

INDUCTION OF RESPIRATION-DEFICIENT YEAST BY METHOTREXATE AND ITS RELEVANCE FOR THE ANTIPSORIATIC EFFECT

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Abstract. It is shown that methotrexate is a potent inducer of respiration-deficient mutants in yeast. This implies that methotrexate acts on the function of mitochondria in yeast and perhaps also on epidermal mitochondria, when used in the therapy of psoriasis. It may therefore be uncertain if the antipsoriatic effect of methotrexate depends only on its inhibition of the synthesis of nuclear DNA. A direct effect on the mitochondria and thereby perhaps also on the differentiation process in the psoriatic epidermis seems possible. There may thus be one feature in common between the effect of methotrexate and dithranol on the psoriatic epidermis.

Methotrexate blocks the enzyme dihydrofolate reductase which catalyses the conversion of folic acid to tetrahydrofolate. The latter compound is necessary for the donation of one carbon unit to deoxyuridine in the formation of thymidine. Methotrexate thus prevents endogenous thymidine synthesis and thereby DNA synthesis. The antipsoriatic effect of methotrexate is very well documented. This effect seems logical as cellular proliferation in psoriatic epidermis is greater than normal. The inhibitory effect of methotrexate on DNA synthesis is thus assumed to diminish cell production in the epidermis, thereby stopping the psoriatic process.

Although the above-mentioned properties of methotrexate seem to be sufficient to explain the antipsoriatic effect of this agent, it cannot be excluded that methotrexate also may function in some additional way.

Christophers & Braun-Falco (1) have shown that maximally stimulated epidermopoiesis in the guinea-pig does not cause parakeratosis, as does stripping of the horny layer. It is not yet proven that a mere slowing down of the epidermal

proliferation in psoriasis will result in healing. The psoriatic process also has a component of deficient differentiation, which need not necessarily be secondary to a rapid cellular proliferation.

Today we have two main potent antipsoriatic agents. One is methotrexate, which has to be used systemically, and the other is dithranol, which is used topically. With regard to dithranol we know that it interacts with DNA (3, 4, 5) and that it induces respiration-deficient (RD) mutants in yeast (2, 7). It has been assumed that dithranol exerts its antipsoriatic effect through a process similar to its induction of respiration deficiency in yeast, i.e. by affecting the respiratory function of mitochondria in the epidermis, the primary process probably being a binding to mitochondrial DNA (6, 7).

Because we feel that for dithranol-like compounds the antipsoriatic effect is strongly correlated to the ability of the compounds to induce RD-strains in yeast, we decided to investigate whether methotrexate may have a similar effect. If that is the case, the antipsoriatic potency of methotrexate may possibly be due to a preferential effect on the epidermal mitochondria.

MATERIAL AND METHODS

The frequency of RD-mutants was studied in a diploid prototrophic strain of *Saccharomyces cerevisiae* selected on minimal medium after crossing two auxotrophic mutants. Cells from an overnight culture in complete medium (7) were used to inoculate the test cultures. Methotrexate was dissolved in 96% ethyl alcohol. From this solution and subsequent dilutions with alcohol, 0.5 ml was added to flasks with 19.5 ml of complete medium. The control

Table I. Effect of methotrexate on growth and RD-induction in yeast

Concentration of methotrexate ^a	Relative growth ^b	RD-mutants (%)
0	100	1.5
1.3×10^{-5}	20	16.3
2.5×10^{-5}	15	28.5
5×10^{-5}	9.1	56
1×10^{-4}	4.8	89
2×10^{-4}	3.0	93

^a Moles per litre of the incubation medium.

^b Expressed as percentage of the growth of the control.

contained the same concentration of alcohol as did the treated series. Each flask was inoculated with 0.2 ml of the original overnight culture and incubated for 18 hours in darkness at 30°C on a shaker. The growth was determined photometrically. After the incubation the cells were washed twice in saline, re-suspended and plated on a medium containing sodium tellurite and incubated for 3 days at 30°C. Colonies of respiration-competent cells are black, while RC-mutants form small, white colonies (7). For each methotrexate concentration, 300–600 colonies were counted.

RESULTS

In a preliminary experiment with methotrexate concentrations differing by factors of 5, it was found that methotrexate induces RD-mutants in yeast and that the most interesting concentration range to study was between 10^{-5} and 2×10^{-4} M. Within this concentration range the experiments were repeated with the various concentrations differing by factors of 2. The results are given in Table I.

The initial inoculum corresponds to about 1% of the control growth after 18 hours. The numbers given in column 2 of the table therefore indicate how many more cells there are after 18 hours of growth. In the control, there are thus 100 times more cells and in the highest concentration of methotrexate, 3 times more cells, after 18 hours of growth.

As seen in the table, the highest concentration of methotrexate strongly inhibited cell division, 1×10^{-4} M allowing only about a fourfold increase of the number of cells, i.e. two nuclear divisions. At the same concentration, nearly 90% of the cells were respiration deficient. This indicates that not only was the formation of new mitochondria inhibited, but that methotrexate also

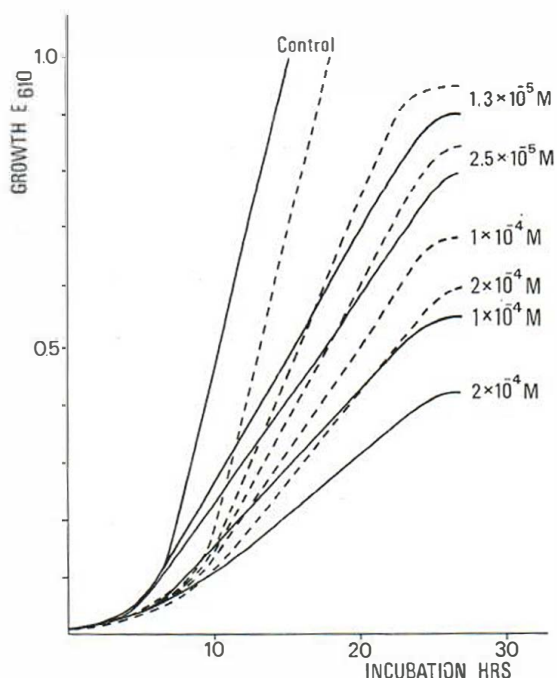


Fig. 1. The growth of respiration-sufficient (—) and respiration-deficient (---) yeast cells at various concentrations of methotrexate. The cells were incubated in complete medium at 30°C. Growth was measured with a Linson spectrophotometer and given as extinction values for 610 nm.

inactivated existing mitochondria. In a reconstruction experiment made to test if methotrexate had a selective effect on RD-cells already present in the inoculum (1.5% in the control) we compared the growth of a culture of the original respiration-sufficient cells with the growth of a culture of RD-cells obtained by inoculation from 10 independent RD-colonies. The growth at different concentrations of methotrexate is shown in Fig. 1. At all the concentrations tested the total production of cells as well as the growth rate were higher for the RD-cultures. However, at 18 hours of incubation the differences are small and cannot be responsible for more than a minor increase of the number of RD-cells. Thus the results support the assumption of an inactivating effect on already existing mitochondria.

DISCUSSION

In a normal cell nearly all genetic information is stored in the nuclear DNA and a small amount

in the DNA of the mitochondria. The DNA of the mitochondria in a yeast cell constitutes about 10% of the total DNA content and has been shown to be responsible for the coding of the inner membrane of the mitochondria but not of the whole organelle.

Dithranol, acridines and some other compounds have the ability of interacting with mitochondrial DNA in such a way that the mitochondria become incomplete or respiration-deficient. If this happens to all mitochondria in a cell, the whole cell becomes respiration-deficient. Yeast cells can do well without the oxidative mechanism of the mitochondria and thus grow by fermentation. Either by changing the information by mutation or by preventing the replication of the mitochondrial DNA, the yeast cells may become respiration-deficient.

In the present investigation, methotrexate has been shown to be a potent inducer of RD-strains in yeast. Thus the function of the mitochondrial DNA seems to be more sensitive than the function of the nuclear DNA to the action of methotrexate.

The concentration of methotrexate inducing a significant percentage of RD-mutants in yeast is somewhat higher than may be expected in the epidermal cells in a psoriatic patient treated with methotrexate. However, to induce complete respiration deficiency all mitochondria have to be knocked out, which may be lethal for a human cell. A considerable effect on the respiration rate or some other effect of the mitochondria may therefore be obtained by much lower concentrations of methotrexate, consistent with the survival of the cells.

Thus, the conclusion that may be drawn from the present investigation is that methotrexate may

act not only by inhibiting the DNA synthesis of the nuclei, as generally believed. An effect on the mitochondria and thereby perhaps also on the differentiation process of the psoriatic epidermis may also be of importance for the anti-psoriatic effect.

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REFERENCES

1. Christophers, E. & Braun-Falco, O.: Mechanisms of parakeratosis. *Brit J Derm* 82: 268, 1970.
2. Gillberg, B. O., Zetterberg, G. & Swanbeck, G.: Petite mutants induced in yeast by dithranol (1,8,9-trihydroxy-anthracene) an important therapeutic agent against psoriasis. *Nature* 214: 415, 1967.
3. Swanbeck, G. & Thyresson, N.: Interaction between dithranol and nucleic acids. A possible mechanism for the effect of dithranol on psoriasis. *Acta Dermatovener (Stockholm)* 45: 344, 1965.
4. Swanbeck, G.: Interaction between deoxyribonucleic acid and some anthracene and anthraquinone derivatives. *Biochim Biophys Acta* 123: 630, 1966.
5. Swanbeck, G. & Zetterberg, G.: Studies on dithranol and dithranol-like compounds. I. Binding to nucleic acids. *Acta Dermatovener (Stockholm)* 51: 41, 1971.
6. Swanbeck, G. & Lundquist, P. G.: Ultrastructural changes of mitochondria in dithranoltreated psoriatic epidermis. *Acta Dermatovener (Stockholm)* 52: 94, 1972.
7. Zetterberg, G. & Swanbeck, G.: Studies on dithranol and dithranol-like compounds. II. Mutagenicity. *Acta Dermatovener (Stockholm)* 51: 45, 1971.

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