Effect of extracted antigens from some pathogenic fungi on the mice skin

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Summary

Adult mice (BALB/c) were inoculated at differen loci of skin with five species of dermatophytes: *Epidermophyton floccosum Microsporm canis* , *M. gypseum* ,*Trichophyton mentagrophytes var. mentagrophytes* and *T. verrucosum* and two species of yeasts (*Candida albicans* and *C.tropicalis*) using scratch technique and dermal injection of purified enzymes (Keratinase and proteinase) with suspensions of these pathogenic fungi.Results showed that the dermatophytes revealed a variations in infectivity as 100% and 50% for *M.gypseum* and *M.canis* : respectively , while no infections symptoms were observed for other examined fungi .The dermal injection by purified enzymes of fungi culture revealed an infection percentages as 87.5% and 62.5% for keratinase and proteinase: respectively .The infected skin of mice showed a remarkable histopathological changes in comparison with non- infected mice.

الخلاصة

لقحت الفئران المختبرية (BALB/c) بطريقة الخدش في مناطق متفرقة من الجلد بخمسة انواع من الفطريات المرضية الجلدية هي *Epidermophyton floccosum* و *Microsporm canis* و *M. gypseum* و *Trichophyton mentagrophytes var.* و نوعين من خمائر (*Candida albicans* و *C. tropicalis*) وكذلك حقنت بالانزيمات النقية (keratinase , proteinase) مع معلق من مزارع هذه الفطريات .

اظهرت النتائج وجود اختلاف في نسب الاصابة بالفطريات حيث بلغت 100% لكل من *T. verrucosum* و *mentagrophytes var.* و 66% *Trichophyton mentagrophytes* و 50% *M. gypseum* بينما لم تشاهد علامات اصابة بالفطريات *Microsporm canis*

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الاخرى .سجلت نسب الاصابة نتيجة الحقن الجلدي بالانزيمات و معلق مزارع الفطريات 87.5% و 62.5% الـ proteinase , keratinase على التوالي نوحظ من خلال الفحص النسيجي وجود علامات مرضية نسيجية في مقاطع الجلد للفئران المصابة مقارنة مع غير المصابة .

Introduction

Dermatophytes and pathogenic yeasts usually invade and parasitize only the non-living keratinized layers of skin , nails and hair(1).This highly developed host - parasite relationship for a multitude of clinical manifestations (2).Many of these host - parasite interactions are dependent on specific moiefies (3) and enzymes production (4,5)

Nevertheless studies on dermatophytes pathogenicity using a reliable animal models are limited and only few reports regarding some histopathological changes by causative agents of candidiasis (6),and dermatophytes(7) are available .

The aim of this study is to examine the capability of some dermatophytes and *candida species* in pathogenesis and the role of enzymes production in pathogenicity using mice as animal model.

Materials and methods

1-Prepation of inoculum : Seven isolates were obtained from the stock culture collection previously isolated from specimen of dermatophytosis and candidiasis patients (table 1) which were identified according to the established taxonomic criteria (8,9)

2-Experimentals: experimental mice (*Mus musculus* , BALB\c) six to eight weeks old were purchased from animal house of pathology dept. medicine college, university of Qadisiya .The mice which were used for breeding cheeked to sure that the animals were free from any infection .

3-Infection process: Experimental mice were inoculated loci of skin (table 2) using scratch technique after clipping and shaving of the loci, with fungal isolates (10),dermal injection of purified enzymes (keratinase and proteinase) extracted from isolate of *Trichophyton mentagrophytes Var. erinacei* (inoculation volume 0.2ml) was also used to inoculate mice (10).The assays were performed with two lots (infected and control)six mice for infected and three for control for each isolate . Inoculated skin were cheeked *daily* during the first week and at weekly intervals therefter for the development for infection during a follow -up period of 35 days using the direct smears with 10% KOH and cultured onto Sabourouds Dextrose Agar of skin scrapings to confirm the presence of fungal infection.

4-Histopathology: At the end of experiment, the animals were sacrficed by decapitation, skin tissues were saved in 10% formalin as a fixatative .following conventional procedures for tissues sectioning at a thickness of 5 Mm. The slides were stained with periodic -Acid schiff (PAS) for histopathological studies (11).

The results obtained in two groups (infected and control) were also statistically analyzed using Chi square (X^2 test).

Results

After 7-14 days of inoculation and throughout the examination of clinical feactures.smears and cultures of skin lesions , the capability of the selected pathogenic fungi to infect the experimental animals were differed according to fungal pathogens and the ecological source of isolate. This difference is statistically significant (P<0.005) (table 2) Results indicated that the dermatophytes revealed a variations in infectivity as shown by infection percentages as 100% for each of *Trichophyton mentagrophytes var. mentagrophytes* and *T. verrucosum* , 66% *Microsporm gypseum* and 50% for *M.canis* , while no infection observed for the other examined fungi by using scratch technique .However , the average precentage of infection was 45%among the tested fungi.

Table 3. shows that the dermal injection using keratinase and proteinase extracted from the *T.mentagrophytes var.*erinacei isolate with fungal suspension of the tested fungi which were not infective alone to the mice when using the skin scratching.percentage were 87% and 62.5% for keratinase and proteinase , respectively .while the average percentage of infection was 75% .this differnce is statistically significant (P<0.005).

Clinical features of infected mice using skin scratch and dermal injection with or without fungi isolates showed that skin lesion is single; small;circular ;eddened ;maculopapule ; areas which advances slowly at the periphery with a tendency forcentral healing after 14 days ;itching is a common symptom with little of hair losses. while the histopathological changes of skin tiusses showed acanthosis (five time greater than the original thickness)para and hyperkeratosis of the epidermis together with local infiltration of the polymorphonuclear (fig.1)

experimental animal models have proven to be valuable tools for studying the routes of infection (1) .However little attention has been paid concering the role of enzymes in pathogenesis of experimental animal models and a remarkable finding established that numerous aggressins such as spreading factors , toxins and enzymes have been implication patholgenicity of fungal diseases (12).The present results indicated that the ability of selected dermatophytes and pathogenic yeasts to produce a disease (when using skin scratching) was clearly dependent upon the fungal sources (anthropophilic ,zoophilic and geophilic) and the presence of a virulence factor (digestive enzymes). consequently , both factors contribute in the skin pathogenesis . However , *Trichophyton* and *Microsporum* species are considered zoophilic and or geophilic pathogens and having a high ability to invade the keratinized tissue and cause the infection when inject with enzymes .This may be due to the presence of moieties such as lipids , phospholipids, polysaccharides and peptides fractions ,as a cell wall extract of dermtophytes , elicited positive allergic skin reaction in the sensitized mice, and this may act as contact sensitizer which are responsible for the skin reactivity induced by some dermatophytes (3) .On the other hand , another factor which faccilitates or induces teh infection is the enzymatic activity produced by pathogenic fungi(4,5) .this enzymes permit or aid the

dermatophytes fungi to utilize a component of keratinized structures (elastin , collagen and keratin), or chemotaxis of polymorphonuclear leucocytes and eventually causing acanthosis and hyperkeratosis (13,14).

the failure of *Epidermophytes floccosum* and *Candida* species using skin scratching without enzymes to induce infection can be related to its anthropohilic source and ability of these fungi to produce enzymes , specially keratinase and proteinase that digest the keratinized structure of animal skin tissue is relatively lower or absent .

these findings support the previous report on the enzymic activity of these fungi (10-15).

Table(1) list of fungal isolates

Fungal species	Source /Site of isolate	Isolate no	Disease caused
Epidermorphyton floccosum (Harz Langeron & Miochevitch)	Anthropohilic / skin	Ef22	Tinea Pedis
Microsporum canis (Bodin)Bodin	Zoophilic	Mc1111	Tinea Capitis
M.gypseum (Bodin) Guiart & Grigorakis	Geophilic /hair	MG 792	Tinea Capitis
Trichophyton mentagrophytes var.mentagrophytes (Robin) Blanchard	Zoophilic / skin	TMM 186	Tinea cruris
T. verrucosum Bodin	Zoophilic / skin	TVE 577	Tinea croporis
Candida albicans (Robin)	Anthropophilic /nail	Calo66	Cutaneous candidiasis
C. tropicalis (Castell) Berk	Anthropophilic /nail	CT 156	Cutaneous candidiasis

Table (2) Mice infection (%) by some pathogenic fungi using skin scratch technique

Fungal species	Inoculation loci	No. of inoculated animals	Positive infection	Infection (%)
E.floccosum	Foot	6	0	0
M.canis	Head	6	3	50
M. gypseum	Head	6	4	66
T.mentagrophytes var. mentagrophytes	Trunk	6	6	100
T.verrucosum	Trunk	6	6	100
C.albicans	Axilla	6	0	-
C.tropicalis	Axilla	6	0	-

X^2 test ($P< 0.005$)

Table (3) Mice infection (%) using dermal injection of enzymes with fungal suspension of *Trichophyton mentagrophytes var.* erinacei for non infected mice by skin scratching .

The inoculm	No. of inoculated animals	Positive cases	Infection (%)
Keratinase	8	7	97.5
Peoteinase	8	5	62.5
Culture mycelium	42	19	45
Control	21	0	0

X^2 test ($P < 0.005$)

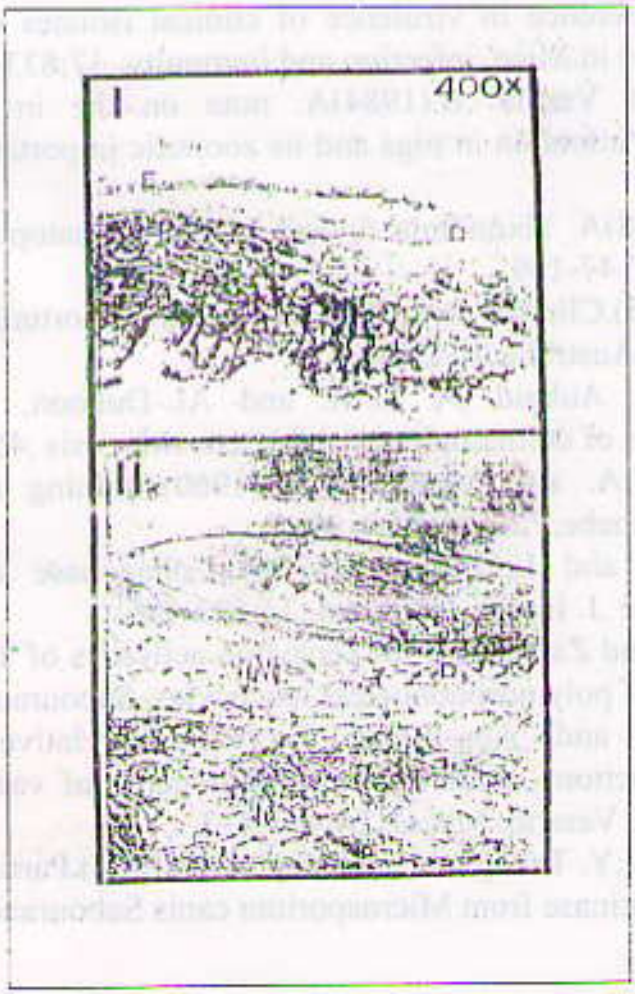

Fig (1) Histopathological changes of infected skin of mice by *Trichophyton mentagrophytes* Var mentagrophytes .
1-uninfected skin (E=Epidermis:D=Dermis):2-infected skin (E=Epidermis :D=Dermis).showing acanthosis of epidermis (arrowod).(400X) .

References

1-Howard, D. (1983).fungi pathogenic for human and animal part B: pathogenicity and detection Vol.3: Marcel Dekker ,USA .Pp.543.

2-Cabo,J.F;Cequiel M.V.L.;Aisa S. and Arriibas ,M.T.V.(1988).Dermatophytosis of pigs by trichophyton mentagrophytes.Mycopathologia ,101:161-164.

3-Aderson,B.A.;Hellgren ,L.and Vincent ,J.(1976) Allergic delayed skin reactions from lipid fractions of Trichophyton .Sabouraudia 14:237-241.

4-Yu,r.j.Harmon ,S.R.;Grapple ,S. F. and F.(1971). Two cell bound Keratinasrs of tichophyton mentagrophytes .j. invest.56:27-32.

5-Das,S.K. and Banegerjee A.B.(1977).Lipolytic enzymes of trichophyton rubrum .Sabouraudia 15:13-323.

6-Wingard I.R.;Dick .I.D.;Merz W.G.;Sandford , G.R.; Saral ,R. and Burns W.H.(1982).Difference in virulence of clinical isolates of candida tropicalis and Candida albicans in Mice .infection and immunity ,37;833-836.

7-Thakur.D.K.and Verma ,B.(1984)A. note on the incidence of Trichophyton mentagrophytes infection in pigs and its zoonotic importance .inter.J.ZOO.,11:123-125.

8-Ajello ,L. (1968)A. taxonomic review of the dermatophytes and related species .Sabouraudia ,6:147-159.

9-Ellis ,D.H.(1995).Clinical mycology :the human opportunistic mycosis .Gillingham printers Pty Ltd Australia .PP.2166.

10-Muhsin, T.M. Aubaid ,A. H.M. and AL-Duboon, A.A.(1998).Extracellular enzyme activities of dermatophytes and yeasts .Mycosis ,45:1-5.

11-Mcmanus ,J.F.A. and Mowry .R.W.(1960).Staining methods :histology and histochemical. Hoebe,P.b.Inc., New York.

12-Rippon .J.W. and Lorinez A.L.(1964).Collagenase activity of strepomyces nocardia madurae .J. Invest. Dermatol.,34:483-486.

13-Davies .R.R. and Zaiani F.(1984).enzymic activities of Trichophyton rubrum and the chemotaxis of polymorphonuclear leucocytes .Sabouraudia ,22;235-241.

14-Ghannoum.M. and Abu-Eiteen K.(1986).Correlative relationship between proteinase production , adherence and pathogenic of various strains of *Candida albicans* .J. med. Veterin. Mycol.,24;407-413.

15-Takiuchi, L. Sei,Y. Takagi , H. and Negi , M. (1984).Partial characterization of the extracellular keratinase from Microsporium canis Sabouraudia ,22;219-224.