# CHANGES IN EPIDERMAL CHALONE ACTIVITY DURING RESPONSE OF THE SKIN TO APPLICATION OF CARCINOGEN AND EPILATION

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A decrease in the biological activity of epidermal $G_2$-chalone, isolated from the dorsal skin of rats, was demonstrated 1-2 days after treatment with the carcinogen methylnitrosourea and one day after epilation. The results of quantitative analysis of mitoses and morphological analysis of the skin which was the source of the chalones showed that this effect is based on different mechanisms.

KEY WORDS: epidermal $G_2$-chalone; proliferation; carcinogen; epilation.

The role of chalones in reparative tissue regeneration has not yet been adequately studied. Only recently have reports been published of a change in the activity of chalones during tissue regeneration [1, 8-10]. However, the mechanisms of this phenomenon are still unexplained. This is largely because no morphological analysis of the tissues acting as the sources of the chalones was undertaken in the investigation of this problem. Comparison of processes taking place in the donor's skin with the biological activity of the chalones isolated from it can evidently shed light on some of the mechanisms of the change in activity of these regulators of proliferation in the course of restoration of the tissues.

In the present investigation two models of regeneration in the skin were chosen: after a single application of carcinogen (methylnitrosourea – MNU) and after epilation. MNU was chosen because of its rapid elimination from the tissue [6] and, as a result, the more convenient analysis of the proliferative response of the epidermis after application of MNU than of other carcinogens which persist in the body for a long time. Also, besides obtaining chalones and testing their biological activity, the state of the donor's skin was investigated histologically and the level of proliferation in it assessed quantitatively.

## EXPERIMENTAL METHOD

Experiments were carried out on noninbred rats and C57BL mice from the "Rappolovo" Nursery, Academy of Medical Sciences of the USSR. The two main groups consisted of 33 rats. The dorsal skin of 15 animals of group 1 was painted once with a 1% solution of MNU in acetone after previous shaving of the hair, while the hair of 15 animals of group 2 in the same region was epilated. The animals of each group were decapitated three at a time at the same time of day (11 a.m.-noon) 1, 2, 3, 5, and 12 days after the procedure. The whole region of skin painted with the carcinogen or epilated was used as the source of chalones, and some of it also was studied histologically. The method of obtaining chalones from the skin of rats was described previously by the writers [2]. The biological activity of the isolated chalones was tested in C57BL mice. The lyophilized material containing chalones was dissolved immediately before injection in physiological saline, so that 5 mg extract in 0.4 ml solvent was injected into each mouse. Undissolved components were removed by centrifugation at 3000 rpm for 15 min. The mice were killed 4 h after intraperitoneal injection of the chalones in groups of 5 or 6 animals at a time. The external ear was taken for histological analysis. Sections were cut and mitoses counted in 6000-8000 basal cells of the epidermis of the ear. The numerical results were subjected to statistical analysis by the Fisher–Student method.

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TABLE 1. Changes in Mitotic Activity of Epidermal Cells of Dorsal Skin of Rats at Different Times after Painting with MNU and Epilation (M ± m)

| Days after beginning of experiment | Number of mitoses, ‰ | | |
|---|---|---|---|
| | normal | painting with MNU | epilation |
| 0 | 8,2±1,31 | — | — |
| 1 | — | 0,99±0.74<br>$P<0.001$ | 29,2±2.00<br>$P<0.001$ |
| 2 | — | 0,73±0.36<br>$P<0,001$ | 14,55±3.64<br>$P<0,05$ |
| 3 | — | 3,96±1.94<br>$P<0,05$ | 14,77±2.78<br>$P<0,05$ |
| 5 | — | 4,96±1.40<br>$P<0,05$ | 8,15±1.28<br>$P>0,1$ |
| 12 | — | 8,98±1.38<br>$P>0.1$ | 10,78±1,59<br>$P>0.1$ |

TABLE 2. Degree of Depression of Mitotic Activity of Epidermal Cells of Skin of Mouse Ear after Injection of Chalones Isolated from Intact Skin after Painting with MNU or Epilation (M ± m)

| Source of Chalones | Time after procedure, days | Mitotic activity after injection of chalones, ‰ | Inhibition of mitoses compared with control (3.43 ± 0.48) |
|---|---|---|---|
| Intact dorsal skin of rats | 0 | 0,37±0,14 | 89 ($P<0.0$ ) |
| Dorsal skin of rat treated with MNU | 1 | 1,33±0,53 | 61 ($P>0,05$) |
| | 2 | 2,08±0,72 | 39 ($P>0,1$) |
| | 3 | 0,75±0,28 | 78 ($P<0,01$) |
| | 5 | 0,75±0,33 | 78 ($P<0,01$) |
| | 12 | 0,62±0,39 | 82 ($P<0,001$) |
| Skin of rats after epilation | 1 | 3,30±1,33 | 0 ($P>0,1$) |
| | 2 | 0,46±0,17 | 87 ($P<0,01$) |
| | 3 | 0,83±0,26 | 75 ($P<0,01$) |
| | 5 | 0,30±0,15 | 91 ($P<0,01$) |
| | 12 | 0,75±0,28 | 78 ($P<0,01$) |

## EXPERIMENTAL RESULTS

Data showing changes in mitotic activity of the epidermal cells of the dorsal skin of the rats at different times after treatment are given in Table 1.

As the summarized data show, 1, 2, 3 and 5 days after a single painting with MNU marked depression of mitotic activity of the epidermal cells was observed; its return to its initial level only after 12 days. Histological analysis of this skin showed that one day after painting with the carcinogen dying cells could be seen in the basal layer, and the few mitoses visible were characterized by distinct fusion of the chromosomes. Signs of inflammation were present in the dermis. After two days, besides a decrease in thickness of the epithelial layer, small regions of hyperplastic epidermis could be seen, with marked basophilia of the cytoplasm of the epidermal cells. The intercellular spaces were widened, evidently on account of intraepithelial edema. The phenomena taking place in the skin during the first two days after treatment with the carcinogen must thus be regarded as the result of its toxic action.

After epilation a marked increase in mitotic activity was observed in the cells of the interfollicular epithelium, and after 24 h it was 3.6 times higher than the control value. Simulation of proliferation of the epidermocytes after treatment of this type is well known [4, 5, 7]. Later, after 2-3 days the proliferative activity began to diminish, and by the 5th day was back to its initial level. Histological examination of the skin in this case revealed only marked basophilia of the epithelial layer 1 and 2 days after epilation, with focal hyperplasia on the third day. Evidence of moderate inflammation was present in the dermis.

The results of investigation of the biological activity of the chalone-containing extracts isolated from the skin after treatment with the carcinogen or epilation are given in Table 2.

It will be clear from Table 2 that chalones isolated from the skin 1 and 2 days after treatment with the carcinogen lost their activity, or their content was sharply reduced in such skin. Be that as it may, the degree

of inhibition of mitosis in the epidermis of the external ear of the mice did not differ statistically from normal. These results are in agreement with those of Rohrbach and Laerum [8], who showed that 1-3 h after the skin was painted with methylcholanthrene, the chalone-containing complex isolated from it had not the power to inhibit mitoses in epidermocytes of normal skin of the mouse ear. These experiments led their authors to suggest that chalones can be inactivated by carcinogens. However, as will be clear from Table 2, as the result of injection of chalones isolated from the skin of rats after epilation, a temporary disappearance of their inhibiting activity also took place (after 1 day). Nevertheless, comparison of the activity of the chalone-containing extracts isolated from the skin after treatment with the carcinogen or epilation (Table 2) with the level of proliferation of the epidermocytes of donors' skin (Table 1) suggests that the mechanisms lying at the basis of the decrease in chalone activity in such cases are basically different. The toxic action of the carcinogen evidently leads to a decrease in protein synthesis in the epidermis, including, perhaps, a decrease in synthesis of chalones. Naturally, therefore, the chalone content in such skin falls temporarily, to be restored later during repair of the epidermis. Conversely, the absence of inhibitory activity of chalones isolated from the skin 24 h after epilation coincides with marked stimulation of the proliferative activity of the cells of the rat epidermis. These results are indirect evidence that cells starting to undergo proliferation lose their ability to synthesize $G_2$-chalone. This chalone is considered [3] to be synthesized by cells of the basal layer, and it is mainly these cells which are stimulated after epilation.

From the writers' point of view the most interesting finding is the loss of biological activity of the chalones under the influence of MNU, from which it can be concluded that tissue-specific control over proliferation of the epidermocytes is weakened during the response of the tissue to the action of the carcinogen.

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