VASCULAR ALTERATIONS IN HAMSTER CHEEK POUCH DURING DEVELOPMENT AND PROGRESSION OF SQUAMOUS NEOPLASIA INDUCED BY DMBA IN ASSOCIATION WITH UV LASER RADIATION

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ABSTRACT - The aim of the present work is to analyze the histological changes on hamster buccal mucosa caused by the topical use of 7,12-dimethylbenzanthracene (DMBA) and exposition to a 220 µJ/pulse nitrogen laser light (@ 337 nm) at an average power of 2,3 mW. Twenty-one hamsters divided into two experimental groups were treated six times with DMBA. One hamster was kept as control. Group I was composed by ten hamsters and was submitted only to DMBA. Group II, also with ten hamsters, received the same treatment as group I and was exposed to the laser radiation. The time duration of each irradiation section was 10 seconds. All the treatment happened in alternated days.
For the histological analysis two biopsies were done. Both experimental groups presented dilatation of vessels, thickening of the epithelial tissue and the presence of inflammatory infiltrates. The preliminary results indicates that, for group II, the number of dilated vessels and its new area are much more significant than for group I.

INTRODUCTION

The classic hamster cheek pouch model of squamous cell carcinoma has been used to simulate human oral carcinoma in the transition from dysplasia to carcinoma *in situ* to invasive cancer[1].

Genetic or molecular changes can be induced in laboratory by chemical substances, for example dimethylbenzanthracene or ultraviolet radiation[2,3]. The application of dimethylbenzanthracene in hamsters can develop hyperplasia, dysplasia and invasive carcinoma.

The association of dimethylbenzanthracene and the ultraviolet radiation was used by Bestak and Halliday to promote squamous cell carcinoma in mice[4].

MATERIALS AND METHODS

Twenty-one adult male Syrian hamsters (*Mesocricetus Auratus*) weighing 200 g to 210 g were housed 5 per cage and fed standard laboratory chow and water *ad libitum.* The animals were divided into three groups. Study groups I and II were composed by 10 animal each; one animal was maintained as a control. The control hamster did not receive any kind of treatment.

The carcinogen 7,12-dimethylbenzanthracen e (DMBA) substance, topically applied in the animals by using a paintbrush, was diluted in

acetone at a 0.5%. The 30 DMBA treatment sections for the animals of Group I and II were done every other day, performing a 60-days treatment period.

After that treatment period, the animals of Group II received 6 applications of UV in alternate days. The UV radiation was provided by a 220 μJ/pulse nitrogen laser (@ 337 nm) at an average power of 2,3 mW. The hamster cheek pouch was everted manually and the laser irradiated the lesion region for 10 seconds.

A proper apparatus for the positioning of the hamsters during the treatments was constructed. All experiments were performed in the hamster´s right cheek pouches, with the animals under anesthesia. To induce general anesthesia Zoletil (Virbac) was injected at 0,1 mg/Kg intramuscularly. Zoletil is a combination of tiletamine and zolazepam.

For the histological analysis two biopsies were done in all animals. The first one was done 72 days after the beginning of the experimentation period, coinciding with the end of the UV treatment of Group II. The second one was done 60 days after the first biopsy. Biopsies were done with the animals under Zoletil anesthesia applied at a dosage of 0,2 mg/Kg.

Slides were evaluated by a blinded observer and histological grade was characterized as normal; hyperplasia; mild / moderate or severe dysplasia; carcinoma *in situ*; invasive carcinoma. In addition, the presence of inflammatory infiltrates was pointed out.

RESULTS AND DISCUSSION

Histological analyses for group I after a total of seventy-two days of treatment presented the following changes: thickening of the epithelial tissue due to the accumulation of keratin (hyperkeratosis), hyperplasia, acanthosis and an exacerbated inflammatory process. It was also observed an increasing of the tissular vascularization. The results of the second biopsy for the same group still present hyperkeratosis, acanthosis and the inflammatory process. The increasing number of vases follows the tissular reaction to the continued aggression.

The obtained results for group II presented hyperkeratosis, acanthosis and a more significant hyperplasia in comparison to the alterations in group I. The cellular atypias as well as the drop-shaped projections characterizes the carcinoma *in situ*. It was also noted the thickening of the epithelial tissue and the disorganization of the basal layer followed by an increased number of vases.

Figure 1 presents the histogram of the vase cross-section for the control hamster. Figure 2 presents the histograms of the vase cross section for (a) the first tissue collection of group I, (b) the first tissue collection of group II, (c) the second tissue collection of group I and (d) the second tissue collection of group II.

All groups presented an increasing in both the number and vase cross section in comparison to the control hamster results. Figure 2 (a) and (b) shows no difference between the group treated with DMBA (group I) and the one treated with DMBA associated to the UV exposition (group II). The vase cross-section distribution shown in figure 2 (d) has broadened as compared to the narrower histogram of figure 2 (b).

The clinical aspect of the control hamster cheek pouch can be observed in figure 3 (a) evidencing normal clinical aspects such as color, texture and vascularization. Figure 3 (b) shows a representative photograph of the treated cheek pouch of a hamster from group I after seventy-two days of treatment with DMBA. Besides the normal texture and color, an increase in the tissular volume due to the epithelial hyperplasia is observed.

A significant change in the vascularization and the presence of a neoplasic lesion with irregular texture are evidenced in the photograph of the treated region for a hamster from the group II (figure 3 (c)).

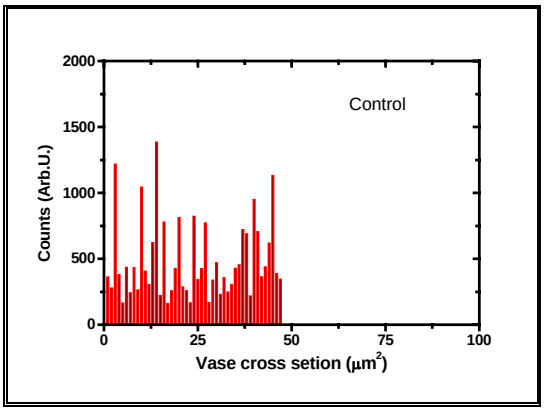

Figure 1: The vase cross-section distribution for the control hamster.

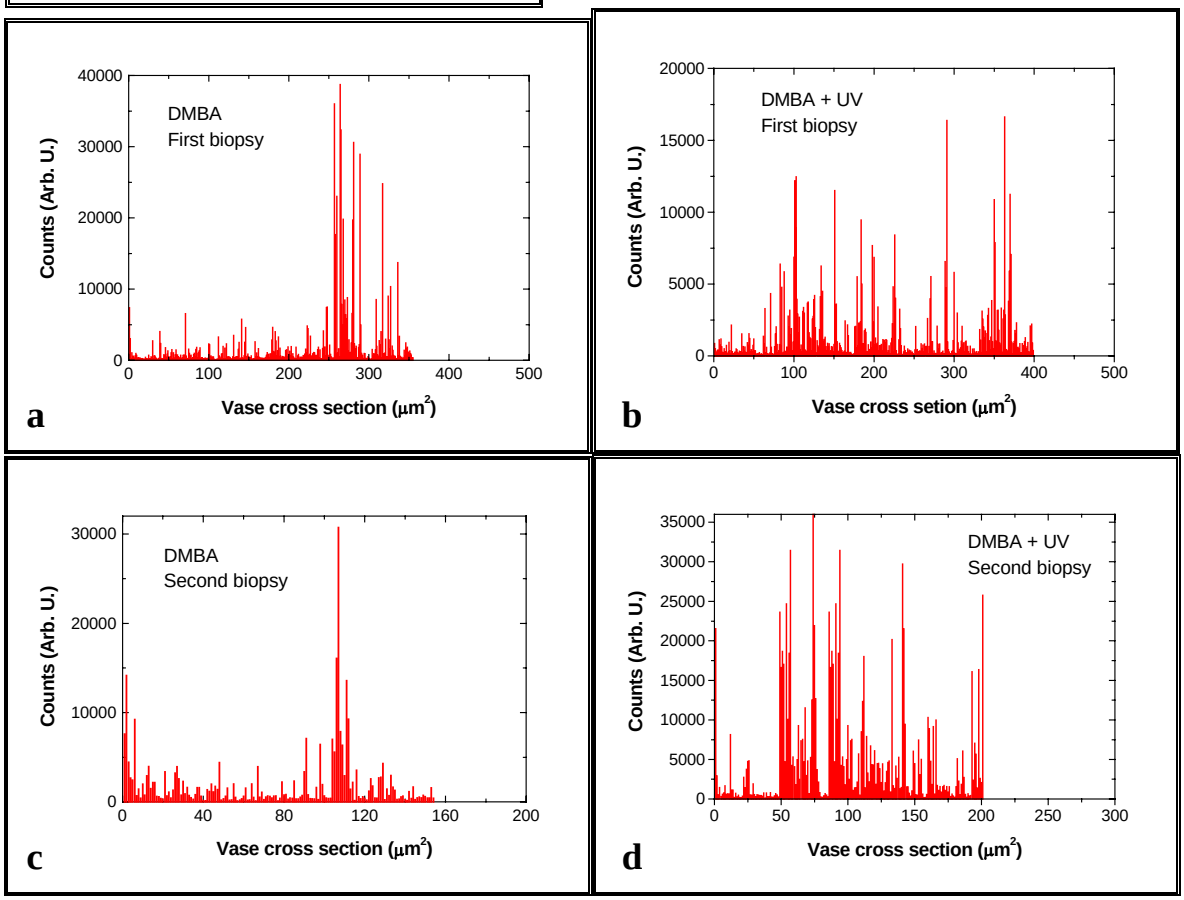

Figure 2: The vase cross-section distribution for each treated group (I and II).

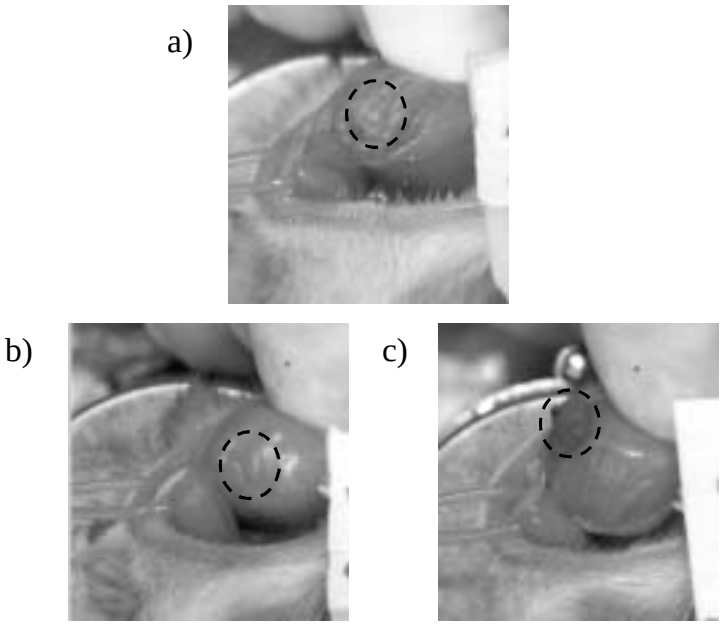

Figure 3: Photographs of the hamster cheek pouches.

CONCLUSIONS

The application of DMBA in the hamster cheek pouch induced hyperplasia and tissular reaction showed up by an inflammatory process characterized by an increase in the number of vases and their cross sections and by the presence of inflammatory infiltrates.

The hamsters treated with DMBA in association with ultraviolet radiation presented a higher degree of changes characterized by reactions similar to those of the previous case. The presence of a more significant hyperplasia with tumoral mass formation was observed histological and clinically. Tissular disorganization and carcinoma in situ were observed for this group.

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