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Short Communication

Incorporation of ^{3}H-Thymidine into DNA: Inhibition by Dithranol and its Di- and Triacetate

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Dithranol was introduced into anti-psoriatic therapy in 1916, but the principle of its activity remains unkown with regard to the quantitative aspects of its efficacy e.g. in enzyme inhibition, DNA interaction and interference with lipid metabolism. In vitro experiments have indicated that dithranol irreversibly inhibits glucose-6-phosphate-dehydrogenase (EC 1.1.1.49) [10] and the incorporation of thymidine into DNA [6].

Compared with dithranol, there have been few investigations of dithranol triacetate and dithranol diacetate; dithranol triacetate has been introduced as an anti-psoriatic (Exolan) [5]). In vitro experiments using dithranol triacetate and dithranol diacetate with human serum have revealed that both molecules are hydrolytically and oxidatively degraded [12]. The different rates of absorption of differently labelled moieties of dithranol triacetate ($^{14}CH_3-CO$, ^{3}H-anthracene) have been explained by (partial) hydrolysis in or on the skin [13], which suggests that this compound has a pro-drug character. On the other hand, both dithranol acetates inhibit glucose-6-phosphate-dehydrogenase [9]. In order to obtain a better comparison between dithranol and its acetates, we determined the influence of these compounds on the incorporation of thymidine into epidermal DNA.

Dithranol and its tri- and diacetate were prepared as has previously been described [12]; dithranol and its triacetate were dissolved in acetone, and dithranol diacetate was dissolved in CH_2Cl_2 (for concentrations, see Fig. 2). For each concentration (Fig. 2), five female mice (Charles River Wiga; 25–30 g) were shaved on the back and both sides; four mice were used for control experiments. In every case, 50 µl of the solution of dithranol and its esters (Fig. 1) was applied to 5 cm^2 of the skin. The animals were kept in single cages to prevent them licking each other, and they had free access to water and nutrition. After 5 h, 25 µCi ^{3}H-thymidine (20 Ci/m*M*; 0.9% NaCl) was injected intraperitoneally, and exactly 1 h later, the mice were killed by cervical dislocation. The treated skin was excised, wrapped in aluminium foil and frozen in liquid N_2. The epidermis and dermis were separated according to the methods of Mufson et al. [8] and Marrs and Voorhees [7], respectively. The frozen skin was dipped into water at 55° C for 1 min and then into an ice bath for a short time. The epidermis was then peeled from the dermis with a scalpel; about 15 mg epidermis was obtained from each mouse. Five-millilitre syringes were loaded with a filter paper, 1 cm washed sand and 1.6 cm (about 0.75 g) hydroxylapatite (DNA grade; Biogel HTP), largely

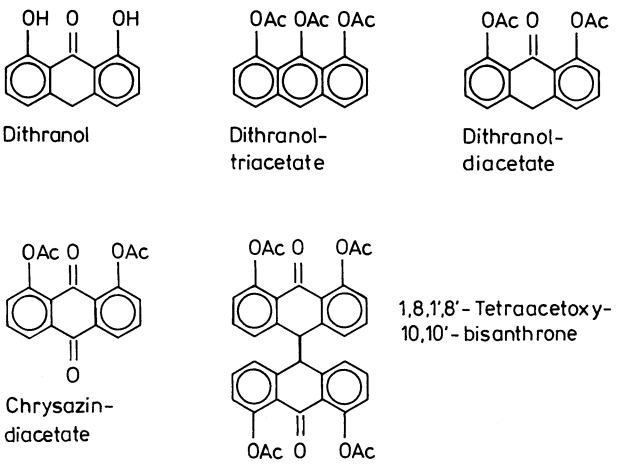

Fig. 1. Dithranol, its acetylesters and their degradation products

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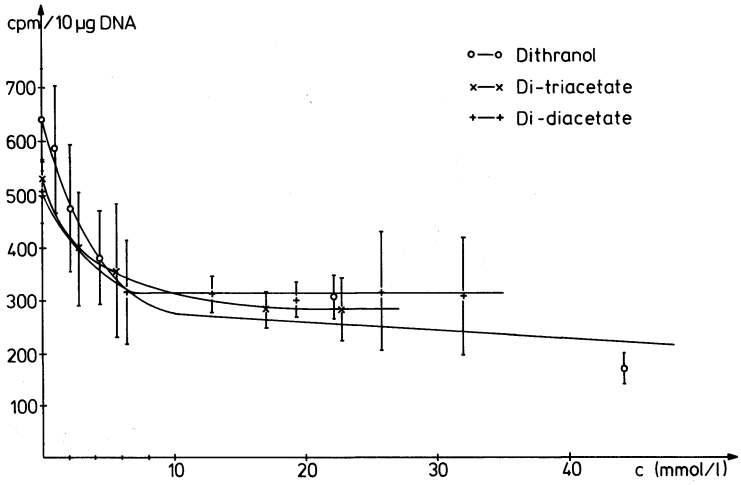

Fig. 2. Decrease of thymidine incorporation into DNA with increasing concentrations of dithranol and its di- and triacetate.
In *brackets:*
mean values — cpm — and
standard deviations for c = 0.
Dithranol, ○ (640.3; 95.8); dithranol triacetate, × (528.4; 39.8); dithranol diacetate, + (504.7; 55.3)

in accordance with the procedure of du Vivier et al. [1, 2][1]

In contrast to the findings of du Vivier et al. [2], the suspension of hydroxylapatite in the starting buffer did not absorb DNA. Therefore, hydroxylapatite was suspended in 0.01 *M* elution buffer and boiled for a few seconds. The gel was stabilized in the syringe by washing twice with 5 ml starting buffer under slight pressure.

Epidermis samples were homogenized with a borosilicate-glass tissue grinder in 2 ml lysing medium, and the suspension was carefully mixed with the hydroxylapatite with a Pasteur pipette. After 15 min, the RNA (Orcinol test [11]) and proteins (Folin-Ciocalteu [14]) were eluted with 3 × 5 ml starting buffer under slight pressure, and the urea was then washed out with 4 × 5 ml 0.014 *M* elution buffer. The DNA was eluted with 0.48 *M* elution buffer; fractions 2 and 3 (out of 4; 1.5 ml each) contained nearly the whole quantity of DNA (modified diphenylamine reagent [4]). Protein contamination was excluded by the relation: $\text{absorption}_{260}/\text{adsorption}_{280} > 1.8$ — $\text{adsorption}_{260} \times 50 = \text{DNA content}$ [3].

As a variation of the method of du Vivier et al. [2], 8 ml instagel (Packard) was added to a solution containing 2 ml H_2O, 2 ml combined DNA fractions and 3 g urea. The mixture was converted to a stable gel by careful shaking. The number of counts per minute per 10 µg DNA was calculated.

Metabolism of Dithranol Triacetate and Dithranol Diacetate

One millilitre of 0.8% solution of dithranol triacetate in acetone and 1 ml 1.0% solution of dithranol diacetate in CH_2Cl_2 were administered to the back and sides of one mouse each. The animals were killed after 6 h, the epidermis was homogenized in 5 ml starting buffer, and the suspension was extracted three times with 5 ml CH_2Cl_2. The organic phase was dried over Na_2SO_4, reduced to a small volume in vacuo and chromatographed on silica sheets 60 F_{254} with toluene/acetone/glacial acetic acid (94/4/2 vol) or toluene/glacial acetic acid (80/20 vol).

Figure 2 shows that dithranol is the strongest inhibitor of thymidine incorporation; the inhibition rate is related to the dose. Dithranol triacetate shows a weaker dose dependence; the inhibition rate is a little lower than that of dithranol at comparable doses (22 m*M*; 2 m*M*). Dithranol diacetate, which is supposed to be more reactive against bionucleophiles because it is a phenylogous mixed anhydride, shows an inhibition rate which is comparable to that of dithranol triacetate.

Even when high doses of the dithranol acetates were administered to the skin of one mouse each, dithranol triacetate was recovered in an unchanged form; however dithranol diacetate was to some extent oxidatively metabolized into chrysazin diacetate and 1,8,1′,8′-tetraacetoxy-10,10′-bisanthrone. Chrysazin diacetate and this bisanthrone also are produced by dithranol diacetate under non-enzymatic conditions. The hydrolysis of the dithranol acetates to dithranol,

1 The buffers were prepared according to the methods of du Vivier et al. [1, 2]. For the "starting buffer", 480.8 g (8 *M*) urea was dissolved to 1 l in phosphate buffer pH 6.8. For the "lysing medium", 480.8 g (8 *M*) urea, 10 g sodium dodecylhydrogenosulfate (1%) and 292.2 mg ethylenediaminetetraacetate (EDTA; acid; 1 m*M*) were dissolved to 1 l in phosphate buffer pH 6.8. The phosphate buffers (0.010 *M*, 0.014 *M*, 0.24 *M*, 0.48 *M*; pH 6.8) were prepared from equimolar quantities of $Na_2HPO_4/2H_2O$ and NaH_2PO_4/H_2O

however, was not observed, which indicates that the inhibition of thymidine incorporation by these compounds is not caused by their conversion to dithranol.

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