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Anthralin: How Does It Act and Are there More Favourable Derivatives?

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Anthralin is still the most effective and safest therapeutic agent for treatment of psoriasis. Our data may assist toward an understanding of its mode of action and introduce new derivatives, more antiproliferative and less toxic than anthralin *in vitro*. Anthralin exerts a direct effect on keratinocytes and leukocytes. In time-lapse studies it significantly prolonged the prophase of mitotic keratinocytes in subtoxic doses and suppressed the expression of keratin 6 mRNA in the immediately suprabasal layer of psoriatic epidermis in vivo. Anthralin inhibits the transformation of lymphocytes and the release of reactive oxygen species from activated leukocytes, *in vitro*. We provide evidence that these effects of anthralin are mediated by protein kinase C. Twelve new hydrophilic derivatives of anthralin, including a 1,8-dimethoxy compound, as well as C-2 and C-10 substituted anthrones were tested on human keratinocytes. The antiproliferative effect of those derivatives bearing lacton rings at a C-10, consisting of 4, 5, or 6 C atoms, exceeded that of anthralin and were equally or less cytotoxic than the parent drug. These compounds had no pro-drug character *in vitro*, since they did not metabolize via anthralin, as shown by HPLC. These data indicate that there may be anthralin derivatives with more favourable properties for topical therapy than anthralin itself. ***Key words: anthralin; analogues; protein kinase C; keratin; neutrophils.***

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PUTATIVE TARGETS OF ANTHRALIN (TABLE I)

Anthralin is an empirical agent whose mode of action is still unknown. It inhibits keratinocyte proliferation. Both the growth factor TGF-alpha and its receptor, the EGF receptor, are overexpressed in psoriatic lesions and are down-regulated by anthralin. By performing time-lapse studies on keratinocytes and titrating anthralin concentration to the lowest effective dose, we demonstrated a roughly sevenfold prolongation of the prophase, compared with untreated controls (1).

Treatment with anthralin also results in a normalization of keratin expression, i.e. increase in differentiation associated keratins K1, K2 and K10 and reduction of the proliferation associated keratins K6, K16, K17. In comparison with other antipsoriatic treatment modalities, anthralin shows the most rapid effect on keratins. Parallel to the reappearance of differentiation keratins, anthralin induces the reappearance of the differentiation marker filaggrin, which is, together with K10, essential for the restoration of the normal alpha keratin pattern in the horny layer. The reappearance of the differentiation keratins and filaggrin may be taken as indicators of clinical remission of psoriasis. On the other hand our findings give some evidence that K6 seems to be a more specific target for anthralin, since it is found elevated in uninvolved skin of psoriatic patients without changes in differentiation keratins and its mRNA reduction precedes by far that of K10 mRNA expression (2).

Another main target of anthralin appears to be T-lymphocyte activation, indicated for instance by the *inhibition* of DNFB-induced contact hypersensitivity, and of the mixed leukocyte/epithelial cell reaction, as well as the reduction of the urine neopterin level during treatment. This effect is evidently bidirectional, since anthralin directly inhibits T-lymphocytes, e.g. E-rosette formation and mitogen stimulation (3) and reduces T-cell activating cells, such as ATPase+ and CD1 (OKT6)+ Langerhans cells as well as Th1+ dendritic cells in mice.

The fourth main effect of anthralin is the inhibition of the chemotaxis of PMN and its intra-epidermal accumulation (4) as well as inhibition of the release of ROS from stimulated PMN. From the molecular point of view PKC is the most likely target for anthralin, since this enzyme is involved in controlling keratinocyte proliferation as well as ROS release from PMN (5).

PROSPECTS FOR IMPROVING THE BENEFIT/SIDE EFFECT RATIO (TABLE II)

The simplest way to improve the benefit/side effect ratio of anthralin is to restrict its application to the lesions only, e.g. by using stiff formulations or occlusive dressings. The rationale of short contact therapy is that anthralin penetrates faster through lesional than perilesional skin. Thus, short contact therapy may be improved by the additional use of penetration enhancers, such as urea, in order to better segregate the lesional antipsoriatic and the perilesional irritative effects. Similar penetration enhancing effects may be expected with microencapsu-

Table I. *Putative anthralin targets in psoriatic skin*

○	Keratinocyte proliferation EGF-receptors
○	Keratinocyte differentiation Keratin
○	T-lymphocyte activation mitogen stimulation, mixed epidermal lymphocyte reaction CD1 + cells, delayed hypersensitivity E-rosette formation
○	PMN chemotaxis and ROS release lipoxygenase
Other cells:	monocytes, mast cells, fibroblasts
Biochemically:	G-6-PDH, serine proteinases, thioredoxin reductase, mitochondrial respiration, PKC, polyamine biosynthesis, cyclic nucleotides

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Table II. *Possibilities (most experimental) to improve benefit/side effects ratio of anthralin treatment*

○	Applications:	short contact, low-dose, occlusion, gradual dose increase
○	Formulations:	plus urea, salicylic acid, tar, glucocorticoids vehicle: 70% o/w cream microencapsulated, liposomes
○	Combinations:	antioxidants, amines, glucocorticoids, lipoxygenase & cyclo-oxygenase inhibitors, serotonin antagonists, antihistamines, PAF antagonists, other anti-inflammatory drugs
○	New analogues:	10-acyl derivatives

lated or liposomal encapsulated anthralin. The problem with the liposomal encapsulated formulations is the instability of anthralin itself (6). To prevent the persistence of an anthralin reservoir in the horny layer, the use of easily washable ointments, anthralin-solvating baths, radical scavengers, or anthralin degenerating agents have been recommended at the end of medication. Moreover we showed that the addition of tar reduces anthralin irritation and leaves the antipsoriatic activity unchanged, provided that the ointment is freshly prepared, stored in a refrigerator, and used within the following 3 weeks (7).

Even though there is no clinical indication for tachyphylaxia it is obvious that the irritability of the perilesional skin abates as the anthralin therapy proceeds. There seems to be an adaptation of the skin to anthralin irritative activity. We found some evidence that danthrone, which may accumulate in the skin during treatment, inhibits NAD(P)H: quinone reductase activity, which is able to protect the cells from oxidative stress (8). Indeed, we also demonstrated in vitro an increasing tolerance of keratinocytes to anthralin after repeated exposures (9).

There are conflicting data concerning the advantage of combining anthralin with glucocorticoids. Bilateral comparison of anthralin treatment with and without one week of pretreatment with betamethasone dipropionate showed a faster response on the glucocorticoid-treated side after one week, but it took longer to achieve the same final result than on the side treated with anthralin alone (10). This is in agreement with a double-blind comparison of dithranol and a glucocoticosteroid-anthralin combination.

The most attractive attempts are those made by synthesizing new anthralin analogues. According to current knowledge, analogues obtained by substitution at the C10 position of anthralin seem to be the most promising: there is e.g. the 10 acylderivative, butanthrone, which is less irritative but also less effective than dithranol in clinical studies. Other 10-acylderivatives of anthralin have been developed, e.g. coupling products of anthralin with retinoids and salicylic acid, all of them inhibiting G-6-PDH. We tested 13 newly synthesized compounds by Wiegrebe on human keratinocytes and found that those derivatives bearing lactone rings at the C10 position had a favourable antiproliferative/cytotoxic ratio in vitro, which exceeded that of anthralin (11). Some of them were also more potent than anthralin in the inhibition of G-6-PDH and the 5-lipoxygenase pathway as indicated by a reduced production of 5-HETE and LTB4 by PMN. In conclusion, we feel that there is a realistic prospect of finding analogues of anthralin with a more favourable benefit/side effect ratio than anthralin itself.

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