Comparison of Immunosuppressive effects of Cyclosporin A and Dexamethasone on invasive aspergillosis in experimental animals

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In this study, 60 female New Zealand white rabbits were chosen and divided into 3 groups. The animals in group 1&2 were immunosuppressed with dexamethazone and cyclosporin A (CYA) respectively. The animals in group 3 (control animals) were not immunosuppressed. In each group, half of the rabbits were infected intratracheally and the other half were infected intravenously with 4×10^5 conidia of Aspergillus fumigatus. None of the 20 control animals showed any clinical symptoms. All the rabbits in groups 1&2, which were infected intravenously, developed clinical signs of invasive aspergillosis. However, of these 2 groups the rabbits which were given the conidia intratracheally, only 7 of 10 animals in group 1 demonstrated clinical signs of aspergillosis. At autopsy there was macroscopic and microscopic evidence of invasive aspergillosis. Histopathological examination revealed hyphae invasion in liver, lung, brain and spleen. We conclude, in natural conditions that aspergillosis is initiated by inhalation of airborn condidia, cortison-treated patients are more susceptible to invasive aspergillosis than individuals who received CY-A.

Key words : Aspergillus Fumigatus, Cyclosporine A, Dexamethasone

The aspergilli are among the most ubiquitous saprophytic fungi in the environment. Conidia from these organisms are easily isolated from decaying vegetation, soil, and air world wide. Among these, ***Aspergillus fumigatus*** is the most common cause of aspergillosis. Aspergillus flavus is the second most common species, particulary in invasive disease of immunosuppressed patients (2).

Aspergillus niger, A. sydowi, A. terreus, A. ustus, A. versicolor, A. amstelodami, A. oryzaem, A. restrictus, A. candidus, and ***A. nidulans*** have also been reported to cause invasive disease (2, 11).

The term " ***Aspergillosis*** " describes many different clinical entities which fall into four main categories: Allergic aspergillosis, aspergilloma, invasive aspergillosis, and chronic necroziting aspergillosis (12).

Invasive aspergillosis is used generally to imply histopathologically demonstrated invasion of tissues. Aspergillosis in the lung arises by endobronchial proliferation of hyphae followed by invasion across bronchi into pulmonary arterioles and lung parenchyma, with subsequent ischemic necrosis (13). The propensity of aspergillus hyphae to invade blood vessels can also result in disseminated lessions with thrombosis, infarction, and hemorrhage of the organs involved (14).

Risk factors predisposing patients to invasive aspergillosis include corticosteroid therapy (12, 18, 22) cytotoxic chemotherapy (12, 18), transplantation, espacially during immunosuppressive therapy (22, 24) and chronic granulomatous disease, a qualitative disorder of neutrophil function, but neutropenia is most predominant (25).

Material and methods

Experimental animals :

Sixty female New Zealand white rabbits, 2-3 Kg, were chosen. To prevent bacterial superinfections, gentamycine, 4 mg daily, was given intramuscularly for 4 days before the start of the experiments. The rabbits were divided into three groups of 20 as follows :

- Group 1 : The rabbits received dexamethazone intramuscularly 3 mg/kg every day for 8 days.
- Group 2 : The rabbits received intramuscular cyclosporin A for 4 days. Days 1-3 the dose was 4 mg/kg and on day 4 the dose was increaed to 10mg/kg.
- Group 3 : The animals in this group (control animals) were not immunosuppressed, but infected.

Preparation of inoculum :

A. fumigatus, was originaly isolated from a patient with pulmonary aspergillosis. It was maintained on sbouraud's glucose agar plates at 37°C. To isolate conidia, 12 days old cultures were flooded with 10ml of sterile distilled water. The plates were sealed and shooked mutually. The water was poured off into tubes and condida were counted by a haemocytometer. The concentration of condida was adjusted to 4×10^5 conidia/ml.

Intratracheal inoculation :

Out of all 3 groups, 10 rabbits were anaesthetized using 25 mg/kg Ketamine and little amount of acepromazine. The anterior neck hair was shaved by a scalpel and the injection site was disinfected. The needle was driven into the trachea. To assure the proper position of the needle, aspiration was performed and if air was easily drawn into syringe, 1 ml of the inoculum, containing 4×10^5 conidia/ml was injected into the trachea. The reduction in respiration was a confirmation of the entrance of inoculum into the lungs.

Intravenous inoculation :

Following disinfection of the ear, using the marginal vein, each rabbit was intravenously infected with 4×10^5 conidia in 1 ml of sterile distilled water.

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Statistical analysis :

The chi-squre test was used to assess statistical differences between the groups.

Results

Clinical signs :

After infection, the rabbits were observed closely for the appearance of the clinical symptoms. In the control group, out of 20 animals, none showed the clinical symptoms. Rabbits, that received Dexamethazone and CyA (group 1&2) and injected the fungus IV, indicated the clinical signs and died after 3 days. But, of these 2 gropus, among 20 rabbits which were injected the conidia intratrachally, only 7 of 10 animals in group 1 demonstrated clinical signs and they also died after 8 days.

Significant differences in clinical signs of invasive aspergillosis were observed between groups 1&2 with respect to route of infection. These differences were significant between control group and group 1, control group and group 2, as well.

In these animals, the clinical symptoms, 2 days after intratracheal inoculation, were anorexia, ataxia, severe movements of the head, tilting of head and neck towards one side. This stage of disease lasts for 5-6 hours and then the afore-mentioned symptoms become more severe. It will be followed by nystagmus and the animals excitability increase fell on the ground and rolled with tense limbs and finally died after severe seizures. In some of the rabbits keratitis and blindness were observed. The dissease lasted, on the average, 24 hours.

Postmortal examination :

We immediately performed necropsy on all the rabbits, which died because of exaperimental infection. 20 days after injection of the fungus, all the rabbits, which survived, were sacrificed and necropsy was performed on them.

There were a lot of granules in the kidneys, lungs and livers of the rabbits, which demonstrated clinical signs and even, in some cases, in the abdominal cavity (Fig. 1).

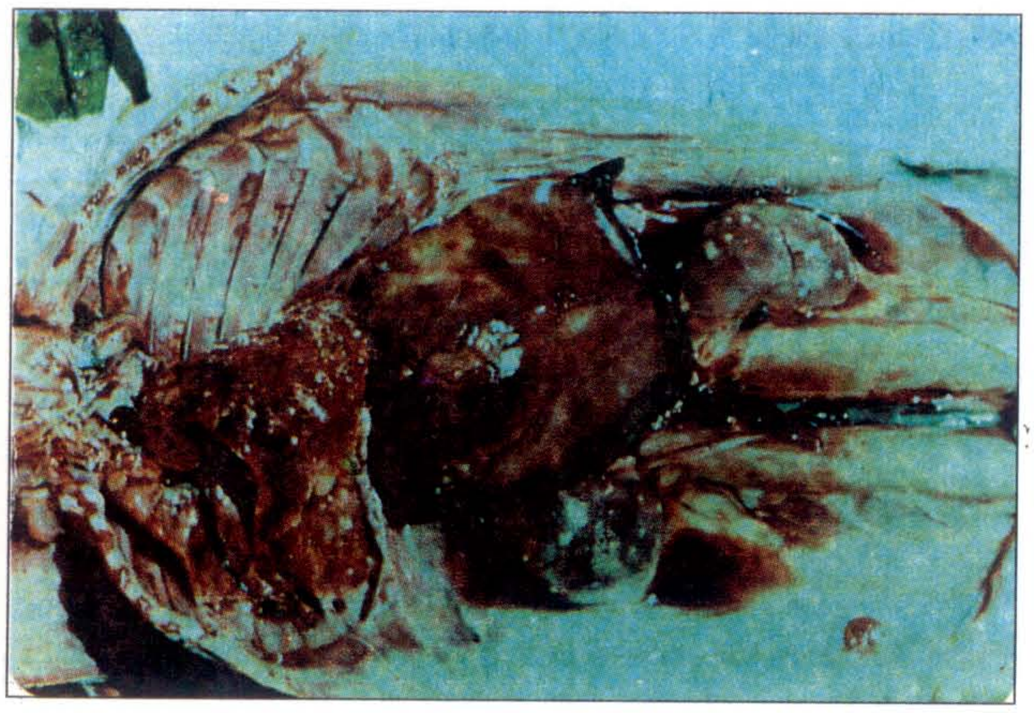

Fig. 1. Fungal granules in the lungs, liver and kidneys.

In necropsy of the rabbits, in group 1&2 which did not demonstrate clinical symtoms, there were not seen any fungal granules but all showed different degrees of pulmonary lesions, including hyperemia, petechial spots, hepatization of some parts of lungs and large spots of hemorrhage in lungs (Fig. 2).

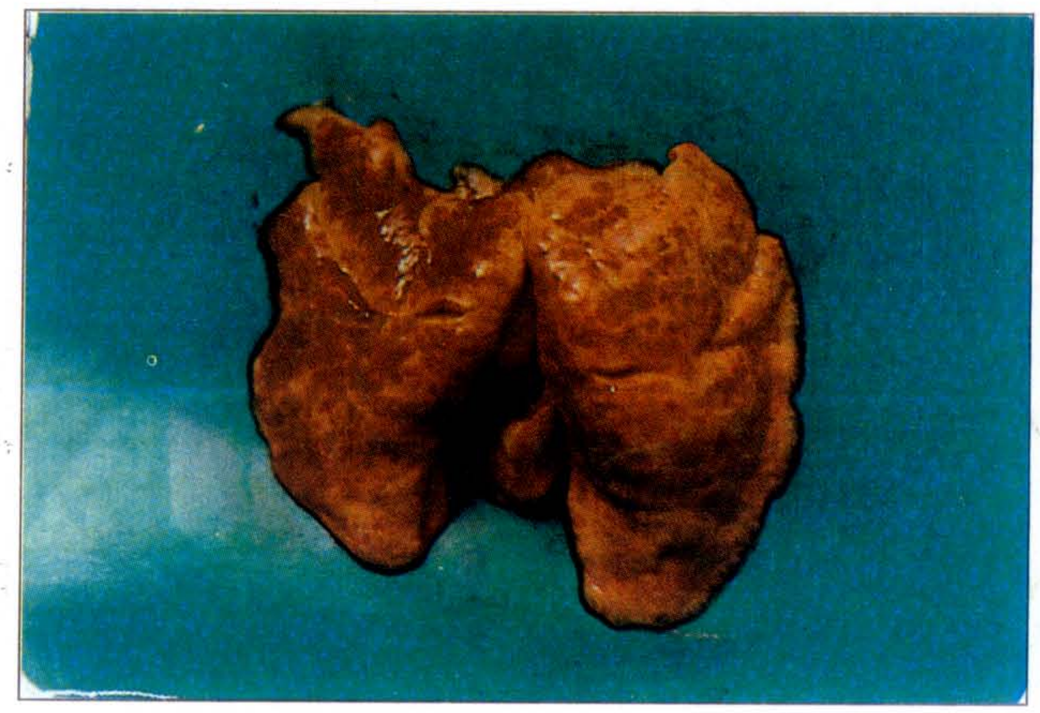

Fig. 2. Petechial spots with hypermia in lung.

In control group, only 1 rabbit, which received aspergillus intravenously showed little fungal lesions in the necropsy and there were not observed any other particular symptom.

Histopathological findings :

Liver :

In liver tissue, hemorrhage and necrosis were observed. There were also several granulomatous lesions, with different sizes, in some cases (Fig. 3).

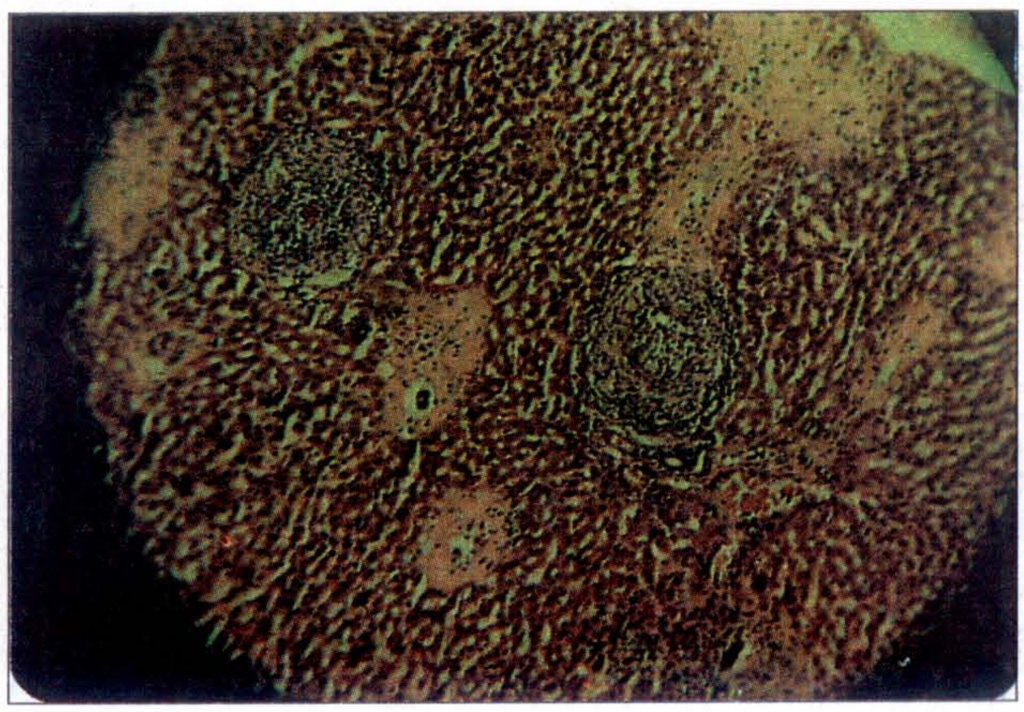

Fig. 3. Granulomatous lesions in liver (×10, H&E).

In these lesions giant cells, fibroblasts and lymphocytes were presented. Several eosinophils, in addition to the afore-mentioned inflammmatory cells, were observed in the infected foci. Granulomatous lesions, were mostly local ones. Fungal hyphae in the liver of the rabbits, which had received aspergillus intravenously, were also seen.

Kidney :

The kidney tissue was severly hyperemic, and in some places, there were hemorrhage and necrotic lesions and inflammation

(Fig. 4). Kidney tubules had been swollen and degenerated. There were large and small lesions obvious in the kidney tissue. In the place of lesion, inflammatory tissue and cell necrosis is largely seen.

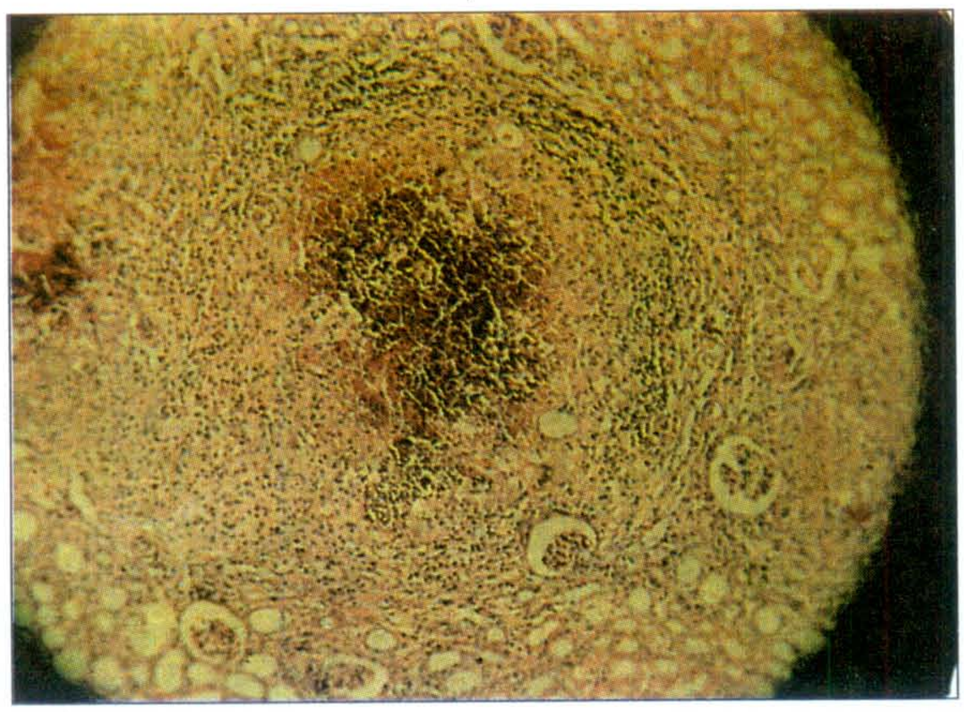

Fig. 4. Necrotic lesion and inflammation in kidney (×10, H&E).

Necrotized tubules, contained dark eosinophilic cytoplasm and picnosed nucli. Ther were pieces of hyphae in the centre of the necrosed foci. Necrotized lesions were non piogenic and were mostly observed focally.

Lung :

In the lung tissue, severe hyperemia, hemorrhage and several necrotic spots were observed (Fig. 5).

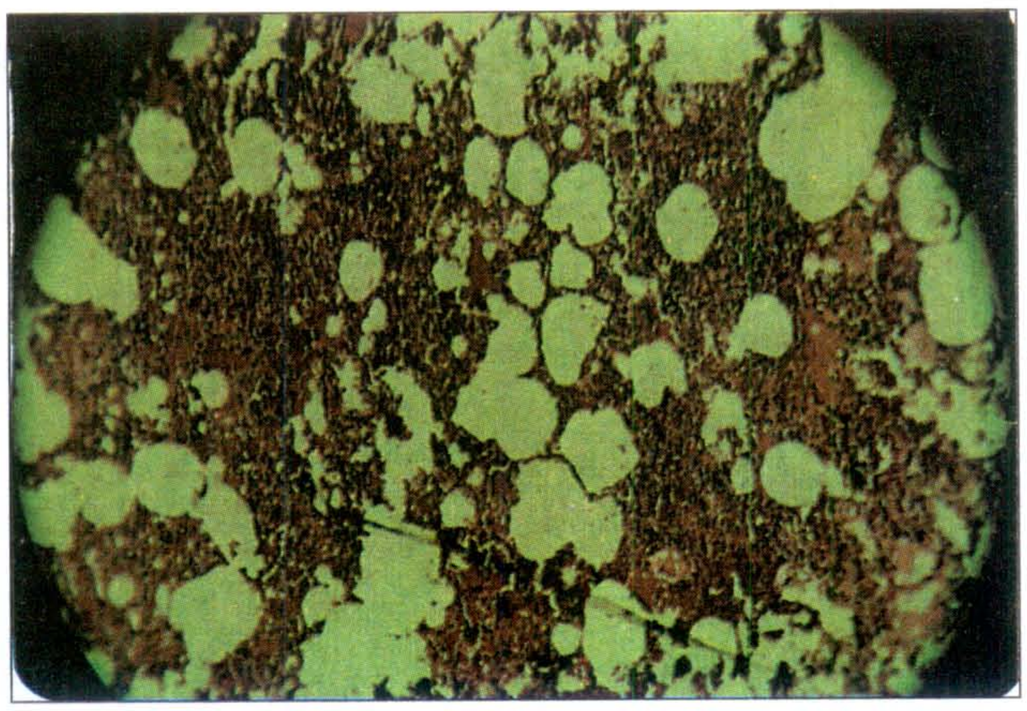

Fig. 5. Pulmonary lesion showing hemorrhage & hyperemia (×10, H&E).

A large portion of the lung alveols had been filled with proteinaceous, eosinophilic and fibrinous material. There were foci consist of inflammatory cells and necrosed lung tissue. In some foci, thrombosis and necrosis of the vessel walls were observed. In most of the necrosed spots, pieces of hyphae were revealed. There were accumulation of mononuclear cells and strong proliferation of epithelloid and macrophages around these lesions. Pulmonary lesions were mostly of acute, non-pyogenic pneumonia and chronic granulmatous pneumonia.

Brain :

In the rabbits received aspergillus interavenously, there were abundant small but scattered hyphae (Fig. 6). There were hyphae in brain and cerebrum. Wide range of necrosis was observed in the brain white matter. In some parts of brain, there were foci of mononuclear cells and especially lymphocytes. The necrosed foci of brain had been replaced by inflammatory cells. In the spaces, around the lesions, demyelination and infiltration of mononuclear cells in the nerve tissue, interaxonal edema and distention of Robin-virchow spaces were observed. In most case, non pyogenic encephalitis and meningitis with lymphocyte accumulation around vessels were indicated.

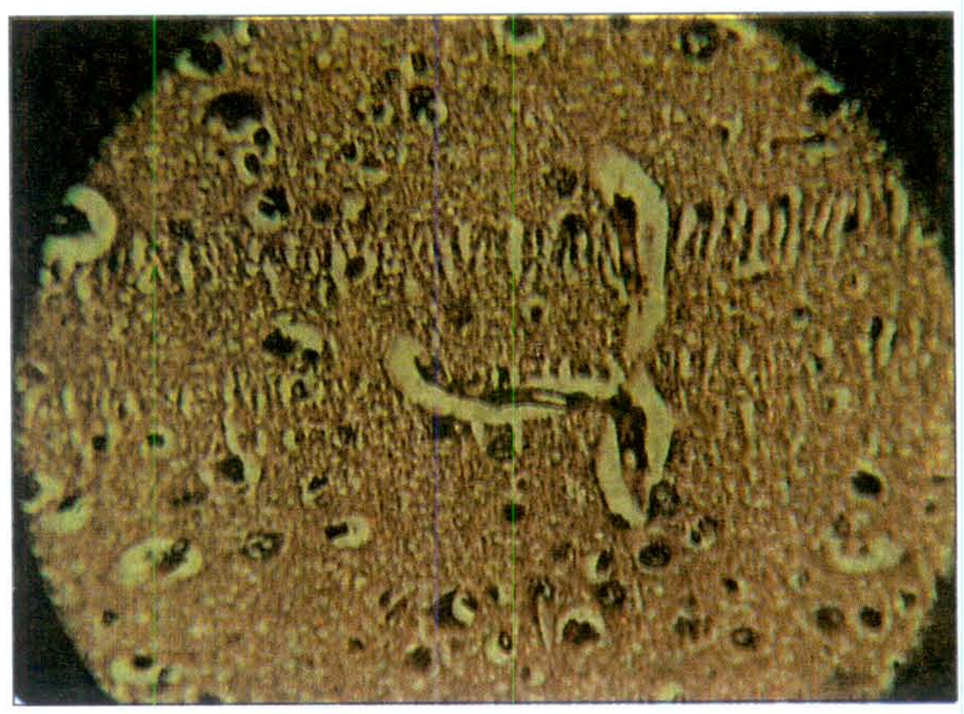

Fig. 6. Lesion in brain, showing viable septate hyphae (×40, PAS reaction).

Spleen :

In the spleen tissue of the animals in groups 1&2, lymphocyte population was largely decreased. Spleen sinusoids were full of red blood cells. The number of and size of lymphatic follicles were fewer than normal. Lymph follicles of the spleen did not show normal concentration, density. Spleen reticular tissue was quite clear, indicating a decrease in the lymphocytic population.

Discussion

Invasive aspergillosis most frequently occurs in the severly immunocompromised patients (14). Local or regional invasive disease is more commonly seen in individuals with metabolic aberrations, cyanotic heart disease, heart prosthesis, and of most importance, in individuals with immunocompetence secondary to drug therapy or irradiation, congenital immunodeficiency, or malignancy (20). Risk factors predisposing patients to invasive aspergillosis include corticosteroid therapy, cytotoxic chemotherapy, transplantation, granulomatous disease (12, 18, 22, 25).

To analyse further the mechanisms that may be involved in resistance to invasive aspergillosis, we decided to use corticosteroid and CY-A. The major effect of CY-A seems to be its ability to inhibit T cell functions by acting at three distinct but related stages in the process of T-cell activation:

1. Inhibiting T cell help of accessory cells for the synthesis of interleukin- 1 (IL-1);
2. Preventing IL-2 producing T cells from expressing receptors for IL-1 and suppressing the synthesis of IL-2;
3. Rendering T cells unresponsive to IL-2 (21-29).

Likewise, CY-A affects natural killer (NK) cells activity (30). In animal models, germination of aspergillus conidia plays an important role in the early pathogenesis of the disease which leads to lethal infection. The alveolar macrophage is the first phagocytic cell to encounter inhaled conidia and its primarry role in defense appears to be prevention of conidial germination (14). Cortisone significantly impairs the ability of macrophages to prevent germination of conidia in vitro and in vivo (31, 33).

Following intranasal inoculation of aspergillus conidia in cortisone treated mice, alveolar macrophages phagocytize conidia. However, unlike the normal macrophages, lysosomal granules of cortisone treated macrophages do not fuse with the phagosomes containing the conidia, and conidia begin to germinate within 2 to 4 h of inoculation (4, 14, 31).

In this study, we have shown that alveolar macrophages play an improtant role in resistance to experimental invasive aspergillosis. Because, among 10 rabbits (group 1), received dexamethasone and were injected the conidia intratracheally, 7 rabbits demonstrated clinical signs. Whereas , in group 2, in which rabbits received CY-A and were injected the conidia intratracheally none demonstrated clinical signs.

On the other hand, the route of infection is another critical factor which determines the relevance of an animal model. In our observation, we have revealed that when the conidia were injected intravenously, all rabbits in group 1&2 showed clinical symptoms. But in control group, the clinical signs were not observe.

Regarding all the above-mentioned points and with attention to the fact that CY-A suppres T cell function and NK cell activity, it seems that cell mediated immunity and NK cell activity could play an inhibitory role in Invasive aspergillosis.

Based on this observation, results of histopathological examination of different organs proves that aspergillus hyphae invade blood vessles and results in, producing thrombosis and tissue necrosis and this way, it will be disseminated to other organs.

In conclusion, this study showed, in natural condition that aspergillosis is initiated by inhalation of airborn condidia, cortison-treated patients are susceptible to invasive aspergillosis. This can not be the case for the individuals who received CY-A.

Acknowledgements

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مقایسه اثرات سرکوب ایمنی سیکلوسپورین A و دگزامتازون در آسپرجیلوزیس تجربی

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در این مطالعه، ۶۰ سر خرگوش ماده انتخاب گردیده و به سه گروه ۲۰ تایی تقسیم شدند. حیوانات گروه ۱ با دگزامتازون و گروه ۲ با سیکلوسپورین A سرکوب ایمنی شدند، اما خرگوشهای گروه ۳ (گروه شاهد) هیچ نوع دارویی دریافت نکردند. در هر گروه، نیمی از خرگوشها از طرق داخل نای و بقیه از راه تزریق وریدی با کنیدیهای آسپرجیلوس فومیگاتوس (۴×۱۰^{۵} کنیدی) عفونی شدند. در گروه شاهد، هیچیک از حیوانات علائم کلینیکی را نشان ندادند. تمام خرگوشهای گروه ۱ و ۲ که از راه وریدی عفونی شده بودند علائم آسپرجیلوزیس مهاجم را ظاهر نمودند، در حالیکه از این دو گروه حیواناتی که کنیدیها را از راه داخل نای دریافت کرده بودند فقط ۷ خرگوش در گروه ۱، علائم کلینیکی آسپرجیلوزیس را نشان دادند. در اتوپسی، مشاهدات ماکروسکوپی و میکروسکوپی نشاندهنده آسپرجیلوزیس مهاجم بود. در آزمایشات هیستوپاتولوژی، تهاجم آسپرجیلوس به کبد، ریه، مغز و طحال مشاهده گردید. نتایج این بررسی نشان می‌دهد که در شرایط طبیعی که آسپرجیلوزیس با استنشاق کنیدیهای قارچی موجود در هوا ایجاد می‌شود، بیمارانی که تحت درمان با کورتیزون می‌باشند، نسبت به افرادی که سیکلوسپورین A مصرف می‌نمایند حساسیت بیشتری در ابتلاء به آسپرجیلوزیس مهاجم خواهند داشت.

واژه‌های کلیدی : آسپرجیلوس فومیگاتوس، سیکلوسپورین A، دگزامتازون