mykosen | mykosen 28 (2) 85–89
Angenommen/Accepted: 26. März 1984

Studies on Haemagglutinins of Microsporum gypseum

Untersuchungen über die Hämagglutinine von Microsporum gypseum

M. Refai, R. Soliman, S. Attia and F. El-Far

Department of Microbiology,
Faculty of Veterinary Medicine, Giza/Egypt

Key words: Haemagglutinins – haemagglutination – Microsporum gypseum
Schlüsselwörter: Hämagglutinine – Hämagglutination – Microsporum gypseum

Summary: 36 isolates of Microsporum gypseum recovered from Egyptian soil were found to be capable of agglutinating red blood cells of cow, buffalo, camel, sheep, chicken, duck, pigeon, Guinea pig and mouse. Also microconidial variants obtained during repeated subculturing had the same property. Haemagglutinins of both the wild and microconidial forms were partly thermolabile and partly thermostable. They resisted the effect of trypsin, concentrated hydrochloric acid and 4N sodium hydroxide, but they were markedly inactivated by potassium periodate (wild form) and chloroform (microconidial form). The specificity of the reaction was confirmed by the haemagglutination inhibition test.

Zusammenfassung: 36 Stämme von Microsporum gypseum, die aus ägyptischem Erdboden isoliert wurden, zeigten die Fähigkeit, Erythrozyten von Rind, Büffel, Kamel, Schaf, Huhn, Ente, Taube, Meerschweinchen und Maus zu agglutinieren. Auch die Mikrokonidien-Variante, die während wiederholter Überimpfungen erhalten wurde, zeigte eine ähnliche Fähigkeit, Hämagglutinine beider Formen waren zum Teil thermolabil und zum anderen thermostabil. Sie widerstanden der Wirkung von Trypsin, konzentrierter Salzsäure und 4N Kalilauge. Sie wurden aber durch Kaliumperjodat (Wildstamm) und Chloroform (Mikrokonidien-Variante) zum größten Teil inaktiviert. Der Hämagglutinationshemmungstest bestätigte, daß die Reaktion spezifisch war.

Introduction

Refai and Shalaby (1975) found that 9 species isolated from man and animals could agglutinate red blood cells of different animals and birds. Refai et al. (1977) proved the specificity of the reaction by the application of haemagglutination inhibition test. They also studied the properties of haemagglutinins of Trichophyton rubrum, T. violaceum, T. schönleinii, Epidermophyton floccosum and Microsporum canis.

The present study is dealing with the nature of haemagglutinins of Microsporum gypseum isolated from Egyptian soil.

Material and Methods

36 Microsporum gypseum isolates recovered from Egyptian soil were tested for their capability to agglutinate red blood cells of different animals and birds. As microconidial variants were obtained from 10 isolates during repeated subculturing, a wild isolate (F-82/37) that produced numerous macroconidia and very few microconidia and its microconidial form (only microconidia and no macroconidia) that showed classical haemagglutination of chicken RBC's were selected for the studies on the nature of haemagglutinins.

The haemagglutination test was carried out in plastic haemagglutination plates. To each cup of the plate 0.05 ml saline were added. An equal amount of 2% fungal suspension was mixed with the saline of the first cup and serial two-fold dilutions were made by a microloop, then 0.05 ml of 0.5% RBC's were added to each cup. The plate was left at room temperature and the result was recorded when the negative control showed a dense button of cells.

RBC's of cow, buffalo, camel, sheep, chicken, duck, pigeon, Guinea pig and mouse were tested, but chicken RBC's were used throughout the work. Cell-free culture filtrate was also tested. Cultures of varying age (one week to 5 months) were examined but 3 weeks old cultures were used throughout the study.

Haemagglutination inhibition test was carried out using M. gypseum antiserum prepared in rabbits from which non-specific inhibitors were removed (Refai et al., 1977).

In order to study the nature of M. gypseum haemagglutinins, 2% fungal suspensions of 3 weeks old cultures of both the wild and microconidial forms were subjected to the following treatments:

1. Heating in a water bath and in an autoclave at 60–120 °C for 1–4 hours.
2. Repeated freezing (-80°C) and thawing (37°C) for 20 times.
3. Ultrasonication at the highest speed for 1 hour.
4. Incubation for 4 hours at room temperature in buffer solutions of pH 3.0–10.0 followed by washing thrice with saline.
5. Adjustment of the pH of the diluent as well as suspensions of RBC's and fungal elements to pH values varying from 5.0 to 10.0.
6. Mixing in equal amounts with chloroform, ether and acetone, then shaking for 30 minutes. After evaporation of the solvents the sediment was washed thrice with saline.
7. Mixing 1 ml fungal suspension with 3.0 ml m/90 potassium periodate and shaking for 60 minutes, then 3.0 ml glycerol saline (1%) were added followed by thrice washing with saline.
8. Mixing in equal amounts with 1% trypsin, incubation at 56°C for 30 minutes to 2 hours then thrice washing in saline.
9. Mixing 0.9 ml fungal suspension with 0.1 ml of either concentrated hydrochloric acid or 4N sodium hydroxide and incubation at room temperature for 30 minutes, followed by neutralization of the pH and thrice washing with saline.

Results

Haemagglutination

All 36 isolates could agglutinate RBC's of different animals and birds within 40 minutes but the various isolates varied in the titre of their haemagglutinins from 2^2 to 2^6. In general, the microconidial suspension had higher titre than the suspension of the wild isolate. The cell-free culture filtrate did not agglutinate the RBC's indicating the absence of soluble haemagglutinins.

Table 1
Effect of age of M. gypseum cultures on haemagglutination

M. gypseum	Titre of haemagglutinins Age of cultures in weeks (w)							
	1 w	2 w	3 w	4 w	8 w	12 w	16 w	20 w
Wild form	2^3	2^4	2^4	2^4	2^3	2^3	2^2	2^2
Microconidial form	2^4	2^5	2^6	2^6	2^4	2^3	2^3	2^3

Table 2
Haemagglutination of different RBC's by M. gypseum

M. gypseum	Titre of haemagglutination of RBC's of								
	Cow	Buffalo	Camel	Sheep	Chicken	Duck	Pigeon	G.pig	Mouse
Wild form	2^3	2^3	2^2	2^3	2^4	2^2	2^3	2^2	2^2
Microconidial form	2^5	2^4	2^3	2^4	2^6	2^3	2^4	2^4	2^3

Table 3
Effect of heating on the haemagglutinins of M. gypseum

M. gypseum	Titre of haemagglutinins after heating							
	Control	60°C 1 h	70°C 1 h	80°C 1 h	90°C 1 h	100°C 1 h	100°C 2 h	120°C up to 2 h
Wild form	2^4	2^4	2^4	2^4	2^4	2^3	2^3	2^2
Microconidial form	2^6	2^6	2^5	2^5	2^4	2^4	2^3	2^3

The use of M. gypseum antiserum inhibited the haemagglutination by either the wild or microconidial form.

Effect of age of the culture

As shown in Table 1, the highest titres were obtained by suspensions made from 2–3 weeks old cultures in case of the wild isolate and 3–4 weeks old cultures in case of the microconidial form.

Haemagglutination of RBC's of different animals and birds

Both the wild and microconidial forms could agglutinate all types of RBC's tested (Table 2). The best result was obtained with chicken RBC's.

Effect of heat

The heating of the fungal suspensions up to 100°C for 2 hours inactivated a part of the haemagglutinins as indicated by the drop of the titre by 2 logs in case of the wild form and 3 logs in case of the microconidial form. However, autoclaving at 120°C for up to 2 hours did not destroy the remaining haemagglutinins (Table 3).

Effect of freezing and thawing or sonication

The repeated freezing and thawing for 20 times or sonication at the highest speed for one

Table 4
Effect of pH on haemagglutination by M. gypseum

M. gypseum	Titre of haemagglutinins at various pH values							
	pH 5.0	6.0	7.0	7.2	7.6	8.0	9.0	10.0
Wild form	ø	2^5	2^4	2^4	2^3	2^2	2^2	2^2
Microconidial form	ø	2^7	2^6	2^6	2^5	2^4	2^3	2^2

Table 5
Effect of chemicals on the haemagglutinins of M. gypseum

Chemicals	Titre of haemagglutinins	
	Wild form	Microconidial form
Untreated control	2^4	2^6
Potassium periodate	2^2	2^6
Trypsin	2^4	2^6
Chloroform	2^3	2^3
Ether	2^3	2^4
Acetone	2^4	2^5
Conc. hydrochloric acid	2^4	2^6
4N sodium hydroxide	2^4	2^6

hour of M. gypseum suspensions had no effect on the titre of the haemagglutinins of both forms.

Effect of pH

When the fungal suspensions were kept for 4 hours in buffer solutions of pH 3.0–10.0 then washed in saline the haemagglutinins were not affected. On the other hand, the titre of haemagglutinins was influenced when the test was done at different pH values, i. e. the diluent as well the suspensions of fungal elements and RBC's were adjusted to pH values of 5.0–10.0. At pH 5.0 the blood cells were haemolyzed. The highest titre was observed at pH 6.0–7.0 and the titre decreased with the rising of the pH values reaching to 2^2 at pH 10.0 (Table 4).

Effect of chemical treatment

As shown in Table 5 the haemagglutinins of M. gypseum (wild and microconidial forms) were resistant to the effect of trypsin, concentrated hydrochloric acid and 4N sodium hydroxide. The treatment with potassium periodate caused remarkable reduction of the haemagglutinating activity of the wild form, whereas the haemagglutinins of the microconidial form were not affected at all by the periodate. On the other hand, chloroform reduced the titre of haemagglutinins of the microconidial form markedly where it dropped from 2^6 to 2^3, while the haemagglutinin titre of the wild form was reduced only by one log. The use of other fat solvents as ether and acetone showed little or no effect (Table 5).

Discussion

It is worthy to note that the agglutination of red blood cells by M. gypseum was specific as it could be inhibited by M. gypseum antiserum. This substantiates the specificity of haem-

agglutination by dermatophytes reported by Refai et al. (1977). On the other hand, this is of practical importance in confirming the identification of M. gypseum especially when the characteristic macroconidia are lacking as it happened in the microconidial variants obtained during repeated subculturing during the present study. This needs however further studies concerning the possibility of cross-haemagglutination inhibition if other dermatophytes or their antisera are used.

The chemical treatments indicate that the haemagglutinins of the wild form are mainly carbohydrates in nature and incorporate a small portion of lipids. On the other hand, the haemagglutinins of the microconidial form seem to consist mainly of lipids.

References

Refai, M. & M. A. Shalaby (1975): Studies on haemagglutination by different fungi. Derm. Mschr. 161, 1022–1024.

Refai, M., M. A. Shalaby & M. S. Saber (1977): Studies on the physical, chemical and serological properies of haemagglutinins of dermatophytes. Mykosen 20, 198–202.

Address: Prof. Dr. M. Refai, Department of Microbiology, Faculty of Veterinary Medicine, Giza, Egypt.