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Effect of Oral Co-administration of Curcumin and Piperine on the Development of Induced Cutaneous Tumors in Mice

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ABSTRACT

Turmeric is a yellow powder from the rhizomes of a herbaceous plant, *Curcuma longa.* Curcumin, a major component of turmeric, is a polyphenol that has anti-oxidant, anti-inflammatory and antiproliferative properties that give it an anti-tumor effect. Administration of oral curcumin appears few effective because of its low bioavailability but adjuvants such as piperine present in black pepper may improve this bioavailability. The aim of the present study is the evaluation the effect of curcumin and piperine-based regimen on the development and growth of chemo-induced cutaneous tumors in Swiss albino mice. A single topical application of 400 nmol of DMBA (7,12-dimethylbenz (a) anthracene) is followed one week later by the application twice a week of 5 nmol of TPA (12-O-tetradecanoylphorbol-13-acetate) during 10 weeks. DMBA/TPA-induced papillomas are evaluated in mice fed a standard diet or curcumin (0.5%) and piperine (0.005%) diet. Curcumin significantly inhibited the tumorigenic effect of DMBA and TPA by decreasing tumor incidence by 50%, tumor multiplicity by 38% and tumor volume by 90%. In addition, the histological study showed that the curcumin and piperine diet attenuated epidermal changes caused by DMBA/TPA treatment such as hyperplasia, cellular atypia and hyperkeratosis. Our study demonstrated that oral co-administration of curcumin and piperine has a significant inhibitory effect on DMBA/TPA-induced cutaneous tumorigenesis. Piperine, by increasing the bioavailability of curcumin, improves its chemoprotective and chemo-preventive efficacy against tumor development.

Keywords: Curcumin, DMBA, Papillomas, Piperine, TPA.

INTRODUCTION

Turmeric is a yellow powder derived from crushed rhizomes of a herbaceous plant native to Southeast Asia, *Curcuma longa* (Zingiberaceae family). It has been used for millennia as a spice and also in traditional Ayurvedic and Chinese medicine [1, 2]. Turmeric is traditionally used for the treatment of various affections such as respiratory disorders, liver disorders, rheumatisms, sprains, ulcers and wounds [2]. Although its medicinal properties have been known for millennia, the mechanism of action of turmeric and its bioactive components have been discovered only recently. Curcumin (diferuloylmethane), a natural polyphenol, is the major component of turmeric [3]. In recent decades, this molecule has been the subject of a large number of studies that have shown, among other things, its antioxidant, anti-inflammatory and anti-proliferative properties [4, 5].

It has been shown that with these properties, curcumin can act as an anti-tumor substance [6]. Preclinical studies have shown that oral administration of curcumin has an inhibitory effect on the development of many types of cancers in rodents; colorectal cancer, cancer of the pancreas, mouth, stomach, aerodigestive tract, liver, breast, prostate, lung, head and neck, skin [7, 8].

The study of the effect of curcumin on carcinogenesis is carried out by various techniques including tumor induction in rodents. In the case of cutaneous tumors, the two-stage model of skin carcinogenesis in mice is particularly effective. This technique is based on the topical administration of a mutagenic initiator followed by a pro-inflammatory promoter. The resulting tumors are benign papillomas that may progress to invasive squamous cell carcinomas [9].

Works based on this method showed that curcumin inhibited the growth of induced cutaneous tumours as well in the case of a topics application as of an oral administration. However, it seems that oral curcumin is less effective because of its low bioavailability mainly due to poor absorption, metabolism and elimination too fast [1]. Piperine, a major plant alkaloid found in black pepper [10], can improve the bioavailability of curcumin by inhibiting hepatic and intestinal glucuronidation [11, 12].

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The aim of this work is to evaluate the effect of concomitant oral administration of curcumin and piperine on the development of epidermal tumors induced in mice by topical treatment with DMBA (7,12-dimethylbenz [a] anthracene) followed by repeated applications of a pro-inflammatory phorbol ester, TPA (12-O-tetradecanoylphorbol 13-acetate).

MATERIALS AND METHODS

Animals

The Swiss albino mice come from a breeding carried out in the Biology Department of the Faculty of Sciences Ben M'Sik. The mice are housed in plastic cages, provided with water and food ad libitum. Male mice aged 10 weeks are selected and quarantined 2 weeks before the start of the experiment. After the quarantine, the mice are distributed into 3 groups of 8 individuals each: Control group, DMBA/TPA group, DMBA/TPA / Curcumin group.

Diet and treatments

The control diet consists of standard pellets (INAAM) composed of (g / kg) 160 g of protein; 20 g of fat; 70 g of mineral matter; 3.5 g of phosphorus; 9 g of calcium and vitamins A, D3 and E. The curcumin diet is obtained by incorporating 0.5% curcumin (Sigma-Aldrich) and 0.005% piperine (Sigma-Aldrich) into the control diet. The curcumin diet is prepared every 2 weeks in our laboratory and stored at -20°C before use.

The Control group and a carcinogen-treated group (DMBA/TPA) are fed throughout the experiment with standard granules. The DMBA/TPA/Curcumin group receives the curcumin diet one week before the start of treatment and throughout the experiment. Each mouse of the DMBA/TPA/Curcumin group receives on average 17.5 mg of curcumin per day.

Treatment with carcinogens is based to the model of carcinogenesis in two stages described by Filler *et al*. [13] and adapted by our team. Two days before the start of treatment, an area of the dorsal skin (4 x 3 cm) of each mouse is shaved with electric clipper. The groups DMBA/TPA and DMBA/TPA/Curcumin are treated with the DMBA [7,12-dimethylbenz(a)anthracene (Sigma-Aldrich)] mutagenic agent (initiation) by a single topical application of 400 nmol of DMBA in 200 µl of acetone and then quarantined for one week. Then, both groups receive topical treatment with the TPA [12-O-tetradecanoylphorbol-13-acetate (Sigma-Aldrich)] promoter (5 nmol of TPA in 100µl of absolute alcohol) twice weekly for 10 weeks. The mice of the control group receive a topical application of 200 µl of acetone for one week and then a twice-weekly application of absolute ethanol (100 µl) for 10 weeks.

The mice are weighed and their skin is thoroughly inspected once a week. As they appear, the papillomas are counted and measured using a vernier caliper. The tumor volume is calculated according to the formula: 0.52 x (width) ² x (height).

The experimental procedures, animal handling, were done according to the "Guide for the care and use of laboratory animals" (NRC, 2011).

Histological study

The animals are anesthetized with ether and the dorsal skin is removed, rinsed in saline and then cut into strips. The skin samples are fixed by immersion in 10% buffered formalin. The pieces are dehydrated in ethanol, clarified in toluene and embedded in paraffin. The samples are cut into histological sections of 5 µm thickness which will be stained with hematoxylin and eosin. The preparations are observed and photographed by a light microscope (Leika DMSA) equipped with a camera (Leica ICC50 HD).

The χ^2 test and the Student test are used for statistical analysis.

RESULTS

Evolution of body weight of animals during experimentation

Figure 1 shows the change in body weight in the three groups of mice. Control mice show a steady increase in weight throughout the duration of the experiment. In the DMBA/TPA group and the DMBA/TPA/Curcumin group, the change in weight is less regular; periods of weight gain are separated by periods of weight loss especially from the sixth week of promotion. There is no significant difference between the two groups of mice treated with carcinogens. Note also that overall the weight of the treated mice is significantly lower than that of the control mice ($P < 0.05$) (Fig. 1).

Effect of curcumin on the incidence of chemically-induced skin tumors

Treatment with DMBA (an application of 400 nmol) followed by TPA (5 nmol, twice weekly) for 10 weeks resulted in the appearance of benign tumors or cutaneous papillomas on the back region of the treated mice of both groups (DMBA/TPA and DMBA/TPA/Curcumin). The tumor incidence and the time of onset of the first tumors, however, vary between the two groups (Fig. 2). The DMBA/TPA group develops the first tumors during the 6th week of promotion while the DMBA/TPA / Curcumin group shows its first tumors during the 7th week of treatment. All of the mice in the DMBA/ TPA group (100%) have tumors as early as the 8th week of promotion against 50% of the mice in the DMBA/TPA/Curcumin group (Fig. 2). Thus, there is a slowdown in the appearance of chemoinduced tumors as well as a significant decrease ($P < 0.05$) of tumor incidence (50%) in the group of curcumin-treated mice.

Effect of curcumin on the multiplicity and volume of chemically induced skin tumors

Papillomas that appeared in the DMBA/TPA and DMBA/TPA/Curcumin groups are counted and measured weekly. The mice of the DMBA/TPA group develop a number of tumors significantly greater than that of the DMBA/TPA/Curcumin group. Indeed, after 10 weeks of promotion, the mice treated without curcumin develop on average 9.25 ± 2.6 tumors whereas the mice that received curcumin develop an average of 5.75 ± 1.65 tumors (Fig. 3 and 5). Curcumin resulted in an inhibition of tumor multiplicity of 38%.

After 10 weeks of promotion, the maximum tumor volume observed is 40 mm3 and was recorded in a mouse from the DMBA / TPA group. In mice treated with curcumin (DMBA/TPA/Curcumin) the maximum recorded volume is 6 mm^3. The mean tumor volume of the DMBA/TPA group is 25.5 ± 6.31 mm^3. It is significantly higher than the tumor volume of the DMBA/TPA/Curcumin group which is 2.48 ± 0.63 mm^3 (Fig. 4 and 5). Treatment with curcumin resulted in inhibition of tumor growth of 90.28%.

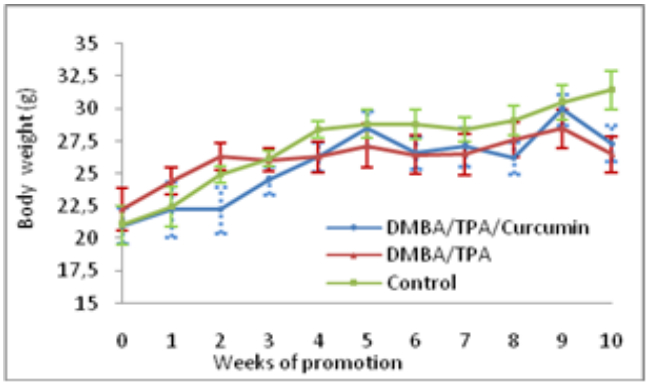

Figure 1: Evolution of body weight of control mice, and mice treated with DMBA/TPA combined with a standard diet or a diet based on curcumin and piperine. Each point represents the mean value ± SD (N = 8).

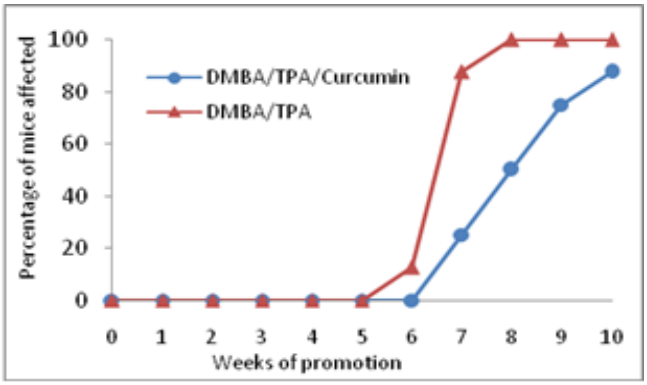

Figure 2: Tumor incidence. Representation of the percentage of mice that developed tumors in DMBA/TPA treated animals in combination with a standard diet or a curcumin-piperine diet (N = 8).

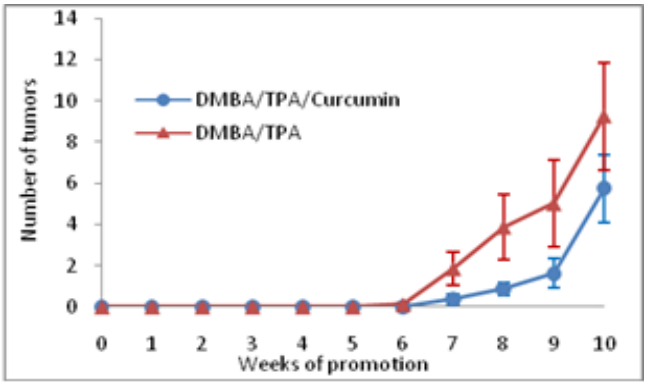

Figure 3: Mean number of tumors per mouse in DMBA/TPA treated animals in combination with a standard diet or a curcumin-piperine diet. Each point represents the mean value ± SD (N = 8).

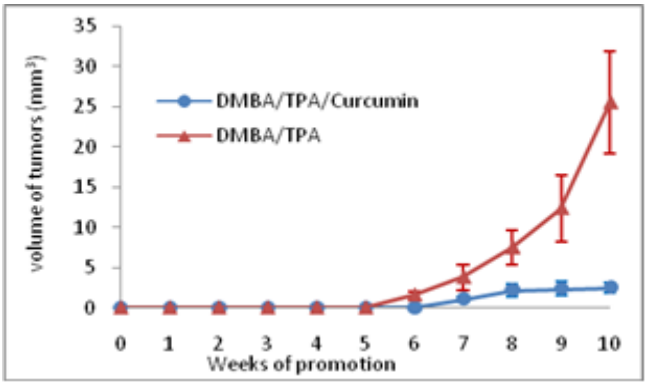

Figure 4: Mean tumor volume per mouse in both treated groups (DMBA/TPA, and DMBA/TPA/ Curcumin). Each point represents the mean value ± SD (N = 8).

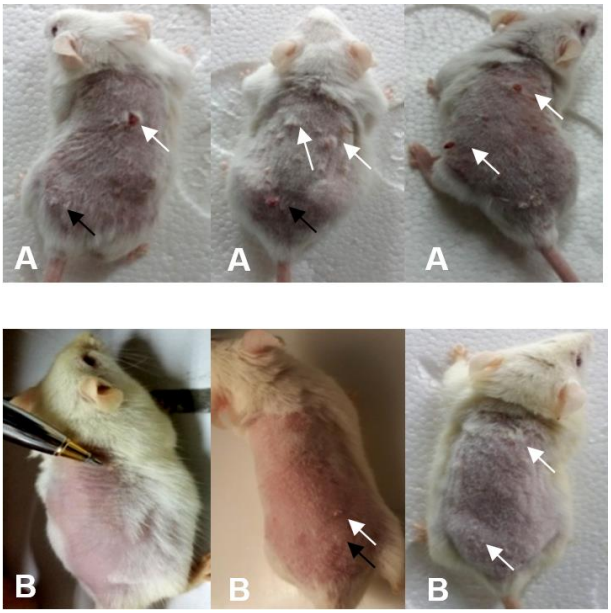

Figure 5: Photographs showing the papillomas (black arrow) developed by mice in the DMBA/ TPA group (A) and the DMBA/TPA/Curcumin group (B).

Effect of curcumin on skin histological changes induced by DMBA / TPA treatment

The back skin of control mice is characterized by a thin epithelium (2-3 cell layers) associated with hair follicles and sebaceous glands supported by connective and adipose tissues (Fig. 6A and 6B).

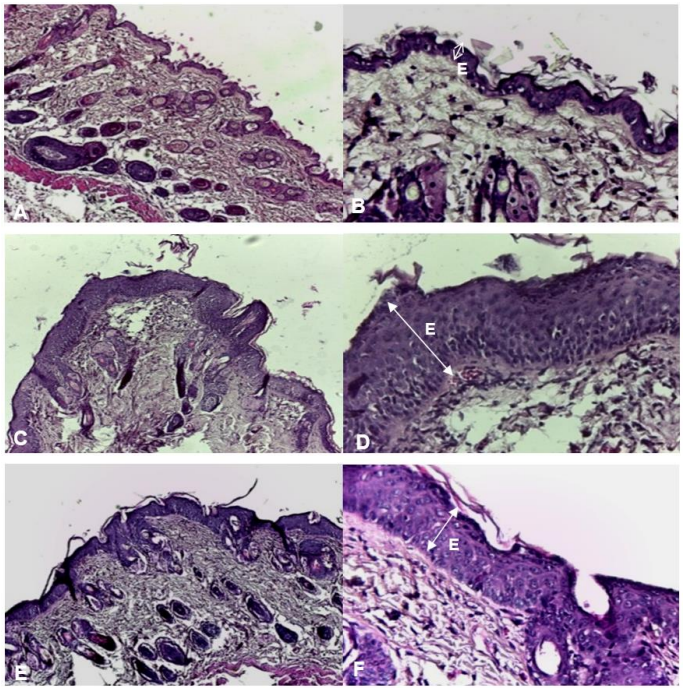

Figure 6: Photomicrographs of cross-sections of mouse skin, stained with Haematoxylin / Eosin illustrating the epidermis (E) in the control group (A and B) and the hyperplasia observed in the group DMBA / TPA (C and D) and in the group DMBA / TPA / Curcumin (E and F); (A, C, E: Gx100, B, D, F: Gx400).

All DMBA/TPA-treated mice show significantly different histological characteristics of the skin than untreated mice. These animals develop epidermal hyperplasia due to repeated applications of the TPA promoter that stimulates the growth of DMBA-initiated cells. This results in an increase in the number of nucleated cell layers and an overall increase in the thickness of the epidermis. It is noted that the hyperplasia is less important in mice of the DMBA/TPA/Curcumin

group who receive curcumin in their diet during skin treatment (Fig. 6C, 6D, 6E, 6F).

The epidermal cells of the two treated groups with DMBA/TPA show abnormalities. In particular, there are cellular hypertrophies, a variability in nuclear size and shape, an increase in the nucleo-cytoplasmic ratio, a hyper-chromatism, poly-nucleoled nuclei, mitoses in the middle and superficial layers of the epidermis and an increase in the number and the size of the blood vessels. The frequency and importance of these abnormalities are less important in the group whose diet contains curcumin (DMBA/TPA/Curcumin) (Fig. 7A and 7B).

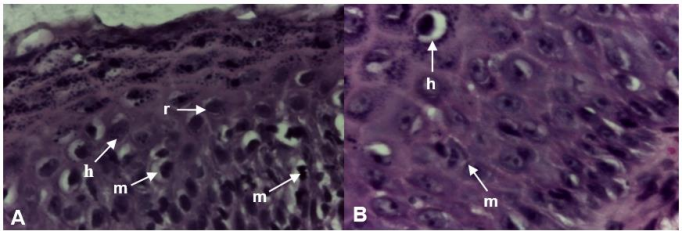

Figure 7: Photomicrographs of Hematoxylin / Eosin stained skin cross-sections illustrating mitosis (m), cellular hypertrophy (h), high nucleo-cytoplasmic ratio (r). A: DMBA/TPA group; B: DMBA/TPA/Curcumin group (G x1000).

Effect of curcumin on chemoinduced papillomas

The treatment of the skin with the promoter agent leads to the development of cutaneous excrescences called epidermoid papillomas. They are benign tumors resulting from the projection of the dermis and the epidermis at the surface consisting of a stromal nucleus surrounded by a hyperplastic epidermis. A dysfunction of keratinocytes that synthesize too much keratin leads to a thickening of the stratum corneum or hyperkeratosis. Chemoinduced papillomas developed in mice consuming curcumin (DMBA/TPA/Curcumin group) have a thinner stratum corneum and a much smaller volume (Fig. 8C, 8D) than papillomas from mice consuming a standard diet (DMBA/TPA group) (Fig. 8A and 8B).

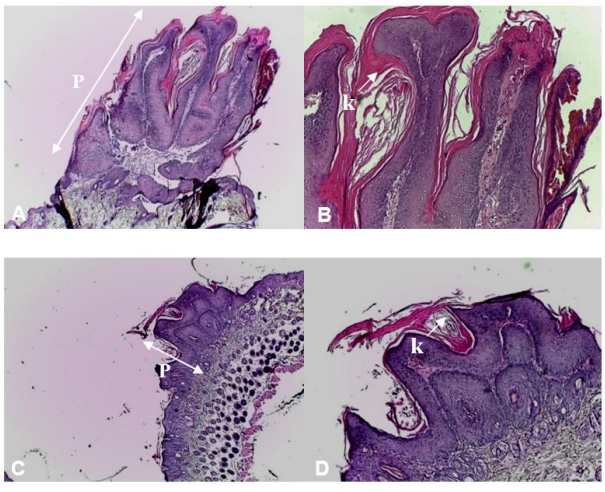

Figure 8: Photomicrographs of Hematoxylin / Eosin stained skin cross sections showing papillomas (P) and hyperkeratoses (k) observed in the DMBA/TPA group (A: Gx40, B: x100) and in the DMBA/TPA/Curcumin group (C: G x 40, D: G x 100).

DISCUSSION

This study was conducted to evaluate the effect of oral co-administration of curcumin and piperine on the development of chemically induced skin tumors in mice Swiss albino by the classic protocol two-step carcinogenesis [9]. Single topical administration of the DMBA mutagenic agent (400 nmol) was followed by repeated applications of the TPA pro-inflammatory agent (5 nmol twice weekly for 10 weeks). One group of treated mice (DMBA/TPA/Curcumin) received a diet with curcumin (0.5%) and piperine (0.005%) while another group (DMBA/TPA) received a standard diet.

Our study showed that oral co-administration of curcumin and piperine significantly reduced the effect of the DMBA initiator and the TPA promoter on the epidermis. We noted a significant decrease in tumor incidence (50%), an inhibition of tumor multiplicity of 38% and a reduction of tumor volume of 90.28% in mice given curcumin and piperine. Thus, with a diet low in curcumin (0.5%), overall our data are comparable with those obtained on the same animal model by a topical administration of curcumin [14]. Note that it has been reported that without adjuvant oral administration of curcumin is less effective than topical use because of its low bioavailability [1]. Our results support the fact that the use of piperine as adjunct to curcumin significantly improves its efficacy [15, 16] by increasing its intestinal absorption and length of stay in tissues [12]. In this sense, authors [17] have demonstrated that concomitant administration of curcumin and piperine increased the serum curcumin concentration by 2000% in humans and 154% in rats.

Otherwise, oral curcumin has been shown to also inhibit xenograft-induced skin tumors [18, 19] and chemically-induced tumors in other organs such as colon in rodents [20, 21]. In addition to oral or topical administration, curcumin can be administered subcutaneously, intravenously, intraperitoneally with more or less satisfactory results [22].

The histological study carried out in this work showed that the skin of the mice of the two groups treated with DMBA/TPA had developed epidermal hyperplasia and papillomas accompanied by cyto-nuclear atypia, angiogenesis and hyperkeratosis as reported by other teams [9, 23]. These histological characteristics are less important in mice fed a diet based on curcumin and piperine. Our results are in agreement with the work of the team Ning *et al.* [24] who demonstrated that curcumin caused a significant decrease in the incidence of papillomas, hyperplasia, dysplasias and angiogenesis in hamster treated with DMBA/ TPA combined with topical administration of curcumin. Curcumin has been shown to have significant anti-angiogenic and anti-metastatic effects both *in vitro* and *in vivo* [25, 26].

Curcumin exerts its anti-tumoral effect by acting on the 3 stages of tumor development: initiation, promotion and invasion. It acts on initiation through protection against mutations and detoxification of carcinogens [7]. It acts on tumor promotion via its anti-inflammatory activity which is exerted notably by the inhibition of the signal activating the NF-κB transcription factor and the expression of cyclooxygenase 2 [27, 28]. NF-κB is known to be induced by promoters such as TPA [29]. Curcumin acts on tumor growth by down-regulating signaling pathways associated with cell growth such as the PI3K / AKT / mTOR pathway, MAPKinase pathways and the JAK / STAT pathway [4, 30].

CONCLUSION

The study described above demonstrated that oral co-administration of curcumin and piperine has a significant inhibitory effect on tumor growth induced by DMBA and TPA treatment. Piperine, by increasing the bioavailability of curcumin, improves its chemoprotective and chemo-preventive efficacy against tumorigenesis.

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