-Biotin blocking kit from Vector Laboratories) at room temperature. The sections were incubated with caspase 3 primary antibody (1:2,000 dilution) for 30 min at room temperature followed by incubation with a biotinylated anti-rabbit secondary antibody for 30 min and incubation with conjugated-avidin solution (ABC ellite kit purchased from Vector Laboratories) for 30 min. Color development was achieved by incubation with 0.02% 3,3′-diaminobenzidine tetrahydrochloride containing 0.02% hydrogen peroxide for 10 min at room temperature. The slides were then counterstained with hematoxylin and dehydrated, and coverslips were added for permanent mounting. A positive reaction was shown as a light brown to dark brown precipitate in the cytoplasm and/or perinuclei of the cells. The percent of caspase 3-positive cells was determined in each lesion. In nontumor areas, the scoring was performed at least 0.5 cm away from tumors.

BrdUrd Incorporation into DNA. BrdUrd, a thymidine analog that is incorporated into proliferating cells during the S phase, is detected by a biotinylated monoclonal anti-BrdUrd antibody and visualized by using streptavidin-peroxidase and 3,3′-diaminobenzidine, which stains BrdUrd-containing nuclei a dark brown (staining kit from Oncogene Research Products, Cambridge, MA). Briefly, all animals were injected with BrdUrd (50 mg/kg) i.p. and killed 1 h later. Endogenous peroxidase was blocked by incubating the tissue sections in 3% hydrogen peroxide in methanol for 10 min at room temperature. The tissue sections were then incubated in a moist chamber with 0.125% trypsin for 10 min at 37°C, rinsed in distilled water, and incubated at room temperature for 30 min with denaturing solution (Oncogene Research Products). The sections were incubated with blocking solution for 10 min at room temperature and covered with biotinylated mouse monoclonal anti-BrdUrd antibody (Oncogene Research Products) at room temperature for 90 min. Sections were rinsed with PBS and incubated with streptavidin-peroxidase for 10 min. Color development was achieved by incubation for 5 min at room temperature with a substrate solution containing 0.02% 3,3′-diaminobenzidine tetrahydrochloride and 0.02% hydrogen peroxide. The slides were weakly counterstained with Mayer's hematoxylin (Sigma) for 2 min, cleared with xylene, mounted with a coverslip, and scored under a light microscope. The BrdUrd-positive cells were expressed as percent positive cells. The percent of BrdUrd-positive cells was determined in each lesion. The entire areas of all tumor sections and focal hyperplastic areas were examined. In nontumor areas, measurements were performed at least 0.5 cm away from the tumor.

Results

Inhibitory Effects of Topical Applications of Caffeine or EGCG on Carcinogenesis in the Skin of UVB-Pretreated High-Risk Mice. UVB-pretreated high-risk mice were treated topically with acetone (100 μl), caffeine (6.2 μmol) in 100 μl of acetone, or EGCG (6.5 μmol) in 100 μl of acetone once a day 5 days a week for 18 weeks, and formation of gross skin tumors was measured. Treatment of the mice with caffeine or EGCG decreased the percent of mice with tumors during the early time intervals, and tumors per mouse and tumor volume per mouse were decreased substantially at all time intervals (Fig. 1). After 12 weeks of treatment, the acetone-treated control group had 4.5 tumors/mouse, and the caffeine- and EGCG-treated animals had 1.3 and 0.8 tumors per mouse, respectively (71–82% inhibition). After 18 weeks of treatment, the vehicle-treated control animals had 6.9 tumors per mouse, and the caffeine- and EGCG-treated animals had 3.6 and 2.5 tumors per mouse, respectively (48–64% inhibition). Histological characterization of the tumors revealed that treatment of the high-risk mice topically with caffeine or EGCG for 18 weeks had little or no effect on the percent of animals with nonmalignant tumors (mostly keratoacanthomas), but the number of nonmalignant tumors per mouse was decreased by 44–55% (Table 1). These treatments decreased the percent of mice with malignant squamous cell carcinomas by 64%, and the number of squamous cell carcinomas per mouse was decreased by 66–72% (Table 1). Treatment of mice with caffeine or EGCG decreased the volume per tumor by 50–56% in nonmalignant tumors, but this treatment did not affect the size of squamous cell carcinomas (Table 2).

Selective Stimulatory Effects of Topical Applications of Caffeine or EGCG on Apoptosis in Tumors but Not in Nontumor Areas in the Skin of UVB-Pretreated High-Risk Mice. Immunohistochemical studies revealed that treatment of mice with caffeine or EGCG once a day 5 days a week for 18 weeks increased apoptosis as measured by the percent of caspase 3 (active form)-positive cells by 87% or 72%, respectively, in nonmalignant tumors, and by 92% or 56%, respectively, in squamous cell carcinomas (Table 3). These effects were selective for tumors because treatment of the mice with caffeine or EGCG had little or no effect on caspase 3-positive cells in focal hyperplastic areas or other nontumor areas of the epidermis (Table 3).

In a separate experiment, we found that treatment of tumor-free high-risk mice topically with caffeine (6.2 μmol) twice a day for 3 or 14 days selectively increased the number of caspase 3-positive cells in dysplastic areas of the epidermis (68% and 74%, respectively), but this increase was not observed in normal appearing areas of the epidermis (data not presented). Topical

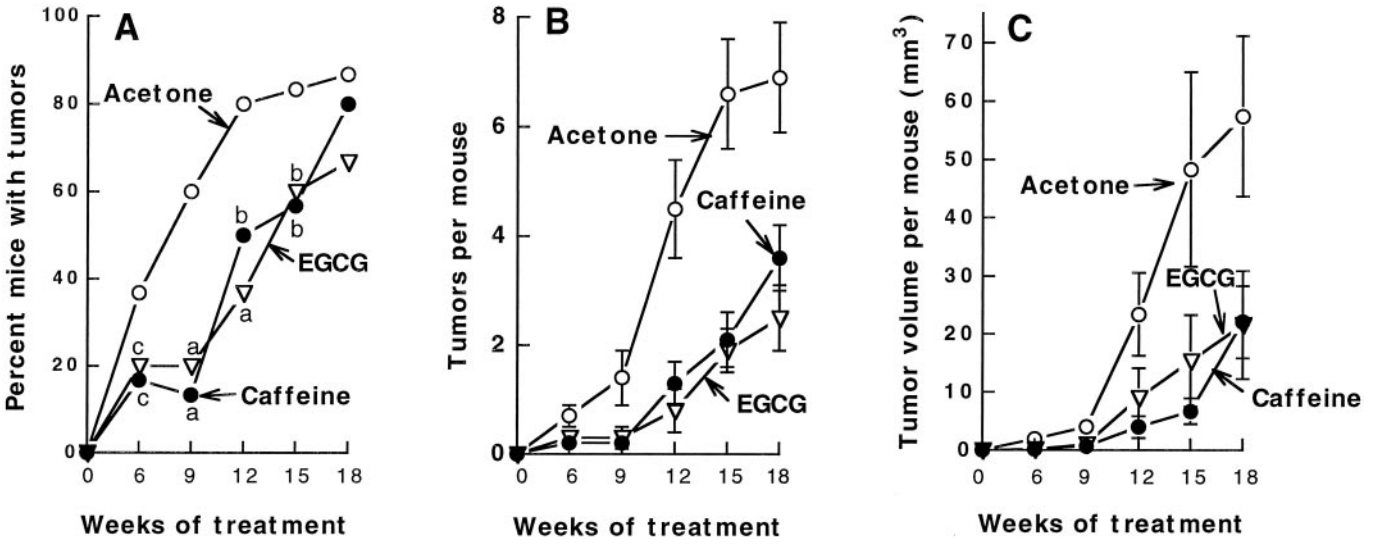

Fig. 1. Effect of topical applications of caffeine or EGCG on tumorigenesis in UVB-pretreated high-risk mice. SKH-1 hairless mice were irradiated with UVB (30 mJ/cm^2) twice weekly for 20 weeks to obtain tumor-free high-risk mice. The mice were then treated topically with 100 μl of acetone, caffeine (6.2 μmol in 100 μl of acetone), or EGCG (6.5 μmol in 100 μl of acetone) once a day 5 days a week for 18 weeks. Mean values ± SE are indicated. (*A*) a, $P < 0.01$; b, $P < 0.05$; c, $P < 0.10$. (*B* and *C*) All points from the groups treated with caffeine or EGCG are statistically different from the comparable points from the acetone-treated control group ($P < 0.01$).

applications of EGCG (6.5 μmol) twice a day for 3 or 14 days had no effect on the number of caspase 3-positive cells in dysplastic or normal appearing areas of the epidermis (data not presented). Typical examples of caspase 3 (active form) immunoreactive positive cell(s) in tumors are shown in Fig. 2.

Inhibitory Effects of Topical Applications of Caffeine or EGCG on Proliferation in Skin Tumors of UVB-Pretreated High-Risk Mice. BrdUrd was injected into the mice 1 h before death and the BrdUrd labeling index was determined. Treatment of the mice with caffeine or EGCG inhibited BrdUrd labeling in nonmalignant tumors by 16–22% ($P < 0.05$, Table 4) and in focal hyperplastic areas of the epidermis by 36–41%, respectively. A similar small inhibitory effect of caffeine or EGCG treatment on BrdUrd labeling was observed in squamous cell carcinomas, but it was not statistically significant (Table 4).

Although oral administration of tea or caffeine to SKH-1 mice markedly decreased the size of the parametrial fat pads and the thickness of the dermal fat layer (8), topical applications of caffeine or EGCG for 18 weeks in the present study had no effect on the weight of the parametrial fat pads but caffeine or EGCG treatments did decrease the thickness of the dermal fat layer (14% or 19%, respectively), when compared with acetone-treated mice (data not shown).

Discussion

Treatment of SKH-1 hairless mice with UVB (30 mJ/cm^2) twice a week for 20 weeks resulted in mice without tumors but with a high risk of developing tumors during the next several months in the absence of further treatment with UVB (high-risk mice; ref. 5). Topical applications of caffeine or EGCG once a day 5 days a week for 18 weeks to high-risk mice inhibit the formation of keratoacanthomas and squamous cell carcinomas (Table 1). The mechanism of the inhibitory effects of topical applications of caffeine and EGCG on tumorigenesis appears to be a strong stimulatory effect of these substances on apoptosis in the tumors (Table 3). In addition, these agents exert a modest inhibitory effect on proliferation in focal hyperplastic areas in the epidermis and the tumors (Table 4). In earlier studies, we found that oral administration of black tea to mice with UVB-induced tumors inhibited growth of the tumors, enhanced apoptosis in the tumors, and inhibited proliferation in the tumors (6). Similar results were observed in tumor-bearing mice treated with green tea (unpublished observations). The results of the present study indicate that topical applications of caffeine or EGCG (constit-

Table 1. Effect of topical applications of caffeine or EGCG on the formation of histologically characterized tumors in high-risk mice

		Nonmalignant tumors		Squamous cell carcinomas	
Treatment	No. of mice	Percent of mice with tumors	Tumors per mouse	Percent of mice with tumors	Tumors per mouse
Acetone	28	82	7.21 ± 1.28	64	1.18 ± 0.25
Caffeine	30	77 (6)	4.03 ± 0.76† (44)	23* (64)	0.33 ± 0.12* (72)
EGCG	30	70 (15)	3.27 ± 0.67*(55)	23* (64)	0.40 ± 0.18* (66)

UVB-pretreated high-risk SKH-1 mice (30/group) were treated topically with 100 μl acetone, caffeine (6.2 μmol) in 100 μl acetone or EGCG (6.5 μmol) in 100 μl acetone once daily 5 days a week for 18 weeks. Nonmalignant tumors were predominantly keratoacanthomas (>95%), and a few papillomas were also observed. The numbers in parentheses represent percent inhibition. Each value represents the mean ± SE.

*$P < 0.01$.

†$P < 0.05$.

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Table 2. Effect of topical applications of caffeine or EGCG on the size of histologically characterized tumors in high-risk mice

		Nonmalignant tumors		Squamous cell carcinomas	
Treatment	No. of mice	Tumor volume per tumor, mm^3	Tumor volume per mouse, mm^3	Tumor volume per tumor, mm^3	Tumor volume per mouse, mm^3
Acetone	28	3.2 ± 0.7	23.2 ± 5.5	161 ± 80	190 ± 95
Caffeine	30	1.6 ± 0.3† (50)	6.4 ± 1.7* (72)	117 ± 81 (27)	39 ± 28‡ (79)
EGCG	30	1.4 ± 0.5† (56)	4.7 ± 1.9* (80)	399 ± 283(−)	160 ± 117 (16)

UVB-pretreated high-risk SKH-1 mice (30/group) were treated topically with 100 μl acetone, caffeine (6.2 μmol) in 100 μl acetone, or EGCG (6.5 μmol) in 100 μl acetone once daily 5 days a week for 18 weeks. Nonmalignant tumors were predominantly keratoacanthomas (>95%) and a few papillomas were also observed. The numbers in parentheses represent percent inhibition. Each value represents the mean ± SE.

*$P < 0.01$.

†$P < 0.05$.

‡$P < 0.10$.

uents of both green and black tea) have direct stimulatory effects on apoptosis in skin tumors, but these treatments do not have an apoptotic effect in nontumor areas of the epidermis.

Selectivity for the effects of caffeine and EGCG are attractive features of these chemopreventive agents. Because most UVB-induced skin tumors have p53 mutations, it is likely that the stimulatory effects of caffeine or EGCG on apoptosis in UVB-induced tumors are by p53-independent mechanisms. In other studies, oral administration of green tea or caffeine for 2 weeks before UVB exposure or a single topical application of caffeine immediately after irradiation with UVB enhances UVB-induced apoptosis in the epidermis without exerting an apoptotic effect in non-UVB-exposed mice (9, 10).

Although other studies have demonstrated a proapoptotic effect of high concentrations of caffeine or EGCG in cultured tumor cells (11–14), the results of the present study and our earlier study (6) demonstrate a stimulatory effect of tea, caffeine, and EGCG administration on apoptosis in tumors of tumor-bearing animals. The effects of orally administered tea or caffeine or topically administered caffeine or EGCG to inhibit UVB-induced carcinogenesis and to stimulate apoptosis in tumors suggest the possibility that oral administration of tea or topical applications of caffeine or EGCG may inhibit sunlight-induced skin cancer in humans. A preliminary study suggests that tea drinkers may have a lower risk of skin cancer than nontea drinkers (15), but additional epidemiology studies are needed. Reports suggesting that women who ingest large amounts of caffeine have an increased risk of osteoporosis are of potential concern (16, 17).

Administration of tea or EGCG to animals has been shown to inhibit chemically induced carcinogenesis in many animal models (18–20). The stimulatory effect of tea, caffeine, or EGCG on apoptosis in tumors of tumor-bearing mice suggests that enhanced apoptosis may be a unifying mechanism by which tea, caffeine, or EGCG inhibit carcinogenesis and tumor growth. Although administration of caffeine inhibits carcinogenesis in several animal models (21–29), carcinogenesis is stimulated in some animal models (30–34). The reasons caffeine inhibits carcinogenesis in some animal models and enhances carcinogenesis in other models are not known.

Table 3. Stimulatory effect of topical applications of caffeine or EGCG on the formation of caspase 3-positive cells in tumors

Treatment	No. of nontumor areas, focal hyperplasia areas, or tumors examined	No. of cells examined	Percent caspase 3-positive cells	Percent increase
Nontumor areas				
Acetone	370	123,000	0.159 ± 0.015	—
Caffeine	271	92,000	0.165 ± 0.027	4
EGCG	276	90,000	0.123 ± 0.024	—
Focal hyperplasia				
Acetone	25	16,000	0.378 ± 0.051	—
Caffeine	19	12,000	0.454 ± 0.079	20
EGCG	14	6,300	0.386 ± 0.051	2
Nonmalignant tumors				
Acetone	202	882,000	0.229 ± 0.017	—
Caffeine	121	426,000	0.428 ± 0.033*	87
EGCG	98	310,000	0.394 ± 0.031*	72
Carcinomas				
Acetone	33	555,000	0.196 ± 0.022	—
Caffeine	10	110,000	0.376 ± 0.056*	92
EGCG	12	227,000	0.305 ± 0.050†	56

High-risk mice (30 per group) were treated topically with acetone (100 μl), caffeine (6.2 μmol), or EGCG (6.5 μmol) in 100 μl acetone once daily 5 days a week for 18 weeks. In nontumor areas, the scoring was done at least 0.5 cm away from tumors. Each value represents the mean ± SE.

*$P < 0.01$.

†$P < 0.10$.

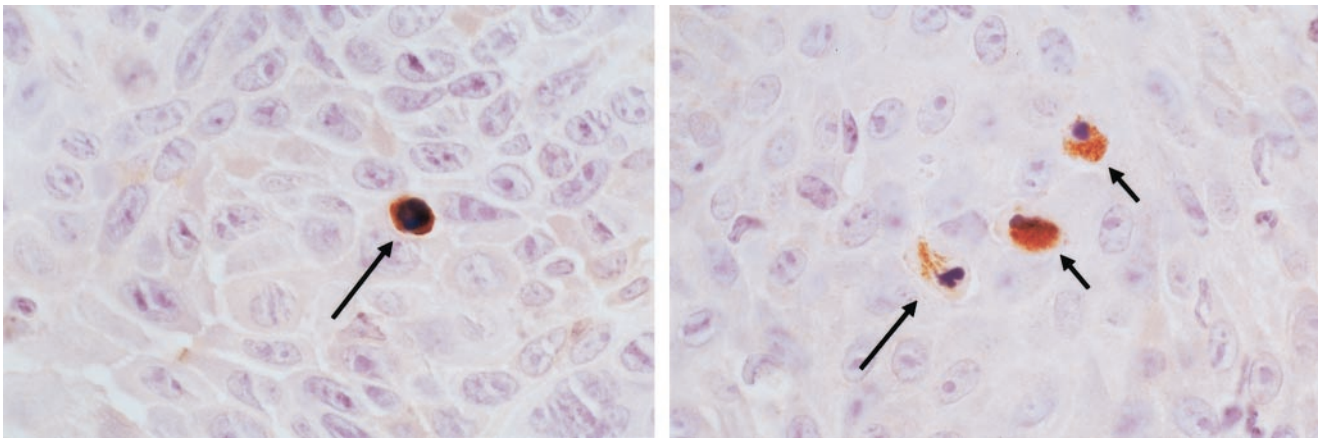

Fig. 2. Morphological characteristics of caspase 3-positive cell(s) in tumors. SKH-1 hairless mice were irradiated with UVB (30 mJ/cm^2) twice weekly for 20 weeks to obtain tumor-free high-risk mice. The mice were then treated topically with 100 μl of acetone, caffeine (6.2 μmol in 100 μl of acetone), or EGCG (6.5 μmol in 100 μl of acetone) once a day 5 days a week for 18 weeks. Most of the cells with caspase 3 (active form) immunoreactive staining had the morphological characteristics of apoptotic cells. The kinds of cells observed are described below. (*Left*) A cell with strong cytoplasmic caspase-3 (active form) immunoreactive positive staining and the characteristics of an apoptotic cell (condensed nuclei and slight cell membrane shrinkage). We hypothesize this represents an intermediate stage of an apoptotic cell. (*Right*) Cells with weak cytoplasmic caspase-3 (active form) immunoreactive positive staining and the characteristics of apoptotic cells (condensed nuclei and obvious cell membrane shrinkage). We hypothesize these cells represent a late stage of apoptosis. (Magnification: ×1,000.)

As indicated above, EGCG increases apoptosis in various tumor cell lines (13), and tumor cells appear to be more sensitive to EGCG-induced apoptosis than their normal counterparts (13). However, the precise mechanism by which EGCG induces apoptosis in tumor cells remains to be elucidated, although some proposals have already been made. These include inhibition of transcription factor NF-κB (35, 36), activation of a tumor necrosis factor α-mediated signaling pathway (37), cell cycle arrest at G_0/G_1 (38, 39) or G_2/M (37), and EGCG binding to Fas, presumably on the cell surface, to trigger Fas-mediated apoptosis (12).

Several studies indicate that low millimolar concentrations of caffeine and other methylxanthines sensitize cultured cancer cells to the toxic effects of radiation or certain chemotherapeutic agents (40–44) possibly by blocking normal checkpoint control of the cell cycle and allowing replication of the caffeine-treated cells (45). It was suggested that caffeine-induced checkpoint defects may be caused by an inhibitory effect of caffeine on ATM and ATR kinase activities that are needed for the phosphorylation of p53 and other proteins that are important for checkpoint control of the cell cycle (45). In another study, combinations of caffeine and radiation caused a synergistic effect on

Table 4. Effect of topical applications of caffeine or EGCG on BrdUrd incorporation in tumors

Treatment	No. of nontumor areas, focal hyperplasia areas, or tumors examined	No. of cells examined	Percent BrdUrd-positive cells	Percent decrease
Nontumor areas				
Acetone	370	24,800	6.8 ± 0.6	—
Caffeine	271	18,500	6.6 ± 0.6	3
EGCG	276	18,200	7.6 ± 0.4	−12
Focal hyperplasia				
Acetone	25	4,200	11.4 ± 2.1	—
Caffeine	19	3,200	6.7 ± 1.2‡	41
EGCG	14	1,600	7.3 ± 1.2‡	36
Nonmalignant tumors				
Acetone	202	153,600	26.4 ± 1.2	—
Caffeine	121	74,200	22.0 ± 1.4†	16
EGCG	98	54,200	20.5 ± 1.3*	22
Carcinomas				
Acetone	33	84,400	37.2 ± 3.3	—
Caffeine	10	16,800	32.8 ± 5.0	12
EGCG	12	34,600	29.6 ± 6.3	20

High-risk mice (30 per group) were treated topically with 100 μl acetone, caffeine (6.2 μmol), or EGCG (6.5 μmol) in 100 μl acetone once daily 5 days a week for 18 weeks. The BrdUrd-positive cells were determined immunohistochemically and expressed as percent positive cells per tumor. The entire areas of all tumor sections were examined. In nontumor areas, the counts were determined at least 0.5 cm away from tumors. Each value represents the mean ± SE.

*$P < 0.01$.

†$P < 0.05$.

‡$P < 0.10$.

apoptosis in p53-defective cells via a p53-independent pathway (46). Recent studies by Nghiem and his colleagues (47) with cultured human osteosarcoma U20S cells indicated that (*i*) caffeine-induced inhibition of ATR (but not ATM) caused premature chromatin condensation and cell death, (*ii*) ATR (but not ATM) prevented premature chromatin condensation, and (*iii*) ATR prevented premature chromatin condensation via Chk-1 regulation. These investigators suggested that cancer cells with a disrupted G_1 checkpoint (such as loss of p53 function) should be sensitized to ATR inhibition and lethal premature chromatin condensation (47, 48). These investigators also pointed out that cancer cells with defective checkpoint control may be selectively affected by ATR inhibitors and suggested that ATR inhibitors that are more potent than caffeine may be useful agents for cancer therapy. The concepts developed by Nghiem and his colleagues on the selectivity of ATR inhibitors for p53-defective cells may help explain why caffeine has a selective apoptotic effect in UVB-induced tumors (previously shown to have p53 mutations) but not in nontumor areas of the epidermis.

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