INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-4, Issue-1, Jan-Mar-2013 Coden : IJABPT Copyrights@2013

ISSN : 0976-4550

Received: 06th Dec-2012 *Revised: 15th Dec-2012* *Accepted: 16th Dec-2012*

Research article

HISTOMORPHOLOGIC STUDY OF EXPERIMENTAL FUNGAL INFECTION WITH IMMUNE MODULATION

Rajashree J Ingin, Vijaykumar L Pattankar and Shagufta Roohi*

Department of Pathology, MR Medical College, Gulbarga.

Correspondence Details: Email: shaguftaroohi@yahoo.com

ABSTRACT

Background and objectives: To study the histomorphological changes in various organs of mice receiving C.albicans inoculation with and without prior corticosteroid injections.

Method: The study consisted of 5 groups. Group I animals received C.albicans intraperitoneally. Group II received corticosteroids prior to intraperitoneal C.albicans inoculation. Group III animals received intravenous injections of C.albicans. Group IV animals received corticosteroids prior to intravenous C.albicans injection. Group V, the control group received normal saline.

Results: Group I animals showed microabscesses on liver, spleen and in omentum. Healing of lesions occurred spontaneously. Group II animals noted increased mortality with more widespread lesions. Involvement of lung and kidney was also noted. In Group III, mortality occurred in all animals within 72hrs. Lesions were also noted in kidneys, heart, and brain.

Conclusion: Corticosteroid administration induces immunosupression resulting in disseminated infections.

Key words: Corticosteroids, Candida Albicans, Microabscess.

INTRODUCTION

Experimental pathology has been used in various fields. Experimental reproduction of infectious diseases was one of the essential Koch's Postulates. With the increasing use of cytotoxic and immunosuppressive agents in the treatment of neoplasm as well as a variety of nonmalignant diseases, use of broad spectrum antibiotics with immuno-suppressant side effects, fungal infections like candidiasis particularly, have become an increasingly important clinical problem. Patients receiving high dosage, long term corticosteroid therapy are particularly at risk for opportunistic infection like disseminated candidiasis. The administration of adrenal glucocorticoids is associated with enhancement of many experimental infections. From many well documented clinical and pathological studies of human systemic candidiasis, it is possible to distinguish three major types of deep seated candidiasis that are accompanied by fungaemia. These are Candida endocarditis, renal candidiasis and acute disseminated candidiasis. Attempts have been made to produce these diseases experimentally. Hurley and Winner (1975) showed that intravenous inoculation of small doses of candida albicans into mice led to chronic progressive fungal disease that was limited to the kidney where as intravenous injections of large inocula produced disseminated mycosis. The purpose of this work was to describe the histomorphological features of experimental candidiasis in healthy mice and to compare the lesions with those in mice given cortisone prior to Candida albicans inoculation.

MATERIALS AND METHODS

Ethical clearance was obtained from the institutional ethical committee. Inbred male Swiss albino mice were used in the experiment. Animals were divided in groups according to the experimental design.

Canadida Albicans: C.albicans laboratory culture originally isolated from a patient with oral thrush was used. For inoculation, saline suspensions of living yeast were prepared from fresh culture grown for 24hrs at 37^0C on pentose agar medium and subcultured on Sabouraud's medium for 24hrs at 37^0C. Single inoculation of 0.5ml consisting of C.albicans in a concentration of $2x10^6$ cells/ml was injected into all the mice except the control group.

Corticosteroids: Dexamethasone with a concentration of 4mg/ml was used. 0.75ml of dexamethasone (3mg) was injected subcutaneously in the nape of neck daily for 6 days.
Histology: Animals which died/sacrificed were autopsied.
Experimental Design: The experiment was divided into five groups.
Group-I: 12 mice received intraperitonial inoculation of C.albicans suspension.
Group-II: 12 mice received subcutaneous corticosteroid for 6 days followed by intraperitoneal inoculation of C.albicans suspensions
Group-III: 12 mice received intravenous inoculation of C.albicans.
Group-IV: 12 mice received subcutaneous corticosteroid injections for 6 days prior to intravenous C.albicans inoculation.
Group-V: 6 mice received normal saline injections, acted as control group
Animals were observed daily for survival, weight, and activity. Animals that died during the experiment were autopsied. Animals that survived were killed at an interval of 5. 10 and 15 days following injection of C.albicans with chloroform inhalation.
Histopathological Examination:
After going through autopsy, representative sections were processed. H &E and PAS stains were performed. Histopathologic changes in various organs of animals belonging to different groups were evaluated and compared. An attempt was made to explain the significance of the observed findings and its possible correlation.

OBSERVATIONS

Group-I:

No apparent illness or altered behavior was noted in the mice immediately after inoculation of C.albicans. At 24hrs only one animal appeared to be sluggishly active which was found dead at 48hrs. The remaining mice were active and no mortality resulted in these animals until they were sacrificed. One animal each was sacrificed on day 5, 10 and 15. The remaining animals survived beyond 1 month.
Autopsy finding: external autopsy were non-specific.
In animal dead at 48hrs:
Liver- showed small creamy white raised lesions measuring 1-2mm in diameter(fig.1). Microscopy showed neutrophils were observed on the capsule and in the subcapsular region(fig.2). The inflammation from the capsular region was seen seeping into the parenchyma through the septum. Dilated and congested central veins were seen with perivenular neutrophilic infiltrate. Portal triditis was also seen. The hepatocytes showed swelling with mild degree anisonucleosis. Pseudohyphae and mycelia of C.albicans were seen in the subcapsular and parenchymal abscesses on PAS stain.

Fig-1 : Gross photograph of liver (Group I) showing exudates on the surface (thin arrow) and a small capsular abscess (thick arrow)

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

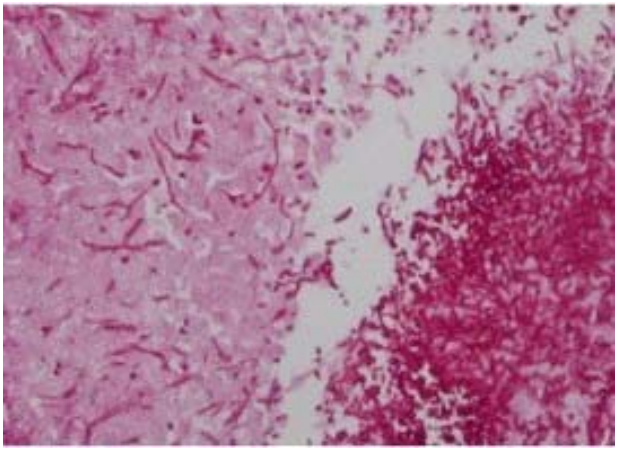

Fig-2 : Photomicrograph of liver showing a subcapsular abscess with multiple hyphae and mycelia of C.albicans (PAS stain x400)

Spleen- similar lesions as seen on the liver were seen. Microscopy showed abscess on the capsular region comprising of neutrophils and few budding yeasts in abscess. Omentum: microabscesses were seen consisting of neutrophils and few lymphocytes with few organisms.
Other organs did not show any specific microscopic changes.
Day 5- showed similar lesions.
Day 10- clearing of lesions were seen the liver with replacement of the neutrophils by lymphocytes and few plasma cells. No organisms were detected.
Day 15- no microabscesses or excess leukocytes in liver parenchyma or portal triad were noted. Other organs were unremarkable.

Group-II:

No change in behavior or weight was noted in mice following corticosteroid injections. No apparent illness was seen after C.albicans inoculation. After 24hrs, few animals were moribund and death resulted at regular intervals. Only one animal survived till day 5, which was sacrificed.
Autopsy findings: Lesions seen in liver and spleen were similar to those encountered in the animals of Group-I but were more extensive. Patchy areas of consolidation were seen in lungs which microscopically showed areas of bronchopneumonic changes. No abscess or yeasts were detected.
Kidneys showed few microabscesses in the cortex and medulla comprising of neutrophils which were seen in the paraglomerular area in the interstitium. Few yeasts were seen within these abscesses on PAS stain.
Day 5- Autopsy of the animal sacrificed showed replacement of neutrophils by lymphomononuclear cells and few plasma cells. Other organs were unremarkable.

Group-III:

No significant change in behavior and appearance was seen immediately after C.albicans. Death occurred in all the animals within 72hrs.
Autopsy findings:
48hrs and 72hrs-
Lungs showed patchy areas of consolidation. Microscopy showed focal areas of bronchopneumonic changes and intra-alveolar hemorrhages. No abscess formation or any organisms were seen.
Brain showed focal areas of gliosis with pseudohyphae of C.albicans(fig.4).
Heart showed features of myocarditis along with many scattered microabscesses consisting of neutrophils and mycelia. Focal areas of endocarditis and pericarditis were observed(fig.5).
Liver- 2-3 microabscesses were seen in liver parenchyma with dilated and congested blood vessels.
Spleen- no fungal lesion were noted in the spleen.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

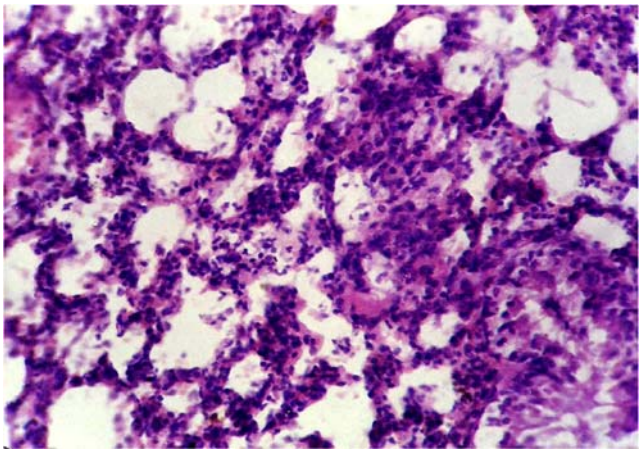

Fig-3: Photomicrograph of lung showing bronchopneumonic changes (H&E x400)

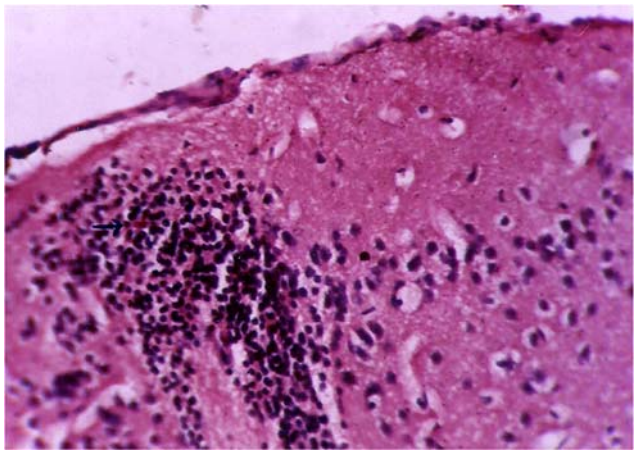

Fig-4: Photomicrograph of brain showing glial nodule with occasional yeast (arrow) (PAS x400)

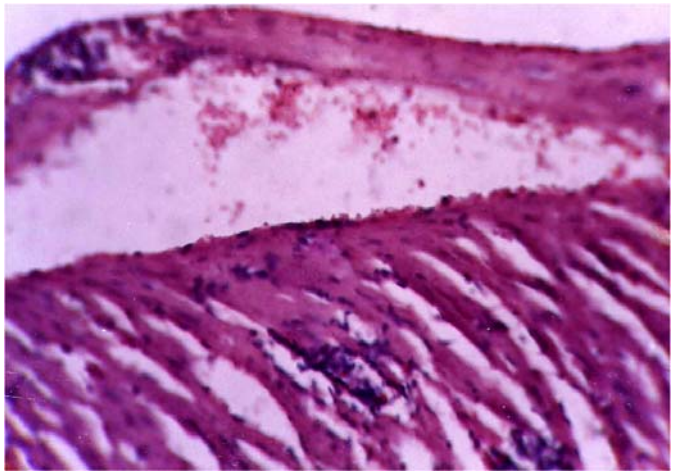

Fig-5: Photomicrograph of heart revealing pericarditis and myocardial abscess (PAS x400)

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Kidneys- were most extensively involved organs. Multiple microabscesses were seen throughout. Lesions were seen in interstitium surrounding some of the tubules and some were periglomerular(fig.6). Glomeruli also showed increased mesangial cellularity due to the presence of neutrophils. Some abscesses showed central area of necrosis. The abscesses were seen extending from the medulla, penetrating the papillae into calyceal system. Some penetrated renal pelvis and the renal capsule. The perinephric fat showed foci of acute inflammatory exudate. PAS stain revealed many proliferating mycelia in the tubules and glomeruli. Only the mycelia within tubules, penetrating the epithelium and basement membrane into interstitium showed marked acute inflammatory response. Papillae also showed budding yeast.
Other organs like GIT, testes, eyes etc. were unremarkable.

Group-IV:
Death was observed in all the animals within 24hrs.
Autopsy findings:
Lungs showed bronchopneumonic changes with occasional demonstrable blastopore in an area surrounded by acute inflammatory cells. Intra-alveolar hemorrhages, dilated and congested blood vessels were seen(fig.3).
Kidneys showed exudate in the perinephric region.
Liver showed multiple microabscesses with foci of necrosis in the parenchyma. Central vein congestion was seen with perivenous acute inflammatory cells. Portal triaditis was also noted.
Other organs were unremarkable.

Group-V:
The control group animals survived normally. The animals sacrificed at 5, 10, and 15 days, did not show any specific change.

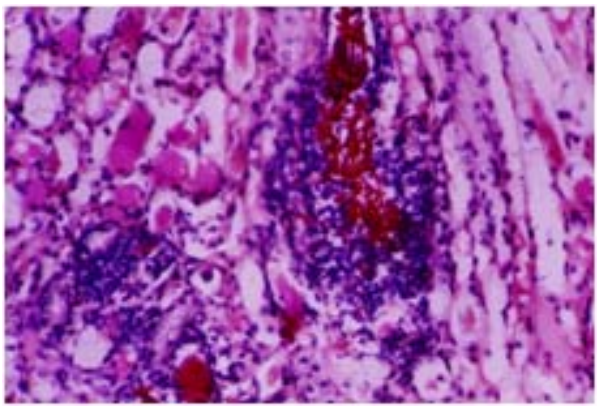

Fig-6: Photomicrograph of kidney exhibiting mycelia of Candida within the tubule penetrating the tubular wall with extensive neutrophilic infiltrates (PAS x400)

DISCUSSION

The present study tries to focus the array of histomorphological changes after Candida injections in albino mice with and without corticosteroid priming, since fungal infections constitute an important hazard after frequent use of steroids, broad-spectrum antibiotics, immunosuppression, transplants and use of bioprosthesis.
In the present study, in Group I animals, which were given intraperitoneal C.albicans injections, only one animal succumbed to death after 48hrs; remaining animals survived until they were sacrificed. Autopsy showed abscesses in liver, spleen, and omentum which contained budding yeast and mycelia.

These lesions healed spontaneously with replacement of neutrophils by lymphomonuclear cells, as evidenced by histopathology of animals sacrificed on day 5, 10, and 15. In an experiment by Hurrley et al. (1975), no mortality resulted in their animals following intraperitoneal injection of C.albicans. Autopsy showed multiple sites of abscess formation in omentum and serosal surfaces of the abdominal wall and liver, but no organisms could be cultured. A similar finding was seen in a study by Winner HI et al. (1960) who studied the effect of intradermal, intraperitoneal and intravenous administration of Candida in guinea pigs.

In Group II animals, which received corticosteroid prior to intraperitoneal injection of C.albicans, death occurred in 11 out of 12 animals within 72hrs. Lesions were seen in kidneys, and lungs along with lesions in liver, spleen and omentum. The fact that cortisone lowers resistance in mice has been described by Claman HN et al. (1972). and Fauci AS et al, (1974).

In the present study death of all the animals within 72 hrs and 24hrs was noted following intravenous injections of C.albicans in Group III and Group-IV animals respectively. The lesions mainly seen in the kidneys, heart and brain were abscesses. The lungs showed bronchopneumonic changes. In experiments by Evan ZA et al. (1977) a greater percentage of pseudohyphae were localized initially in lungs and later in the liver. They proposed that the liver may be less effective than the lungs in killing of Candida. Kidneys were identified as the organs most severely affected during systemic candidiasis. Our findings are consistent with the above observation.

Mycotic lesions were seen in heart in studies by Hurley DL et al.(1975). The myocardial abscesses showed proliferating mycelia in Group III and Group IV animals. Focal endocarditis and pericarditis were also noted.

Glucocorticosteroid was used as an immunosuppressive drug in the present study. All the animals succumbed to death within 24hrs after intravenous C.albicans infection in dexamethasone treated mice in Group IV. Lesions were more extensive when compared to Group III animals. The administration of adrenal glucocorticoids was associated with enhancement of many experimental infections.

It is well known that glucocorticoids exert profound effect on specific host immune responses. Several immunosuppressive effects of these agents have been observed to occur in man, including mild lymphocytopenia (Coburg AJ et al, 1970), decreased immunoglobulin production (Butler WT, Rossen R, 1973), and impaired expression of cutaneous delayed hypersensitivity responses (Bovornkitti S et al, 1960). It is now clear that glucorticoids inhibit the production by multiple cells of factors that are critical in generating the inflammatory response. Glucocorticoids decrease the release of vasoactive and chemoattractive factors and decrease extravasation of leukocytes to areas of injury. Cytokines play an essential role in the integrated action of macrophages, and lymphocytes in mounting immune responses to various pathogens. Cytokines like interferon, granulocyte monocyte colony stimulating factor, interleukins and tumour necrosis factor-α are inhibited by glucocorticoids (Knudsen PJ et al, 1987). This explains the dissemination of C.albicans following intraperitoneal/intravenous injection in cortisone treated mice with decreased clearance of the fungus by the inflammatory cells.

That cytokines play a major role in host defence against systemic Candida infections has been evaluated (Louria DB et al, 1994). Also human lung macrophages have Candida killing capacities (Edwards JE et al, 1978). In addition neutrophils, monocytes and eosinophils also ingest and kill Candida (Jensen J, Warner T, Balish E, 1994).

CONCLUSION

1. Intraperitoneal injections of C.albicans produced lesions confined to the serosal surfaces of liver, spleen and omentum which heal spontaneously.
2. Intravenous injections of C.albicans caused widespread dissemination of candida with the lesions in various organs like kidneys, heart, brain, lungs and liver.
3. Mice receiving corticosteroid injections prior to intraperitoneal/intravenous injections of C.albicans showed increase in the severity of infection and increase in mortality
4. Possible explanations for these findings have been presented.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

REFERENCES

Butler WT, Rossen R. (1973). Efect of methyl prednisolone on immunoglobin metabolism in man. Feb Proct,; 32; 1028

Bovornkitti S, Kangsadal P, Sathirapat P and Oosombatti P. (1960). Reversion and reconversion rate of tuberculin skin reactions in correlation with use of prednisone. Dis Chest, 35, 51-53

Claman HN. (1972). Corticosteroids and lymphoid cells. N Engl J Med. Aug. 287(8): 388-397

Coburg AJ, Gray SH, Katz FH, Penn I, Halgrimson C, Starzl TE. (1970). Disappearance rates and immunosuppression of intermittent intravenously administered prednisolone in rabbits and human beings. Surg Gynecol Obstet,; 131: 933-942.

Hurley DL, Balow JE, Fauci AS. (1975). Experimental disseminated candidiasis. II.Administration of glucocorticosteroids, susceptibility to infection and immunity. J Infect Dis. Oct. 132(4):393-398.

Evans ZA, Mardon DN. (1977). Organ localization in mice challenged with a typical Candida albicans strain and a pseudohyphal variant. Proc Soc Exp Biol Med Jun; 155(2): 234-238.

Fauci AS, Dale DC. (1974). The effect of in vivo hydrocortisone on subpopulations of human lymphocytes. J Clin Invest. Jan, 53(1): 240-246

Jensen J, Warner T, Balish E. (1994). The role of phagocytic cells in resistance to disseminated candidiasis in granulocytopenic mice. J Infect Dis. Oct. 170(4): 900-905

Knudsen PJ, Dinarello CA, Strom TB. (1987). Glucocorticoids inhibit transcriptional and post transcriptional expression of interleukin I in U937 cells. J Immunol Dec; 139(12): 4129-4134.

Louria DB, Baltch AL, Smith RP, Franke MA, Ritz WJ, Singh JK, Gordon MA. (1994). Tumor necrosis factor alpha has a protective role in murine model of systemic candidiasis. Infect Immun Jul. 62 (7): 2761-2772.

Winner HI, Hurley R. (1964). Candida Albicans: Little Brown,; 193-213.

Winner HI. (1960). Experimental moniliais in the guinea-pig. J Pathol Bacteriol Apr; 79: 420-423.