Passive immunization with candida albicans antiproteinase as a prophylactic tool against the candidiasis

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Summary:

Background: *life threating fungel disease is now a frequent substantial of the immunocomprimised host population . Candida infection has raised throughout the era of antibiotics & immunosuppressive chemotherapy.*
Aim of study : *to highlight the need for alternative immunprophlactic tool .Therefore the need to develop prophylactic or therapeutic immunolglobulin against candidacies*
Materials & Methods: *12.5 mg/ml. of Candida albicans proteinase enzyme was injected S/C into a group of five rabbits weighing 3 Kg. with complete Freund's adjuvant . Antisera was collected from the animals after 28 days . 0.2 ml of this antisera given I/V into two group of rabbits ; followed by 0.1 ml of the antiserum one of thses groups as control . the 2nd group challenged with 2.8*10^7 cfu/ml of actively growing C. abicans for three days . the 3rd group received only 2.8*10^7 cfu/ml of C. albicans for three days .*

Results: *Test rabbits , which received both antisera and C.albicans , were completely protected when examined after six weeks . No symptoms of the diaease ; no pathological lesions could be seen in the organs (Kidneys, liver and spleen). Culture for C.albicans from these organs were all negative . In contrast to rabbits received challenged dose of Candida; they become sick , visceral organs showed profuse microabscesses . Candida was isolated from minced organs on culture .*
Conclusion : *the findings draws attention to an important fact that the passive immunizention can be applied to protect patients with leukemia and others immunocompromised individuals from serious infection of Candida .This discovery calles for application to open anew hope for these patients at least to diverse their suffer for sometime.*

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Introduction

Life threatening fungal disease is now a frequent substantial of the immunocomprmised host population. Candida infection has risen throughout the era of antibiotics and immunosuppressive chemotherapy. Patients suffering from AIDS and other underlying diseases particularly lymphoma , Hodgkin's and other leukemia have a predilection to candida infection .1,2,3

Identification of candidal vaccine must take into account the diversity of candida diseases . Antigens to be considered in this contest , are members of asparryl proteinase (SaP2) and the 65-KDa mannoprotein (MP65) antigens (4) .On the other hand toxicity of Amphoteracin B to mammalin cells and cyoreductity therapy undergone by cancerous and AIDS patients make the drug less active and even ineffective sometimes (5) .

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All these , highlight the need for alternative immunprophylactic tool . Therefore the need to develop prophylactic or therapeutic immunoglobulin against candidiasis is a clear priority .

Materials and methods

Candida albicans species used in this work was isolated from leukaemic patient, who showed candidemia . The organism was tested by conventional methods to show typical characteristics of C.albicans according to Emmons (1970)(6).

Candida proteinase enzyme was prepared according to Odds (1988)(7) .

Preparation of immune sera was done according to Methew at al (1986)(8) Five rabbits with a weight of 3 Kg were injected with 12.5 mg/ml of proteinase in saline mixed with one ml of Freund's complete adjuvant subcutaneously . this dose was repeated two weeks later .Blood was collected from injected rabbits after 28 days of the last injection . Sera were

prepared by centrifugation at 100*g for ten minutes. Immune sera was tested for direct agglutination Once a drop of intact serum and another diluted (1:10) were used with one drop of broth culture of C.albicans in log phase , separately . Passive immunization of rabbits was performed according to Mohymen et al (2000)(9) three groups of rabbits (two rabbits in group I,II and four rabbits in the group III weighing 3Kg) were used in this trial . the first group was injected with 0.2 ml of immuned serm at day one , followed by 0.1ml of immuned serum for next two days . the challenged dose of C.albicans (2.8*10^7 cfu/Kg according to Binbouzid et al (1984)(10), was given I/V two hours after receiving the test dose of immuned serum , Once a day for three days . The 2nd group of animals received immuned serum only . as in the dose of group 1(control) .the 3rd group , (four rabbits) received 1/ V injuction of C. albicans (2.8*10^7 cfu/Kg) once a day for three days .

The animls were kept at R^0 and under close observations for any cutaneous , mucoutaneous lesions . Animls sacrificed after six weeks . Gross examination of kidneys, liver and spleen were done looking for microabcesses or other lesions. Minced parts from these prgans were cultured for C. albicans.

Results:

Group I the two rabbits that have been passively immunized and challenged with C.albicans were sacrificed after six weeks . they remained normal and healthy until day of sacrificing , visceral organs i.e. kindays , liver and spleen remained normal in size and appearance . there were no abscesses or microabscesses ; no pathological changes were seen . Direct examination and culture of minced organs were negative for any growth of the yeast .

Group II (animals received immuned serum 0.2 ml at day one , followed by 0.1 ml for two days) as a negative control ; they remained healthy and normal during the course of the trail . Gross examination of kidneys , liver and spleen were normal in texture and shape . Culture of minced organs failed to support any growth of the yeast .

Group III four animals which received 2.8*10^7 cfu / Kg of C.albicans once a day for three days (positive control) showed different lesions beginning at the first week of the experiment . Cutaneous, mucocutanrous , paraonychia and onychomycosis were prominent lesions beside conjunctivitics . When the animles of the group were sacrificed after six weeks they were fairly sick with all the above mentioned symptoms. Gross examination of kidneys , liver and spleen showed hypertrophy and microabscesses all over the surface of the organs . Minced organs i.e. liver, kidney and spleen wrer cultured on Sabouraud's agrar at 37^0 C , yielded heavy growth of C . albicans .

Discussion:

Frequency of candidiasis raised from 20% in leukemic patients (Body 1966)(11) to 40% (Esley et al 1982) (12) and 42% (Ibrahim et al 2002) (13) . candida septicemia is an increasing encountered incidence , particlurly as a terminal event to many underlying diseases , such as leukemia , neoplasia etc. (Rippon 1988)(1).As it is shown in this work aspartyl proteinase play a major role in the pathogenicity of candida . Inoculation of candida was able to produce systemic infection in the rabbits of Group III (positive control) including microabcesses in large number in kidneys, liver and spleen . All these organs gave positive culture for the yeast .

On the other hand rabbits in group 1 (test group)which recived anti proteinase antibodies and chanllenged with candida albicans i.e. protected animals remained normal and healthy throughout the period . Cultures made from minced organs and other samples were negative for the candida growth . It is obvius that the enzyme aspartyi proteinase play major role in the pathogenicity of Candida ; in turn anti-aspartyi proteinase antibodies was able to block the pathogenicity of the yeast , consequently the yeast were killed in the protected animals . This clearly shows that colonization of C.albicans and it's overgrowth has been banded by the circulating antibodies in passively immunized animals . The evidence of that came from the fact that we failed to detect yeast cells in the body fluid and tissues of test animals , both by direct examination and culture . The finding draws attention to an important fact that the passive immunization can be applied to protect patients with leukemia and others immunocompromised individuals from serious infections of candida as previously described .this discovery calls for application to open a new hope for these patients at least to diverse their suffer for sometime .

References:

1. Rippn , J.W.(1988),Med.Mycology ;3rd Ed. Pp 555-560 .W.B Saunders Philadelphia .
2. Fridkin . S. and Javeis , W.R.(1996).Epidem . of nosocomial fungal infection .Clin .Micro.Reviews Oct.1966 pp499-511.
3. Richardson, M.D. and Kokki, M. (1999); diagnosis and prevention of fungal infection, Blood Rev.12:241.
4. Antonio C.(2000)Proteinase antigens and mechanism of anticandida . Nippon Tshinkin Gakkai Zassi 41:219(abstract).
5. Janina ,B.and Jaques B.(1996) Carriers effect on biological activity of Amphoteracin B. Clin.Micro.Reviews Oct 1996.
6. Emmons C.W.,Binford , C.H.and Uptz , J.P.(1970) Medical mycology pp 180 churchil livingstone INC N.Y.
7. Odds,F.C (1988)Candida and Candidiasis . Pp 1-3 second Ed. Bailliere Tindal London .

8. Mathew , R.C.Burrlie J.P.and Tabaqchali,s. Isolationof immunodominant antigens from sera of patients with systemic candidiasis . J.clin.Microbiology . 14:23-237.
9. .Mohymen , N.A.,AL-Khashall,S.S,and Raoof , N.(2000);Adaptive transfer of immune responses to Brucella infection .IBN-AL-Hathiam J.for pure and applied sciences 13:1-5.
10. Benbouized , A., Guinedi,R.,and Cloppet , H.(1984); purification of soluble specific antigens of system candidiasis. J.Chromatography 306:667-687.
11. Bodey . G.P.(1966); Fungal infection complication acute Leukemia . J.Chronic disease 19 :667-687.
12. Eslry E.H.,Keating , M.I, McCredie , K.B.,Body, G.P and Frierech E.H.(1982) ; Causes of remission induction failure in acute myelogenous Leukemia Blood 60:309-315.
13. Azhar A.Ibrahim (2002) Ph.Dthesis Dept.of Micro.,Iraqi Med college Nahrin University .