THE EFFECT OF ANTHRALIN AND ITS DERIVATIVES ON EPIDERMAL CELL KINETICS

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An attempt was made to clarify the effect of anthralin on epidermal cell kinetics. Commercial anthralin powder was separated by column chromatography into three components: pure anthralin, 1,8-dihydroxyanthraquinone, and anthralin dimer. The effect of these on the diurnal mitotic variation and the cell cycle of the hairless mouse epidermis was studied. Neither anthraquinone nor the anthralin dimer had any influence on these parameters. Both the chromatographically pure and commercial anthralins had similar effects. Mitotic activity was considerably reduced, although not to zero, with concomitant elimination of the diurnal peak. The G1 and S phases of the cell cycle were approximately doubled in length. Application of the pure and commercial anthralin also resulted in some irritation not seen with either the anthraquinone or dimer.

The first use of anthralin (1,8,9-trihydroxyanthracene) for topical therapy of psoriasis was derived from a report on chrysarobin, a related compound, in 1878 [1]. In treating psoriatic lesions with commercial anthralin, two side effects have been documented: staining of skin and clothing, and irritation of surrounding normal skin. This report is, in part, directed toward determining whether the therapeutic effect and side effects of commercial anthralin are caused by the pure anthralin or a contaminant. Ponec-Waelsch and Hulsebosch [2] have suggested that anthralin oxidation occurs in the presence of an adsorbing surface in a moist environment; resulting in a pink to violet discoloration and an ineffective system, i.e., anthralin would be speeding up DNA synthesis in psoriatic cells. However, irritation is a possible source of error since this has been shown to cause an increase in mitotic activity of the epidermis [12; Fisher, unpublished data]. In the following work, the known anthraquinone and dimer [13] were separated from pure anthralin in the commercial product to determine which of these may be responsible for any effect on the epidermal cell cycle.

MATERIALS AND METHODS

Purified anthralin, anthraquinone, and anthralin dimer were separated from commercial crude anthralin by silica gel column chromatography. Silica gel G was suspended in heptane and poured into a 1-inch column so that the settled height was 4 inches. The column was then equilibrated with a mixture of benzene, chloroform, and heptane (1:1:1). One hundred milligrams of crude anthralin dissolved in 2 ml of chloroform was placed on the column and the fractions were separated using the chloroform–benzene–heptane mixture. The pale, yellow-colored anthralin moved most rapidly, followed by the orange anthraquinone and deeper yellow dimer. A brown–violet colored contaminant remained at the top of the
column. When the pure anthralin layer reached the end of the column, the column was dismantled, the various gel bands removed, and the compounds were eluted from the gel with hexane. The various eluates were dried under nitrogen and resuspended in ethyl acetate. The purified compounds were identified by their absorption spectra and by thin-layer chromatography according to the method of Segal et al [13].

Adult male hairless mice, age 3 to 4 months, were used in this study. All animals were injected with 30 μc of tritiated thymidine (27.0 mCi/mg) at 6:00 AM ± 15 min. One group of 39 animals had no further treatment, while 24 μg of commercial anthralin in 0.1 ml of ethyl acetate (EtAc) was pipetted onto the backs of a second group immediately after thymidine injection. In a subsequent experiment (later date, same time of day), groups of 27 animals were treated with the same quantities of either purified anthralin, anthralin dimer, or anthroquinone. This was not observed with either the commercial or the purified anthralin beyond 24 hr after application. This was not observed with the 34-hr duration of the experiment, although no statement can be made regarding the stability of the compounds beyond the period of these experiments, although no statement can be made with regard to the situation on the animals' skins. However, over a period of several weeks in the test tube, the purified anthralin, anthroquinone, and anthralin dimer converted to a brown-violet compound although this color staining was not observed on the mice during the experimental period.

**RESULTS**

A review of the histology of the animals showed that the doubling of epidermal thickness, probably indicative of irritation, occurred with both the commercial and the purified anthralin beyond 24 hr after application. This was not observed with the quinone and the dimer. Gross observation of the skin showed slight orange-yellow staining with the pure anthralin and marked orange staining with the quinone. The chromatographically purified compounds were stable in vitro extending beyond the period of these experiments, although no statement can be made with regard to the situation on the animals' skins. However, over a period of several weeks in the test tube, the purified anthralin, anthroquinone, and anthralin dimer converted to a brown-violet compound although this color staining was not observed on the mice during the experimental period.

**Diurnal Mitotic Activity**

Those animals which had been treated only with ethyl acetate showed no difference from the typical diurnal mitotic variation normally seen in untreated animals (Fig. 1a), i.e., a peak mitotic activity was found at 24-hr intervals occurring at approximately 8:00 AM. Animals whose skins had been treated with either the commercial anthralin or the pure anthralin showed a profound inhibition at 4 hr followed by some variability about the control values for the next 14 hr (Fig. 1a). However, the expected peak, seen in the control animals at around 8:00 AM, did not occur. Mitotic activity fell to a minimum at approximately 10 hr after drug application and no significant recovery from this value was seen for the 34-hr duration of the experiment. This difference was significant at p <

![Figure 1](image-url)

Fig. 1. The diurnal mitotic cycle of hairless mouse epidermis. a. Δ—Δ, Untreated animals; ⋄, treatment with 0.1 ml ethyl acetate (EtAc); ○——○, treatment with 0.1 ml purified anthralin/EtAc; Δ——Δ, treatment with 0.1 ml commercial anthralin/EtAc. All treatment applications at time 0. b. Δ—Δ, Untreated animals; ○——○, treatment with 0.1 ml anthroquinone/EtAc; ⋄——⋄, treatment with 0.1 anthralin dimer/EtAc.
Those animals which had been treated with the quinone and also the dimer showed no significant difference as compared with the control groups (Fig. 1b) except at 4 hr where some mitotic inhibition was seen.

Cell Cycle Analysis

Those animals whose skins had been treated with ethyl acetate alone (Fig. 2a) showed no difference in cell cycle analysis from that of the untreated controls. The duration of the S phase as calculated from the 37.5% level of the peak was approximately 6 hr. Animals treated with either the commercial or the purified anthralin, however, showed an S phase increased to approximately 12 hr (Fig. 2a). The peak of labeled mitoses was also delayed as compared with the control. This suggested an increase in the G2 duration from 3.5 hr to 6 to 9 hr. However, the anthroquinone and anthralin dimer caused no change in cell cycle kinetics as compared to the untreated animals (Fig. 2b). Since the data could be interpreted to indicate two effects—some cells being completely blocked and others only slowed in their passage through the cell cycle—it is possible that, for some reason, the latter received less anthralin than the former. An attempt was made to reduce mitotic activity to zero by applying anthralin 3 times at 3-hr intervals starting, as in earlier experiments, at 6:00 AM. Animals were killed at 3:00 PM, i.e., 12 hr after the first application. The results in the Table show that this treatment did not produce any greater inhibition than a single application. In fact, the mitotic activity was higher after 3 applications, which could possibly be explained on the grounds of irritation.

DISCUSSION

Since an approximate doubling of epidermal thickness was seen after anthralin treatment, this might have artificially produced a decrease in mitotic activity. It is, however, unlikely since the hyperplasia was not apparent until about 24 hr after treatment, at which time the fall in mitosis was well established. With the protocol used in this experiment, any alteration of epidermal cell kinetics appears due to the effect of the anthralin as such, and not to either of the two "impurities" found in the commercial product. Apparently epidermal cell production is slowed in two ways: Firstly, both the S and G2 phases of the cell cycle are lengthened and this could explain the very early fall in mitotic index seen in Figure 1a. Secondly, the cells producing the diurnal mitotic peak are prevented from dividing by a single anthralin application. The data in the Table indicate that anthralin is acting as a complete cell cycle block on one portion of the proliferative pool while only slowing the remainder during G2 and S.

**TABLE. The effect of multiple anthralin applications on the mitotic activity of hairless mouse epidermis**

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Mean ± 1.91 ± 0.39 S.E.

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**TABLE. The effect of multiple anthralin applications on the mitotic activity of hairless mouse epidermis**

- **a** 24 µg anthralin in 0.1 ml EtAc applied at 6:00 AM.
- **b** Three applications of anthralin at 6:00 AM, 9:00 AM, and 12:00 noon 0.1 ml EtAc applied at 6:00 AM to controls.

Control vs 1 application p < 0.05.

Control vs 3 applications p = 0.05.
These results could also explain why little or no effect was seen in our earlier treatment of normal human skin with anthralin [6]. Although anthralin does indeed depress mitotic activity, the minimum seen after treatment does not fall significantly below the normal daily minimum. The time at which specimens were taken in our treatment of human skin was at approximately 3:00 PM, which is the normal daily minimum for human skin. It is thus possible that our finding in this instance was due to the use of inappropriate timing. This work will have to be pursued on human material for confirmation. Preliminary (unpublished) data indicate that anthralin may be having a similar effect on psoriatic lesions, i.e., there is mitotic inhibition at 8:00 AM but not at 4:00 PM after prior application of the drug in ethyl acetate.

Any effect on epidermal cell kinetics, and therefore possibly any therapeutic effect in psoriasis, is due to anthralin and not to either the quinone or dimer which are also found in the commercial product. Also the oxidation products of anthralin, which showed no biologic activity in the system described here, are much more intensely colored than the purified drug. This provides a reasonable explanation for the finding of Comaish et al [15] that discolored anthralin pastes (possibly containing large amounts of the quinone) are ineffective in treating psoriasis.

REFERENCES


