EXPERIMENTAL ANAEROBIC BRAIN ABSCESS WITH INOCULATION OF BACTEROIDES FRAGILIS: THE EFFECTS OF METRONIDAZOLE AND DEXAMETHASONE

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SUMMARY :

Despite advances in diagnosis and treatment, a brain abscess caused by anaerobic micro-organisms still poses a major clinical problem. An experimental model of anaerobic brain abscess in rat was developed to determine the effects of metronidazole and dexamethasone on the abscess thus formed. Rats were inoculated with. B. fragilis and treated intraperitoneally each day with either metronidazole (15 mg/kg), dexamethasone (0.25 mg/kg) or both.

It was histopathologically shown that a stage of cerebritis preceded capsule formation. When groups were compared, it was observed that the therapeutic doses of metronidazole administered together with dexamethasone was the best treatment.

KEY WORDS:

Bacteroides fragilis, Brain abscess, Dexamethasone, Metronidazole.

INTRODUCTION

As a result of the recent advances in anaerobic culturing techniques, anaerobic micro-organisms have emerged as the most common cause of brain abscess (3-5, 8,11,12,13,15,22). The frequency of anaerobic brain abscess has prompted the need for an experimental model both for histopathologic research and for the investigation of the effects of various treatments.

An experimental brain abscess was first formed by Homen in 1913 with direct inoculation in rabbits and guinea pigs. In the years that followed, Essich, Greff and Falconer formed a brain abscess by direct inoculation (7). Molinari et al (17) used septic cerebral embolism and Wood et al (27,28) discussed the differences of experimental traumatic and metastatic brain abscess.

Clinical evidence supports that 89 % of bacteria isolated from the brain abscess anaerobic and Bacteroides fragilis is the most frequent among them (7,10,12).

During the clinical course of a brain abscess, severe vasogenic brain edema develops, which in turn causes elevation of ICP and the deterioration of the patient (2,18). Although it has adverse effects on antibiotic penetration, immune responses of the host and collagen capsule deposition, dexamethasone is the most frequently used agent for the treatment of edema and mass effect associated with the brain abscess (2,20,21,23,24,29).

The present study was designed to develop an aerobic brain abscess model in rats, to investigate the various histologic stages of the abscess thus formed and to evaluate the effects of metronidazole and dexamethasone.

MATERIALS AND METHODS

Inoculum preparation: Bacteroides fragilis (NCTC 9343) was inoculated into a brain/heart infusion medium containing thioglucolate and L-sistein and was incubated in anaerobic media at 37°C for 48 hours. The cultures were centrifuged and the precipitation was suspended in 1/3 NaCl solution and collected under carbon dioxide flow into vials. Final concentrations of viable organisms were essayed by serial dilutions of 10^{-1}, 10^{-2}, ... 10^{-8} and was found to be 1.2×10^{8} cologny forming units (CFU)/ml.

Abscess production:

A total of 77 male albino rats each weighing 220-225 g were anaesthetised with intraperitoneal pentobarbital 40 mg/kg. The animals heads were shaved and the scalps prepared with antiseptic solu-

tion and a 1-1.5 cm median incision was made. Following the model described by Winn et al (28), a 2 mm burr hole was placed 4 mm to the right of midline just posterior to the coronal suture. The dura was punctured and a sterile No. 27 needle was introduced 2 mm into the brain. 0.05 cc of B. fragilis was slowly, inoculated and after being held in place for 30-40 seconds, the needle was withdrawn. The scalp wound was closed single layer with surgical silk.

Experimental Groups:

The animals were randomly divided into 7 groups, each containing 11 animals, according to planned treatment and dates of sacrifice.

Eleven animals were selected as controls (Group 1) and were intracerebrally injected with 0.05 cc of 1/3 NaCl solution. Groups 2, 3 an 4, after inoculation with Bacteroides fragilis, were sacrificed consecutively after 2, 7 and 15 days. The remaining three micro-organism inoculated groups received treatments and were sacrificed after 15 days. Group 5 was given metronidazole (15 mg/kg) intraperitoneally each day from Day 4 until sacrifice. Group 6 was given dexamethasone (0.25 mg/kg) and Group 7 received a treatment of both metronidazole and dexamethasone (same dosage) during the same period. The animals were sacrificed by an overdose of pentobarbital.

Macroscopic and Microscopic Examination of Abscesses:

The animals were sacrificed on appropriate days and the brains were carefully removed. The brains were examined macroscopically for hemispheric edema and venous congestion with a surgical microscope (Zeiss Op Mi I). They were then fixed in 10 % formalin for seven days and embedded in paraffin. Sections through the abscesses were made with microtome and slides obtained were stained with hematoxylin and eosin, Masson's trichrome and silver impregnation for reticulin.

The sections were examined to determine the pathologic stages of the anaerobic abscess. Necrotic zones, the inflammatory response, reticulin and collagen formation and neovascularisation were compared in treated and untreated groups.

RESULTS

Control Groups: Gross examinataion revealed slight edema and hyperemia at Day 2, which subsided completely by Day 7. Microscopic examination showed gliosis surrounding the needle tract.

Group 2: (sacrificed 2 days after inoculation) - Hemispheric edema and venous congestion was observed. Sections showed marked edema and shifting. The abscess had a central necrotic zone containing becteria and debris, surrounded by an inflammatory infiltrate. The infiltrate consisted of polymorphonuclear leukocytes, mononuclear cells, and plasma cells. The lesion had no collagen capsule and the surrounding brain had marked edema (Fig 1).

Fig 1 : Early cerebritis on Day 2 (Hematoxylin-eosin × 40)

Group 3: (7 days after inoculation) - Hemispheric edema and congestion persisted. In sections, fibroblasts started to show up in the zone of inflammatory cells. Reticulin synthesis by fibroblasts had begun and this process demarcated the abscess from the surrounding brain. (Fig 2).

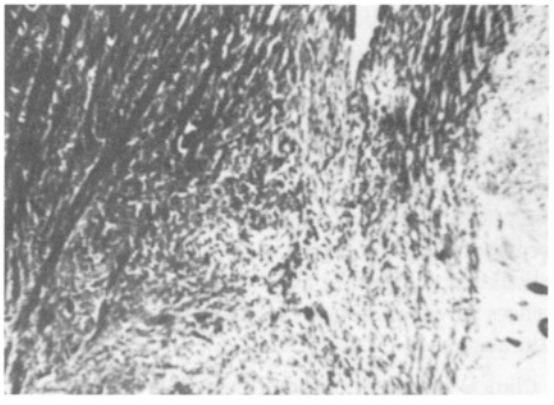

Fig 2 : Formation of collagen capsule on Day 7 (Masson's Trichrome × 200)

Group 4: (Day 15) - The collagen capsule formation was complete and dense (Fig 3). A layer of neovascularisation formed outside the capsule. The abscess was fully developed with all the zones.

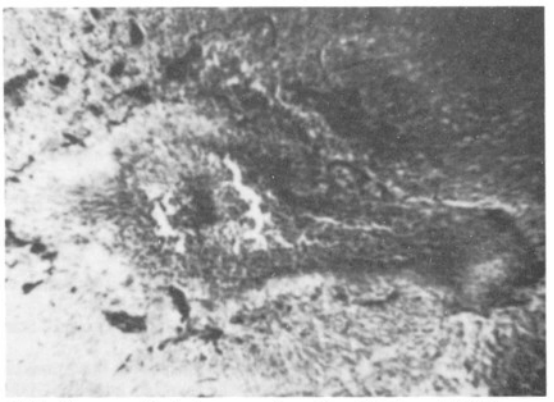

Fig 3 : Abscess with distinct capsule on Day 15 (Masson's Trichrome × 100)

Group 5: (those receiving metronidazole) - There was slight edema and hyperemia. A small central necrotic zone was surrounded by scarce inflammatory cells and collagen capsule. No bacteria were seen (Fig 4).

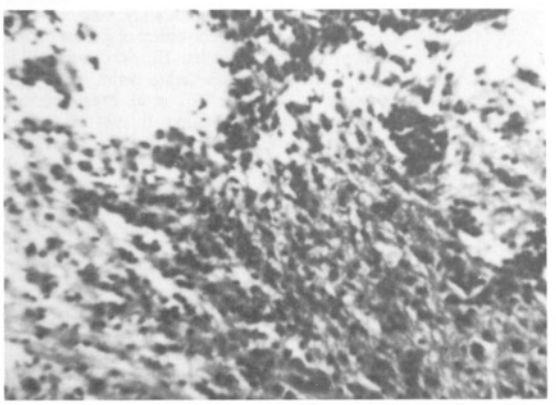

Fig 4 : Abscess treated with metronidazole (Hematoxylin-eosin × 200)

Group 6: (Dexamethasone receiving group) - Cerebral edema was markedly reduced. No capsule was seen. Neovascularisation was slight and peripheral brain looked normal. Inflammatory cells were few and the central necrotic zone harbored bacteria (Fig 5).

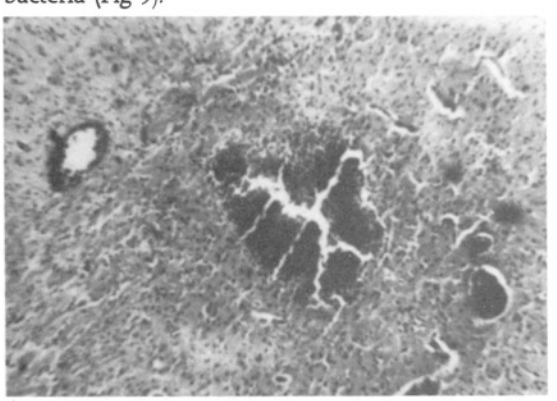

Fig 5 : Dexamethasone receiving abscess showing reduced inflammation and gliozis (Hematoxylin-eosin × 200)

Group 7: (the group receiving metronidazole and dexamethasone) - There was no brain edema. Examination of the slides revealed bacteria in the central zone. There was slight inflammatory response, capsule formation and neovascularisation. Perivascular infiltration was rare and the surrounding brain was normal (Fig 6).

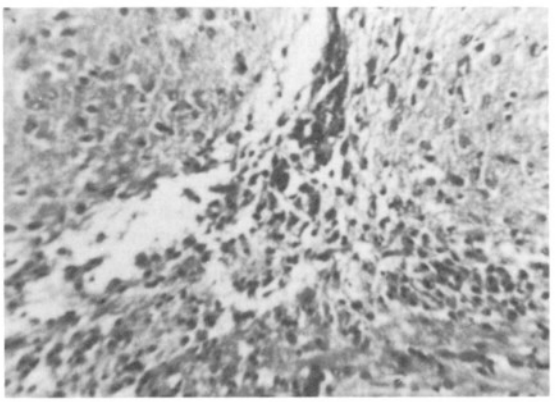

Fig 6 : Metronidazole and dexamethasone given together reveal slight inflammation, relatively normal looking peripheral brain (Nematoxylin-eosin × 100)

DISCUSSION

The need for an experimental model in brain abscess is an undisputed opinion of various authors (1,7,17,23,26,28,29). A good experimental model should be practical and cheap. Compare to our method, Falconer et al (7) have used a two-staged more time-consuming one. Considering the documented ischemic complications of septic embolisation, of foreign body reactions associated with injection of agarose, the method of direct inoculation gave better results (17,19,23,27).

In this experimental study, we found cerebritis on Days 2 and 7, and an abscess with distinct collagen capsule was observed on Day 15. The mature abscess had five zones which were the innermost necrotic center zones surrounded by a zone of inflammatory cells, macrophages and fibroblasts. Enveloping these zones were a dense collagen capsule and a layer of neovascularity. The outermost zone consisted of reactive gliosis and brain edema. These findigs comply with the recent works of Britt and Britt et al (1,2) and Yıldızhan et al (29).

Steroids caused dramatic reduction in brain edema by both restoring normal permeability of vascular endothelial cells and stabilization of neuronal membrane (14,16,18). This results in neurologic improvement of the patient. However, evidence from recent studies shows that steroids have potential

adverse effects in infectious processes. They cause a reduction in the numbers of polymorphonuclear leukocytes, macrophages, mononuclear leukocytes and fibroblasts and delay the clearance of virulent bacteria. Reductions of the capillary permeability by steroids impedes the antibiotic penetration through the blood-brain barrier. Inhibition of neovascularisation reduces the number of migrating fibroblasts, thus hampering production of reticulin an collagen. This delays encapsulation and containment of the infection (2,19,23,24,25). Nevertheless, the excellent results obtained from the treatment of cerebral edema justifies the use of steroids in treating cerebral abscess. We similarly found that rats receiving dexamethasone had less inflammatory response and no collagen capsule. A reduction in neovascularisation and reduction in surrounding brain edema and a delay in the clearance of bacteria was also observed. This may account for the adverse role of steroids in the development of opportunistic infections.

Metronidazole is effective against B. fragilis and diffuses well into the cerebro-spinal fluid and brain tissue (4,6,9,12). The group receiving metronidazole showed a more subsided histopathologic picture and no bacteria was seen. This supports the fact that appropriate antibiotics given early in the course of infection gives better results.

In the group receiving both metronidazole and dexamethasone, the histologic examination showed a very small abscess with bacteria in the necrotic center. There was minimal inflammatory response and less capsule formation. The surrounding brain tissue had very slight edema and looked comparatively normal. The persistence of micro-organisms in this group supports the ongoing argument that steroids have detrimental effects on antibiotic penetration through the blood-brain barrier (14,19).

This study suggests that metronidazole is very effective against cerebral abscess caused by B. fragilis. Dexamethasone has adverse effects on collagen capsule formation, inflammatory responses of the host and delays clearance of bacteria. Nevertheless, dexamethasone, with excellent antiedema properties, should be used in combination with suitable antibiotics, when there are clinical signs and symptoms of elevated intracranial pressure and should be tapered off as the condition of the patient improves.

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