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Prostaglandins, Histamine and Platelet Activating Factor: Different Mediators in Dithranol-Induced Skin Damage

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ABSTRACT

Dithranol is a potent agent in treating psoriasis but its adverse effects on intact skin have limited its usage. There are many proposed mediators for its adverse effects including prostaglandins, histamine, platelet activating factor and free radicals. In this study we examined the effect of different agents (diazepam, terfenadine, indomethacin and garlic extract) on dithranol-induced skin damage in mice. Animals were treated with different drugs before local application of dithranol cream on the right buttock. Skin biopsy was performed after 48 or 96 hours. Diazepam (3 mg/Kg), terfenadine (20 mg/Kg), indomethacin (25 mg/Kg) and garlic extract (1% and 2%) were all capable of reducing dithranol-induced skin damage. It seems that the role of prostaglandins, histamine and platelet activating factor in the pathogenesis of dithranol-induced skin damage is inevitable. Garlic extract effect might be mediated by platelet activating factor but it needs further work to be proven.

Keywords: *Dithranol, Prostaglandin, Histamine, Platelet activating factor, Garlic*

Dithranol has been used as an antipsoriatic drug since 1916 but its irritative side effects have limited its usage [1]. Its application results in irritation and staining of the underlying skin. These irritative side effects are more severe in normal skin surrounding the psoriatic lesions, compared to the lesions themselves [2]. Despite numerous clinical and experimental investigations, the mechanism of action of dithranol has not yet been firmly established.

Dithranol acts via several mechanisms including inhibition of cell proliferation [3], inhibition of granulocyte function [4] and suppression of immune system [5]. Its antiproliferative effect is exerted through affecting DNA duplication and repair [6-8], mitochondrial respiration [8-9], G6PD [10], polyamines [11], lipoxygenase [12-14], calmodulin [15-16] and the balance of cyclic mononuleotides [17]. Dithranol side effects are mediated by prostaglandins [18], histamine [19-20], platelet activating factor (PAF) [20-22] and free radicals [23-24]. Dithranol is a highly reactive agent and is readily oxidized by light, trace concentrations of metal ions and oxygen [25]. Its oxidized metabolites cause formation of reactive oxygen species (ROS) and these two play an important role in the mode of action and induction of side effects of this agent [23].

It seems that mitochondria are the major subcellular site of action for dithranol. Dithranol acts as an electron donor to inner mitochondrial membrane associated redox components and inhibits the electron transport chain [9]. Garlic is a herbal drug and contains several chemicals including glycosides (allicin, ajoene), a variety of enzymes, vitamins and fatty acids26. Garlic extract has been used in several medical conditions such as thrombotic processes [27-30], hyperglycemia [27], hypertension [31], hyperlipidemia [32] and so on.

In this study we examined the effect of indomethacin (inhibitor of prostaglandin synthesis), terfenadine (histamine receptor 1 blocker), diazepam (anti platelet activating factor) [33], as well as garlic extract on dithranol-induced skin lesions.

MATERIALS AND METHODS

Animal

Male BALB/c mice (22 to 25 grams) were kept in separate cages with free access to food and water. They were divided into 14 groups each containing 8 mice

(Table 1). The right buttock region was shaved three days before the experiment.

Method

Intraperitoneal diazepam (3 mg/kg), terfenadine (20 mg/kg), indomethacin (25 mg/kg) or topical garlic extract (1% or 2% v/w) were administered 2 hours before the topical administration of dithranol (1%) cream. Dithranol or garlic extract was applied on a surface area of 1cmx1cm of right buttock. Then after 48 or 96 hours, skin biopsy was performed from the treated skin and fixed in formalin (10%). The tissues were stained using haematoxylin and eosin method and evaluated for detection of microscopic damages.

Microscopic evaluation

Four parameters were checked during microscopic evaluation of tissues (vascular infiltration, vascular congestion, Tissue edema and the ratio of polymorphonuclear leukocytes to mononuclear leukocytes). The first three parameters were graded from 0 to 4, in a blind fashion, as follows: 0: no damage; 0.5: very mild damage; 1: mild damage; 2: moderate damage; 3: severe damage; 4: very severe damage.

The fourth parameter was declared as a ratio number with the nominator and denominator referring to polymorphonuclear leukocytes and mononuclear leukocytes respectively (PMN/MN).

Drugs

Diazepam, terfenadine and indomethacin were administered intraperitonealy while dithranol and garlic extract were administered topically. Diazepam was purchased from Profarmaco, Italy. Terfenadine was purchased from Gedeon Richter Ltd, Hungary. Indomethacin was purchased from Esteve Quemica SA, Spain. Dithranol was purchased from Sigma Chemical, St. Louis, MO, USA. Garlic water extract was purchased from Kosar Pharmaceutical Company, Iran.

Statistic evaluation

For each group, mean and standard deviation were determined for each of the parameters. Then different groups were compared with each other using Mann-Whitney's Mean Rank test. The difference between all groups with *P* value of less than 0.05 was declared as significant.

RESULTS

The control group (receiving cream vehicle) showed almost no microscopic damage in skin biopsies performed 48 or 96 hours after topical administration of control cream.

Skin biopsies performed 48 hours after the topical administration of dithranol (1%). Dithranol (1%) resulted in high scores of vascular infiltration, vascular congestion and tissue edema. It also shifted the PMN/MN ratio toward the dominancy of polymorphonuclear leukocytes (Table 1). Diazepam (3 mg/kg), terfenadine (20 mg/kg), indomethacin (25 mg/kg) and garlic extract (1% and 2%) all reduced the severity of dithranol-induced skin damage as well as polymorphonuclear dominancy. No significant difference was seen between 1% garlic extract and 2% garlic extract in reducing dithranol-induced skin damage.

Skin biopsies performed 96 hours after the topical administration of dithranol (1%). The results were similar to the ones obtained from 48 hour biopsies. Dithranol (1%) caused skin damage and it was prevented by diazepam (3 mg/kg), terfenadine (20 mg/kg), indomethacin (25 mg/kg) and garlic extract (1% and 2%). Again there was no significant difference between 1% garlic extract and 2% garlic extract in reducing dithranol-induced skin damage (Table 1).

DISCUSSION

In this study it was shown that dithranol-induced skin damage can be prevented by diazepam, terfenadine, indomethacin and garlic extract.

There are many proposed mediators for dithranol-induced skin damage including prostaglandins [12, 18, 34], histamine [19-20], platelet activating factor [20-22] and free radicals [23-24]. Kemeny et al reported that indomethacin as an inhibitor of prostaglandin synthesis is capable of preventing ear edema induced by dithranol20. Muller [24] showed that dithranol inacti-

Table 1. Study groups and results of skin biopsies performed 48 or 96 hours after administration of dithranol. Drugs were administered 2 hours before topical administration of dithranol cream. Skin biopsy was performed 48 hours (groups 1 to 7) or 96 hours (groups 8 to 14) after administration of dithranol.

Group	Infiltration [a]	Congestion [a]	Edema [a]	PMN/MN [b]
Control (cream vehicle)	0	0.5	0	0.75
Dithranol (1%)	3	3	3	2.875
Garlic (1%) + Dithranol (1%)	1	1	1	2.125
Garlic (2%) + Dithranol (1%)	2	1	1	2.125
Diazepam (3 mg/kg) + Dithranol (1%)	1	1	1	2
Terfenadine (20 mg/kg) + Dithranol (1%)	1	1	1	1.75
Indomethacin (25 mg/kg) + Dithranol (1%)	0.5	0.5	0.5	1.75
Control (cream vehicle)	0	0	0	0.688
Dithranol (1%)	3	3	3	2.875
Garlic (1%) + Dithranol (1%)	2	2	1	1.25
Garlic (2%) + Dithranol (1%)	2	1	1	1.063
Diazepam (3 mg/kg) + Dithranol (1%)	1	2	1	1.5
Terfenadine (20 mg/kg) + Dithranol (1%)	1	0.5	1	1.625
Indomethacin (25 mg/kg) + Dithranol (1%)	0.5	0.5	0.5	0.938

[a] Pathological score.
[b] Ratio.

vates 12 lipoxygenase and thus changes the balance of arachidonic acid metabolism [35]. The data in our study also support the prostaglandin hypothesis. Indomethacin was able to significantly reduce the adverse effects of dithranol and it seems that prostaglandins specifically and arachidonic acid generally play a role in mechanism of action of dithranol.

Histamine is also a mediator and previous works have proved its role in dithranol-induced skin damage. Kemeny et al showed that clemastin (an H1 and H2 blocker) inhibited the inflammatory effects of dithranol [20]. Our study also supports this hypothesis, because terfenadine (a specific H1 blocker) reduced the dithranol-induced skin damage.

There has been growing evidence that platelet activating factor also play a part in this field [20-22]. PAF is secreted by neutrophils, monocytes and mast cells and results in vasodilation, extravasation and platelet aggregation. Platelets can then secrete the content of their granules which results in inflammation and leukocyte adhesion to vascular wall. Treatment with antagonist of PAF (BN 52021) was successful in reducing dithranol-induced skin damage [21].We used diazepam as an anti PAF agent [33] and the result was consistent with the previous studies.

Surprisingly, garlic extract was also able to reduce the extent of dithranol-induced skin damage. Garlic has many constituents with a wide spectrum of activity. It has a role in thrombotic processes [27] and is used as an anti platelet aggregating agent. It is suggested that garlic may act through inhibition of PAF [28-30]. If this was correct, then one could propose that the preventing effect of garlic on dithranol-induced skin damage might be mediated by PAF. Of course this will not rule out other possible mechanisms.

Other studies have revealed the role of protein kinase C [36], lipid peroxidation in membranes [37], keratinocyte TGF alpha expression and EGF receptor [38] in the mechanism of action of dithranol, but it seems that further work is needed to find out the final answer.

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