Induced Lesions by Dimethyl Benzanthracene Subcutaneous Inoculated in Mouse

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Abstract. Dimethyl benzanthracene subcutaneous administration in mice leads to appearance of some necrosis zones with about 1 cm diameter at inoculation place. At necropsic examination effected to 15 months from experiment star were observed: necrosis zones and cutaneous punctiform erosions in dorsal region, in animals from lots with subcutaneous inoculation with dimethyl benzanthracene (lots 1, 2 and 3); increasing of adipose tissue layer in animals whose food was with carotenoids (lots 4 and 5). Histopathologically were put into evidence the epidermis hyperplasia and hypertrophy in animals of lot with dimethyl benzanthracene subcutaneous inoculation (lot 1); images of dysplasia and metaplasia, changes considered preneoplasic. In animals of lots 2 and 3 (with carotenoid supplement in food), histopathologically were observed only small epithelial proliferations as buttons’ shape and thickening of dermis papillae basis on fibrous connective tissue. In animals from the lot with subcutaneous dimethyl benzanthracene administration, without carotenoid supplement in food (lot 1), histopathologically appears esophagus gastric mucous membrane hyperkeratosis, aspect that is not present in case of animals from the lots with carotenoids in food (lots 2, 3, 4 and 5). The protector effect of carotenoids is observed both on skin of inoculated places with dimethyl benzanthracene and also on esophagus type gastric mucous membrane level. We underline the first time demonstration of protector and healing effect of rodoxanthin.

Key words: dimethyl benzanthracene, rodoxanthin, canthaxanthin, induced lesions, mice

INTRODUCTION

Dimethyl benzanthracene (DMBA) is a hydrocarbon often met in environment, which results from deficient combustion of fuels. DMBA accumulates in air and water from where can reach over plants and superior organisms, as is the human. As chemical point of view, DMBA is an aromatic hydrocarbon, formed by four carbon rings. In benzanthracene group are part three compounds: benzanthracene; 7,12 dimethyl benzanthracene and 9,10 dimethyl-1,2-benzanthracene.

In the condensed cigarette smoke was found a quantity of 0.03 to 4.6 µg/gram of benzanthracene. The benzanthracene was also detected in soil 390-1500 µg/kg, in drinking water (1-23.2 µg/1000 m³) and in surface waters (4.3-185 µg/1000 m³).

The benzanthracene content in aliments depends on preparation method. In bacon and smoked sausages were found 0.4-9.6 µg/kg of benzanthracene, the smoked fishes contained 0.02-2.8 µg/kg of benzanthracene, and in the very smoked bacon until 12 µg/kg of benzanthracene. In potherbs were found next benzanthracene quantities: salad 4.6-15.4 µg/kg; tomatoes 0.3 µg/kg; spinach 16.1 µg/kg; different uncooked or refined vegetable oils 0.5-13.5 µg/kg of benzanthracene (Suciu D., 1980).

The benzanthracene produces cancer in experimental conditions. There observed apparition of intestinal adenomas, hepatomas, tumors of gastro-intestinal tract, epitheliomas,
sarcomas and carcinomas in mouse after administration or painting with benzanthracene (Suciu D., 1980).

Other authors obtained: cutaneous papillomas, carcinomas with squamous cells, basal cell tumors, keratoacanthomas and adenomas of sebaceous glands. Before neoplasmas’ apparition we can observe non-neoplastic lesions: inflammations, ulcerations, hyperkeratosis, acanthosis and cutaneous necrosis (National Toxicology Program, 1986).

**Rodoxanthin** is a carotenoid pigment less studied, in specialty literature being found only summary information about it. As chemical point of view it is part of xanthophylls, near of canthaxanthin and astaxanthin.

The antitumoral effect of rodoxanthin was not yet studied, but taken into consideration the structural similarities the physical and biochemical characteristics could have antineoplastic effect.

The levels where it can intervene in the antitumoral process could be: having antioxidative properties the rodoxanthin is capable to protect DNA by distortions given to oxygen free radicals, stimulates antineoplastic immune system that is capable to prevent tumor proliferation at any level, improves and controls intercellular communication at gap junction level.

**Canthaxantine** is a natural pigment from xanthophylls group, which are oxygenated derived, normally without any A vitamin function (Simposon K.L., 1982). The benefic functions of canthaxantine are: benefic antioxidant effect in some cardiovascular diseases, of immune system, neuro-degenerative affections and anti-inflammatory effects (www.classes.yale.edu/chem221b/probsets/ps-302/ps2-srp02/ps2.pdf).

Per os administration of canthaxanthin or of β-carotene, daily during 14 days, reduced significantly the size of cutaneous papillomas induced by 9,10-dimethyl-1,2-benzanthracene, fact experimentally demonstrated (Katsumura N et al, 1996). The canthaxanthin and retinol-palmitate modulate cellular response and tumor growing (Rybski J.A. et al 1991).

In present work paper we proposed to study the phases precursory of tumor transformation of skin structures. To monitoring the tumor development we try to reproduce experimentally skin tumors administrating subcutaneous in lab animals a substance with known carcinogen properties, as could be the dimethyl benzanthracene. The preneoplasic changes’ and first tumor development stages emphasis is useful also to study the effect of some substances with antioxidant action and with antitumoral effects, substances which exert a protector effect especially on epithelia, as are the carotenoids pigments. Thus, we propose also to study the effects of some substances from carotenoid pigment group, used more and more frequent in treatment of some lesions especially those epithelial ones. From carotenoid category pigments we chose to study protection effect of epithelia exerted by: canthaxanthin and rodoxanthin.

**MATERIALS AND METHODS**

For this experiment were used 40 mice, females, with average weight of 20 g and age about 50 days. The mice were distributed in 5 lots thus:

- **lot 1**: ten mice, subcutaneous inoculated with 0,1 ml sol. dimethyl benzanthracene;
- **lot 2**: ten mice, subcutaneous inoculated with 0,1 ml sol. dimethyl benzanthracene, and per os get daily 0,2 ml canthaxantin;
- **lot 3**: ten mice, subcutaneous inoculated with 0,1 ml sol. dimethyl benzanthracene, and per os get daily 0,2 ml rodoxanthin;
- **lot 4**: five mice, which get daily per os 0,2 ml canthaxanthin, witness lot for canthaxanthin;
- **lot 5**: five mice, which get daily per os 0,2 ml rodoxanthin, witness lot for rodoxanthin.

At the experiment start the mice were clinically healthy. During experiment the animals were fed with bread, powder milk and barley and wheat seeds. The animals from lots 2, 3, 4 and 5 get daily carotenoids as food supplement.

The used carotenoid pigments were rodoxanthin and canthaxanthin. They were dissolved in vegetal oil (soy oil), daily dose being 40 \( \mu \text{g/kg} \) body weight, that means 0.2 ml/animal. The carotenoid administration in food was done before a week cancerigenic substance inoculation, to be accumulated in tissues. The carotenoid pigments were daily administrated during experiment process.

Cancerigenic substance, dimethyl benzanthracene powder, was dissolved in 45.6 mg/10 ml acetone then was subcutaneous administrated. There were done two administrations of dimethyl benzanthracene, at 1 month interval, dose being of 0,1 ml/mouse/administration. The cancerigenic substance administration was done in dorsal region, submitted to local cleaning before each administration.

Animals from experimental lots were clinically examined during entire experiment at regulate time intervals.

After 15 weeks from the first cancerigen inoculation from each experimental lot were slaughtered 5 mice. It was effected necropsic examination, collecting skin and other tissues’ fragments for histopathological examination.

The skin fragments were collected form the zones in which were done inoculations, then were fixed in 10% buffered formaldehyde. To obtain histopathologic preparations the samples were processed by paraffin technique in seriated sections of 4-6 \( \mu \text{m} \), and then were stained by Tricrom Masson and Haematoxylin-Eosin method.

**RESULTS AND DISCUSSION**

At 15 weeks after first inoculation was done slaughtering from each lot.

The observed lesions were next ones:

- **Animals from lot 1**, whom was administrated only cancerigenic substance, the skin from scapula region presents small necrosis focuses, subcutaneous was observed light edema of connective tissue and musculature had watery aspect. At inoculation place were observed skin adherences to subjacent tissues. The spleen was lightly increased with capsule tense and rounded edges.

- **Animals from lot 2**, whom was administrated subcutaneous cancerigenic substance and canthaxantine in food, along backbone, one side to another one, present cutaneous punctiform erosions, the skin was thickened on a surface of about 0.2-0.5 cm.

- **Animals from lot 3**, whom was administrated cancerigenic substance and rodoxanthin in food, were observed skin erosions and necrosis, relatively profound along backbone. The subcutaneous connective tissue had jelly aspect. The spleen was light increased in volume.

- **Animals from lots 4 and 5**, whom only the food was supplemented with carotenoids, had uniform, short, smooth and very shiny hair. Subcutaneous they presented a very abundant adipose tissue. There not observed lesions of tissues and organs, neither difference of shape, color, size, consistence or reports between organs.

After examination of histopathological preparations were observed next:

- **Animals from lot 1**, whom was administrated cancerigenic substance, we observed changes at skin level:
epidermis: appears a zone thick because of hyperplasia and hypertrophy of stratum spinosum cells, in this zone is present basal membrane. In some microscopic fields appears an almost total disorganization of epidermis, where is observed the basal membrane interruption, and epidermal epithelium cells are anarchic disposed, without possibility to differentiate the epithelium layers. The epithelial cells appear light increased in volume, with big, clear, round or oval nuclei, cytoplasm light stained, intercellular connections from stratum spinosum are not very evident, without possibility to delimitate clearly the basal stratum. In some places the epidermis cells infiltrate dermis, being present even young epithelial cells disposed in dermis. dermis: is formed by thick collagen fibers, and cellular population is represented mainly by mature connective cells, respectively fibrocytes.

dermis: do not present changes.

- Animals from lot 2, whom was administrated subcutaneous cancerigenic substance and canthaxantine in food, presented next changes at skin structures’ level:
  epidermis: is thin, but in some places appear proliferation images of epithelial cells as small button shapes or even papillary ones formed only by epithelial cells. These cells appear more clear, bigger, with bigger oval nuclei. In zones with normal epithelium, this one is very thin, with flattened nuclei and cells, formed by 2-3 cell rows.
  dermis: do not present structural changes.
  hypodermis: contains in its structure well developed adipose tissue.

- Animals from lot 3, whom was administrated subcutaneous cancerigenic substance and rodoxantine in food, present next changes at skin structure level:
  epidermis: do not appear remarkable changes of epidermis.
  dermis: in some places appear thickening of dermal papillae through fibrous connective tissue. Were observed focuses more or less circularly, formed by fibrous connective tissue.
  hypodermis: without lesions, adipose tissue being well developed.

- Animals from lots 4 and 5, whom was supplemented the food with carotenoids, presented normal aspect of skin, with very thin epidermis, dermis formed by a fine loose connective tissue, and the hypodermis formed by a very well developed adipose tissue.

Dimethyl benzanthracene produces cancer in experimental conditions. It was observed apparition of intestinal adenomas, hepatomas, tumors of gastro-intestinal tract, epitheliomas, sarcomas and carcinomas in mice after administration or painting with dimethyl benzanthracene (35). The experimental protocols are extremely different, the result depending on quantity of administrated cancerigenic substance, administration place, manner and experiment duration.

Dimethyl benzanthracene in case of repeated and prolonged contact with organism is capable to induce methaplasia and cellular displasia, followed by neoplastic cell and tissue formation. The most frequent met neoplasias are those cutaneous ones, papillomas, carcinomas with squamous cells, basal cell tumors, keratoacanthomas, adenomas of sebaceous glands etc. (Homma W et al, 1983; Katsumura N. et al 1996; Larkin H.A., 1994; National Toxicology Program, 1986) Before neoplasma apparition we can observe non-neoplastic lesions: inflammations, ulcerations, hyperkeratosis, achantosis and cutaneous necrosis (National Toxicology Program, 1986). In our experiment case was observed that according to specialty literature the benzanthracene produces in cutaneous level initially macroscopic visible non-neoplastic lesions, respective ulcerations and cutaneous necrosis (lots 1, 2 and 3), lesions considered as being precursory for neoplasmas’ apparition.

More evident cutaneous lesions appeared only in mice, which were subcutaneous inoculated with dimethyl benzanthracene without getting carotenoid supplement in food (lot 1). In these animals at cancerigenic inoculation place appeared extensive cutaneous necrosis
zones, which were very evident after each administration and their evolution to heal was very slowed. In animals from the lots subcutaneous inoculated with dimethyl benzanthracene and whose food was supplemented with carotenoids (lots 2 and 3), both after first and the second inoculation appeared cutaneous necrosis zones with fast evolution to heal.

Histopathologically, at 15 weeks after inoculation only in animals from lot 1 were observed hypertrophy, hyperplasia and even metaplasia of epidermis cells on a more extensive skin portion. In animals from lots subcutaneous inoculated with dimethyl benzanthracene and whose food was supplemented with carotenoids, the microscopic images reveal only discrete epithelial hyperplasia as small papillae or buttons’ shape (lot 2), respective thickness of dermis papillae due to proliferation of subepithelial fibrous connective tissue (lot 3).

In last days, the carotenoids and their metabolites are used more and more to prevent and treat some affections especially the epithelial cancer and to heal the scars (Tyler V. et al, 1993). The carotenoid role to heal scars was observed in animals inoculated with cancerigenic substance and whose food was supplemented with carotenoids (lots 2 and 3). In these animals the zones with cutaneous lesions (ulcerations, necrosis, denudations) appeared after subcutaneous administration of cancerigenic substance were rapidly reduced to a size equal to that of a needle head, while in animals from lot 1 (subcutaneous administration of DMBA without carotenoid supplement in food) the zones with lesions were reduced to 3-4 mm in diameter; histopathologically, the images show discrete changes in case of animals from lots 2 and 3 (with carotenoid supplement) comparatively to images obtained from animals, which get not canthaxanthin or rodoxanthin.

The protector effect exerted by substances from carotenoid pigment group on epithelia can be put into evidence also by aspects met to esophagus type gastric mucous membrane level. Thus, is remarked the mucous membrane hyperkeratosis from this zone in animals from lot 1 (with subcutaneous inoculation with dimethyl benzanthracene) comparatively to the same mucous membrane type from animals of lots 2 and 3 (subcutaneous inoculated with dimethyl benzanthracene and carotenoid supplement in food), which presented uniform stratum corneum very adherent ti epithelium, without exfoliation tendencies.

The carotenoid pigments are stocked at adipose tissue level of animals and this aspect was observed in the mice of lots 4 and 5, which get carotenoids in food and which presented very well developed subcutaneous adipose tissue.

The antitumoral effect of rodoxanthin was not yet studied, taking into consideration the structural, physical and biochemical similarities with other pigments of xanthophylls group, as is canthaxantine (Andrei Sanda et al, 2003) and it is possible that also this pigment to have antineoplasia effect. From observations done by us, from the experiment start until present days the rodoxanthin protector effect on epithelium is identical with that one of canthaxanthin.

**CONCLUSIONS**

Dimethyl benzanthracene subcutaneous administration in mice leads to appearance of some necrosis zones with about 1 cm diameter at inoculation place. At necropsic examination effected to 15 months from experiment star were observed: necrosis zones and cutaneous punctiform erosions in dorsal region, in animals from lots with subcutaneous inoculation with dimethyl benzanthracene (lots 1, 2 and 3); increasing of adipose tissue layer in animals whose food was with carotenoids (lots 4 and 5).
Histopathologically were put into evidence the epidermis hyperplasia and hypertrophy in animals of lot with dimethyl benzanthracene subcutaneous inoculation (lot 1); images of dysplasia and metaplasia, changes considered preneoplastic.

In animals of lots 2 and 3 (with carotenoid supplement in food), histopathologically were observed only small epithelial proliferations as buttons’ shape and thickening of dermis papillae basis on fibrous connective tissue.

In animals from the lot with subcutaneous dimethyl benzanthracene administration, without carotenoid supplement in food (lot 1), histopathologically appears oesophagus gastric mucous membrane hyperkeratosis, aspect that is not present in case of animals from the lots with carotenoids in food (lots 2, 3, 4 and 5).

The protector effect of carotenoids is observed both on skins of inoculated places with dimethyl benzanthracene and also on esophagus type gastric mucous membrane level. We underline the first time demonstration of protector and healing effect of rodoxanthin.

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