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Inhibition of UVB-induced nonmelanoma skin cancer: A path from tea to caffeine to exercise to decreased tissue fat

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Abstract

Oral administration of green tea, black tea or caffeine (but not the decaffeinated teas) inhibited ultraviolet B radiation (UVB)-induced skin carcino-genesis in SKH-1 mice. Studies with caffeine indicated that its inhibitory effect on the ATR/Chk1 pathway is an important mechanism for caffeine's inhibition of UVB-induced carcinogenesis. The regular teas or caffeine increased locomotor activity and decreased tissue fat. In these studies, decreased dermal fat thickness was associated with a decrease in the number of tumors per mouse. Administration of caffeine, voluntary exercise and removal of the parametrial fat pads all stimulated UVB-induced apoptosis, inhibited UVB-induced carcinogenesis and stimulated apoptosis in UVB-induced tumors. These results suggest that caffeine administration, voluntary exercise and removal of the parametrial fat pads inhibit UVB-induced carcinogenesis by stimulating UVB-induced apoptosis and by enhancing apoptosis in DNA-damaged precancer cells and in cancer cells. We hypothesize that tissue fat secretes antiapoptotic adipokines that have a tumor promoting effect.

Introduction

Sunlight-induced nonmelanoma skin cancer is the most prevalent cancer in the United States with more than 2 million cases per year (more than the number of cases for all of the other cancers combined) [1], and the number of nonmelanoma skin cancer cases has been increasing in recent years [2,3]. Possible reasons for the increasing incidence of nonmelanoma skin cancer are increased recreational exposure to sunlight, increased use of "sun tanning salons", and depletion of the ozone layer. We also wonder whether the increasing incidence may be related to the use of certain moisturizing creams [4].

Inhibitory effects of green tea and caffeine on UVB-induced carcinogenesis

In an early study, we found that oral administration of green tea inhibited the formation of UVB-induced nonmelanoma skin cancer in SKH-1 mice, but decaffeinated green tea was

inactive [5] (Table 1). Oral administration of caffeine had a strong inhibitory effect on UVB-induced carcinogenesis, and adding caffeine to the decaffeinated green tea restored its inhibitory activity [5] (Table 1). Similar observations were made with black tea [5]. Our results indicate that caffeine is a biologically important component of tea.

In additional studies, we irradiated SKH-1 mice with UVB (30 mJ/cm^2) twice a week for 20 weeks and then stopped UVB irradiation. These UVB-pretreated mice have no tumors but develop tumors over the next several months in the absence of further UVB irradiation (high risk mice) [6]. Treatment of these UVB-pretreated high risk mice with oral or topical administration of caffeine inhibited tumor formation (Table 2) [6,7]. These results parallel epidemiological studies indicating that people ingesting regular coffee had a decreased risk of nonmelanoma skin cancer, and decaffeinated coffee was inactive [8,9].

Oral administration of green tea (6 mg tea solids/ml) or caffeine (0.4 mg/ml) as the sole source of drinking fluid during irradiation of SKH-1 mice with UVB twice a week for 20 weeks inhibited UVB-induced formation of mutant p53 positive patches in the epidermis by ~40% [10]. Oral administration of green tea (6 mg tea solids/ml) as the sole source of drinking fluid or topical applications of caffeine (6.2 μmol) once a day 5 days a week starting immediately after discontinuation of UVB treatment enhanced the rate and extent of disappearance of the mutant p53-positive patches [10]. Topical applications of caffeine to the dorsal skin of mice pretreated with UVB for 20 weeks resulted in enhanced apoptosis selectively in focal basal cell hyperplastic areas of the epidermis (putative precancerous lesions), but not in areas of the epidermis that only had diffuse hyperplasia [10]. These studies indicate that the chemopreventive effect of caffeine or green tea may occur by a proapoptotic effect preferentially in early precancerous lesions.

Mechanism studies

Mechanistic studies indicated that caffeine has a sunscreen effect [11] and also enhances UVB-induced apoptosis [12,13]. The stimulatory effect of oral administration of green tea, coffee, and caffeine on UVB-induced apoptosis is shown in Figure 1 [14]. In other studies, topical application of caffeine immediately after UVB irradiation also enhanced UVB-induced apoptosis [13], and the stimulatory effect of topical caffeine on UVB-induced apoptosis occurred by p53-dependent and p53-independent mechanisms [12,15]. Application of caffeine after UVB irradiation avoided the potential sunscreen effect of caffeine. Studies on the p53-independent pathway suggested that oral or topical caffeine administration enhanced lethal mitosis in UVB irradiated mice by inhibiting the ATR/Chk-1 pathway in the epidermis [16] and in tumors from UVB-treated mice [17]. In addition, inhibition of the ATR/Chk-1 pathway by caffeine was associated with enhanced UVB-induced apoptosis in primary human keratinocytes [18].

Additional evidence for the importance of blocking the ATR/Chk-1 pathway for inhibition of UVB-induced carcinogenesis came from finding that genetic inhibition of epidermal ATR kinase resulted in inhibition of UVB-induced carcino-genesis [19]. To test the effect of genetic inhibition of the ATR-Chk-1 pathway on UVB carcinogenesis, transgenic FVB mice were prepared that expressed a kinase dead form of human ATR (ATR-kd) under a human

keratin-14 promoter. These mice were crossed into $Xpc^{-/-}$ mice with a global repair deficiency. UVB-induced carcinogenesis was determined in ATR-kd transgenic mice and transgene-negative littermate controls. Formation of UVB-induced skin tumors was markedly decreased in ATR-kd transgenic mice when compared with UVB-induced tumor formation in transgene-negative controls indicating that genetic inhibition of the ATR/Chk-1 pathway inhibits UVB-induced carcinogenesis (Figure 2) [19].

The results of mechanistic studies indicate that caffeine can inhibit UVB-induced carcinogenesis by exerting a sunscreen effect, by stimulating UVB-induced upregulation of wild-type p53, and by inhibition of the ATR/Chk-1 pathway.

Effects of oral administration of tea, decaffeinated tea and caffeine on tissue fat and skin carcinogenesis in UVB-pretreated high-risk mice

We found that oral administration of green tea or black tea (6 mg tea solids/ml) for 23 weeks to UVB-pretreated high risk mice in the absence of continued treatment with UVB decreased the number of tumors per mouse by 66–68%, the size of the parametrial fat pads by 32–54%, and the thickness of the dermal fat layer by 39–53% [20]. Administration of the decaffeinated teas had little or no effect on any of these parameters, and adding caffeine (equivalent to the amount in the regular teas) to the decaffeinated teas restored their inhibitory effects [20]. Administration of caffeine alone (0.4 mg/ml) decreased the number of tumors per mouse by 61%, decreased the average size of the parametrial fat pads by 56%, and caused a substantial decrease in the thickness of the dermal fat layer [20].

We observed that the dermal fat layer was much thinner under tumors than away from tumors in all experimental groups [20]. For instance, in UVB-pretreated high risk mice given only water as their drinking fluid for 23 weeks, the thickness of the dermal fat layer away from tumors was 162 μm but was only 60 μm directly under tumors. In high risk mice given 0.6% green tea for 23 weeks, the average thickness of the dermal fat layer away from tumors was 100 μm but was only 28 μm directly under tumors. Administration of caffeinated beverages decreased the average thickness of the dermal fat layer directly under tumors by 36% for small tumors (≤0.5 mm diameter), by 57% for tumors 0.5–1 mm in diameter, by 70% for tumors 1–2 mm in diameter, by 90% for tumors 2–3 mm in diameter and by 97% for tumors >3 mm in diameter. In addition to the effect of caffeine to decrease the thickness of the dermal fat layer under tumors, our results suggest that tumors may be utilizing dermal fat as a source of energy or that tumors are secreting substances that enhance lipolysis.

Relationship between the thickness of the dermal fat layer away from tumors and tumor multiplicity

In the above study with UVB-pretreated high risk mice treated with water, green tea, black tea, decaffeinated green tea, decaffeinated black tea, decaffeinated green tea plus caffeine, decaffeinated black tea plus caffeine or caffeine alone, all mice at the end of the study were analyzed histologically for tumors, and 152 of these mice had a total of 689 tumors and 27 mice had no tumors. The relationship between the thickness of the dermal fat layer away from tumors (possible surrogate for total body fat levels) in individual mice and the number

of tumors per mouse in all 179 mice was evaluated [20] (Table 3). Fourteen mice with a very thin dermal fat layer (≤ 50 μm) away from tumors had an average of only 1.6 ± 0.7 tumors/mouse whereas seven mice with a thick dermal fat layer (>250 μm) away from tumors had 7.4 ± 1.8 tumors/mouse. Regression analysis was performed with data from all 179 mice to assess the relationship between the thickness of the dermal fat layer away from tumors for each mouse and the number of tumors per mouse. There was a highly significant positive linear association between the number of tumors per mouse and the thickness of the dermal fat layer away from tumors (p=0.0001).

Effects of topical applications of caffeine on apoptosis in tumors during carcinogenesis in UVB-pretreated high risk mice

Tumor-free high risk mice (30 mice per group) were treated topically with 100 μl of acetone or with caffeine (6.2 μmoles) in 100 μl of acetone once a day 5 days a week for 18 weeks, and all tumors in the treated areas of the mice were counted and characterized by histological examination. The treatments with caffeine decreased the number of nonmalignant tumors (mostly keratoacanthomas) and squamous cell carcinomas by 44 and 72%, respectively (Table 2), and tumor volume per mouse was decreased by 72 and 79%, respectively [7].

The results of immunohistochemical staining of tumors described in the above study indicated that topical applications of caffeine to high risk mice enhanced apoptosis in the tumors but not in areas away from the tumors (Table 4) [7]. These results suggest that the inhibitory effect of caffeine administration on tumorigene-sis in high risk mice may be caused in part by enhanced apoptosis in small tumors during their formation and growth.

Effects of running wheel exercise on UVB-induced apoptosis, UVB-induced carcinogenesis, and apoptosis in tumors

During the course of our studies, we observed that mice treated orally with green tea or caffeine had increased locomotor activity and decreased tissue fat [21]. Because of these observations, we studied the effect of voluntary exercise (running wheel in the cage) on UVB-induced apoptosis, UVB-induced carcinogenesis and apoptosis in UVB-induced tumors. An inhibitory effect of voluntary exercise on UVB-induced tumor formation and a stimulatory effect of voluntary exercise on UVB-induced apoptosis and apoptosis in tumors was observed [22,23]. These results are similar to those observed for animals treated with caffeine.

Effects of a combination of running wheel exercise together with oral caffeine on tissue fat and UVB-induced apoptosis

Treatment of SKH-1 mice orally with caffeine (0.1 mg/ml in the drinking water), voluntary running wheel exercise, or a combination of caffeine and exercise for 2 weeks (a) decreased the weight of the parametrial fat pads by 35, 62 and 77%, respectively, (b) decreased the thickness of the dermal fat layer by 38, 42, and 68%, respectively, and (c) stimulated the formation of UVB-induced caspase 3 (active form) positive cells in the epidermis by 92, 120

and 389%, respectively [23]. No effects of voluntary exercise or oral caffeine administration (alone or together) on apoptosis in the epidermis were observed in the absence of UVB irradiation. The plasma concentration of caffeine in mice ingesting caffeine (0.1 mg/ml drinking water) is similar to that in the plasma of most coffee drinkers (1–2 cups/day). The results of our studies indicate a greater than additive stimulatory effect of combined voluntary exercise and oral administration of a low dose of caffeine on UVB-induced apoptosis. In an additional study, oral administration of caffeine (0.1 mg/ml in the drinking water), voluntary running wheel exercise or the combination to SKH-1 mice irradiated with UVB (30 mJ/cm^2) twice a week for 34 weeks inhibited the formation of tumors (tumors/mouse) by 25, 35 and 62%, respectively.

Stimulatory effect of fat removal (partial lipectomy) on UVB-induced apoptosis in the epidermis of SKH-1 mice

Since administration of caffeine or running wheel exercise decreased tissue fat and enhanced UVB-induced apoptosis, we evaluated the effect of removal of tissue fat on UVB-induced apoptosis. Surgical removal of the two parametrial fat pads 2 weeks before UVB irradiation enhanced UVB-induced apoptosis in the epidermis by 107% at 6 h after irradiation when compared with the effect of UVB on apoptosis in sham-operated control mice [24]. In control studies with mice that did not receive UVB irradiation, partial lipectomy had no effect on the small number of apoptotic cells in the epidermis. Our results suggest that tissue fat may secrete anti-apoptotic substances that enhance carcinogenesis by inhibiting the death of DNA-damaged precancer cells and cancer cells as hypothesized in Figure 3. According to this hypothesis, factors that decrease tissue fat will decrease cancer risk by decreasing the amount of antiapoptotic adipokines, thereby enhancing apoptosis in DNA-damaged precancer cells and in cancer cells. Anti-apoptotic adipokines associated with tissue fat may help explain why obese individuals have an increased risk of cancer.

Surgical removal of the parametrial fat pads decreases serum levels of TIMP1 and other adipokines

Feeding SKH-1 mice a 40% kcal high fat diet rich in omega-6 fatty acids as described earlier [25] or a 60% kcal very high fat diet for 2 weeks increased the serum levels of TIMP1 (tissue inhibitor of metalloproteinase 1) and several other adipokines. TIMP1 was reported to enhance cell proliferation and to inhibit apoptosis [26] suggesting that it has tumor promoting activity. TIMP1 was also reported to be a useful indicator of cutaneous cancer invasion and progression [27]. Removal of the parametrial fat pads from mice on a high fat diet resulted in a marked decrease in the serum level of TIMP1 and other adipokines when compared with the sham-operated control mice. Our results suggest that a high fat diet increases adipokines that have tumor promoting properties and that partial lipectomy decreases the serum levels of these adipokines.

Surgical removal of the parametrial fat pads inhibits UVB-induced formation of skin tumors in mice fed a high fat diet

Our previous studies showed that a 40% kcal high fat diet rich in omega-6 fatty acids enhanced UVB-induced skin tumor formation when compared with mice fed a diet rich in omega-3 fatty acids [25]. We investigated the effect of lipectomy on UVB-induced skin tumorigenesis in mice fed either a high fat diet rich in omega-6 fatty acids or a low fat Chow diet.

SKH-1 mice were given a high fat diet and other mice were given a low fat Chow diet for 2 weeks. Mice on each diet were then divided into two groups. One group of mice had their parametrial fat pads removed, and the other group of mice was a sham-operated control. The average weight of the removed parametrial fat pads from the mice that were fed a Chow diet or the high fat diet was about 15% of total body fat. All animals were treated with UVB (30 mJ/cm^2) once a day, twice a week for 33 weeks.

Surgical removal of the parametrial fat pads markedly inhibited UVB-induced skin tumorigenesis in mice fed the high fat diet, but this effect was not observed in mice fed the low fat Chow diet. Although there was no difference in body weight between lipectomized mice and sham-operated control animals fed the high fat diet, histopathology examination indicated that removal of the parametrial fat pads decreased the number of keratoacanthomas and squamous cell carcinomas per mouse by 75–80%, when compared to the sham-operated controls. Partial lipectomy decreased the tumor volume per mouse for keratoacanthomas and carcinomas by ~90%, when compared to the sham-operated controls.

Immunohistochemical analysis of the tumor samples indicated that lipectomy increased the percentage of caspase 3 (active form) positive cells in areas away from the tumors by 48%, in keratoacanthomas by 68% and in carcinomas by 224%, respectively, and proliferation was also inhibited in lipectomized mice when compared with sham-operated mice. These results indicate that inhibition of UVB-induced carcinogenesis may have resulted from an increase in apoptosis and an inhibition of proliferation in tumors and in precancerous areas away from tumors. Our proposed effect of caffeine administration, exercise, low fat diet, and partial lipectomy to decrease tissue fat and associated antiapoptotic adipokines is shown in Figure 3.

It was of considerable interest that compensatory fat appeared in the peritoneal cavity of partially lipectomized mice near where the parametrial fat pads had been removed. Biochemical properties of the compensatory fat in lipectomized mice at the end of the above tumor study was compared with the biochemical properties of the parametrial fat pads in sham-operated control mice at the end of the tumor study in mice fed the 40% high fat diet. It was found by RT-PCR that mRNAs for TIMP1, Serpin E1 and MCP1 were 50- to 80-fold higher in the parametrial fat pads than in the compensatory fat. Our results suggest that the parametrial fat pads secrete pro-inflammatory/tumor promoting adipokines that are not secreted in appreciable amounts by the compensatory fat.

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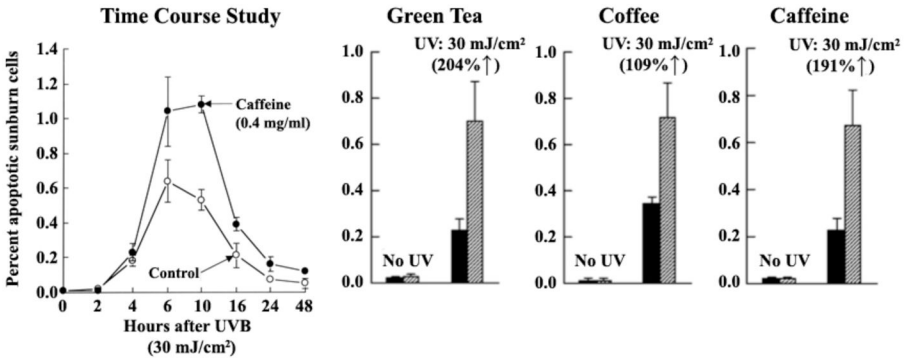

Fig. 1. Stimulatory effect of oral administration of green tea, coffee or caffeine on UVB-induced apoptosis

The time course for the effect of oral caffeine (0.4mg/ml) for 2 weeks on UVB-induced apoptosis in female SKH-1 mice is shown in the first panel. In additional studies, SKH-1 female mice were treated with green tea (6 mg tea solids/ml), coffee (10 mg coffee solids/ml) or caffeine (0.4 mg/ml) as their sole source of drinking fluid for 2 weeks. The mice were irradiated with UVB (30 mJ/cm^2) and killed 10 h later. Apoptotic sunburn cells in the epidermis were determined. The solid bars represent control animals treated with water. The dashed bars indicate treatment with green tea, coffee or caffeine as indicated. (Taken from refs. 12,14.)

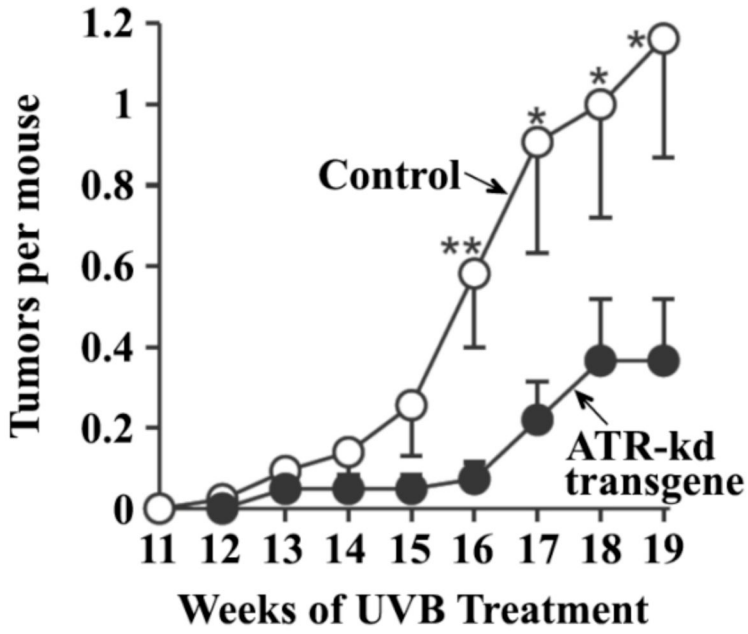

Fig. 2. ATR-kd transgene delays tumor onset and suppresses UV tumorigenesis

ATR-kd transgene suppresses UV-induced tumor development. Mean number of tumors per mouse is shown up to 19 weeks when some mice with advanced tumors were sacrificed and the cohort was no longer complete. Error bars represent SEM. Statistical significance in mean number of tumors per mouse between the groups was as shown at the indicated time points: * $P \leq 0.05$, ** $P < 0.01$. (Taken from ref. 19.)

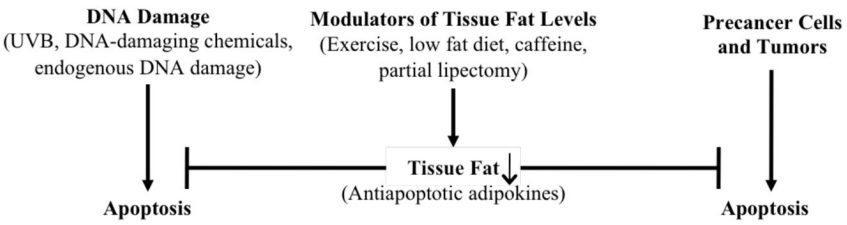

Fig. 3.

Proposed inhibitory effect of tissue fat on DNA damage-induced apoptosis in pre-cancer cells and in tumors.

Table 1

Effect of oral administration of green tea, decaffeinated green tea or caffeine on UVB-induced complete carcinogenesis.

Treatment	Number of keratoacantho- mas per mouse	Number of squamous cell carcinomas per mouse
Water	5.75 ± 1.04	1.17 ± 0.27
Green tea	2.21 ± 0.46[a]	0.52 ± 0.18[a]
Decaf. green tea	4.58 ± 0.64	1.35 ± 0.29
Caffeine	1.81 ± 0.44[a]	0.63 ± 0.14[a]
Decaf. green tea + caffeine	2.53 ± 0.43[a]	0.47 ± 0.11[a]

Female SKH-1 mice were treated with UVB (30 mJ/cm^2) twice weekly for 44 weeks. Tea leaf extracts (1.25 gm tea leaf/100 ml hot water; ~4 mg tea solids/ml) or caffeine (0.36 mg/ml) were administered as the drinking fluid. Each value is the mean ± S.E. from 24–30 mice.

[a] $p<0.05$ (Taken from ref. 5.)

Table 2

Inhibitory effect of oral administration or topical applications of caffeine on tumor formation in UVB-pretreated high risk mice.

		Keratoacanthomas		Squamous cell carcinomas	
Exp.	**Treatment**	**Tumors per mouse**	**Percent decrease**	**Tumors per mouse**	**Percent decrease**
1	Water	4.00 ± 0.47	–	1.82 ± 0.30	–
	Oral caffeine	1.70 ± 0.48[a]	57	0.63 ± 0.31[a]	65
2	Acetone	7.07 ± 1.27	–	1.18 ± 0.25	–
	Topical caffeine	3.93 ± 0.74[a]	44	0.33 ± 0.12[a]	72

In Experiment 1, UVB-pretreated high risk SKH-1 mice (30/group) with no observable tumors were given caffeine (0.44 mg/ml) as their sole source of drinking fluid for 23 weeks. The number of tumors per mouse is expressed as the mean ± S.E. In Experiment 2, high risk UVB-pretreated SKH-1 mice (30/group) were treated topically with 100 µl acetone or caffeine (6.2 µmol) in 100 µl acetone once daily 5 days a week for 18 weeks. Each value represents the mean ± S.E.

[a] $p<0.01$ (Taken from refs. 6,7.)

Table 3

Relationship between the thickness of the dermal fat layer (away from tumors) and tumor multiplicity.

Thickness of dermal fat layer (μm)	Number of mice	Number of tumors per mouse
≤50	14	1.6 ± 0.7
50 – 100	63	2.9 ± 0.4
100 – 150	68	3.8 ± 0.6
150 – 200	17	5.5 ± 1.0
200 – 250	10	7.8 ± 1.4
>250	7	7.4 ± 1.8

UVB-pretreated high risk SKH-1 mice were given water, green tea, black tea, decaffeinated green tea, decaffeinated black tea, caffeine, decaffeinated green tea + caffeine or decaffeinated black tea + caffeine for 23 weeks. The thickness of the dermal fat layer in areas away from tumors or in mice with no tumors was determined. Each value represents the mean ± S.E. p=0.0001 (from the Pearson correlation coefficient) for the thickness of the dermal fat layer away from tumors vs. the number of tumors/mouse for all 179 mice. (Taken from ref. 20.)

Table 4

Stimulatory effect of topical applications of caffeine on apoptosis in tumors.

Treatment	Number of tumors examined	Percent caspase 3 positive cells	Percent increase
	Non-tumor areas		
Control	–	0.159 ± 0.015	–
Caffeine	–	0.165 ± 0.027	4
	Keratoacanthomas		
Control	198	0.229 ± 0.017	–
Caffeine	118	0.430 ± 0.034[a]	88
	Carcinomas		
Control	33	0.196 ± 0.022	–
Caffeine	10	0.376 ± 0.056[a]	92

High risk mice (30 per group) were treated topically with acetone (100 μl) or with caffeine (6.2 μmol) in 100 μl acetone once daily 5 days a week for 18 weeks. Each value for the percent of caspase 3 positive cells represents the mean ± S.E.

[a] $p<0.01$ (Animals are from Table 2, Exp. 2.) (Taken from ref. 7.)