The Effect and Mechanism of Hyaluronidase Action in Experimental Brain Abscess

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Abstract: Brain abscess formation was studied experimentally in rats to determine the effect and the mechanism of hyaluronidase action in the treatment of brain abscess by applying different combinations of hyaluronidase, Mycobacterium bovis (BCG), ascorbic acid and antibiotic sensitive to the inoculated bacteria in various stages of classical abscess development.

The results showed that combined therapy with antibiotic and hyaluronidase started the day before inoculation a bacteria averted the formation of brain abscess and the same therapy started after encapsulation, effectively eliminated the organism and resolved the infection leaving a glial scar. But this therapy, only started at the cerebritis stages, caused an increase of cerebritis. We suggest that it is possible that hyaluronidase may act in a manner similar to its local action, and additionally may activate the immune system. Therefore, the concept of management with hyaluronidase and appropriate antibiotics might be a promising and effective chemotherapeutic method in encapsulated brain abscess for selected high-risk patients.

Key words: Brain abscess, Antibiotics, Hyaluronidase, Mycobacterium bovis, Ascorbic acid.

INTRODUCTION

The introduction of computerized tomography (CT) has seen a significant advance in localizing suspected intracranial infection. Accordingly, it has modified the management of patients with brain abscess (6,7,8,16,21, 22,26,30,31,33).

Although brain abscess is usually treated surgically; there are circumstances in which surgical intervention may not be feasible (5,32) and alternative treatment seems to be the only hope for such patients. However, antibiotics alone have in general been ineffective in improving the morbidity and mortality rates associated with frank brain abscess (17) and results of experimental studies and clinical reports of nonsurgical management using antibiotics alone are not conclusive (2,3,14,19,23,40).

In previous studies we found that antibiotics alone had no effect on prevention or resorption of brain abscess (29,36,39). However, in a recent study (30) we have shown that application of a combined therapy with antibiotic and hyaluronidase after encapsulation, effectively eliminated the organisms in animals and resolved the infection.

The purpose of this study is to reevaluate the role of hyaluronidase in the treatment of experimental brain abscess and to consider how hyaluronidase can affect the abscess.

MATERIALS AND METHODS

Swiss Albino rats, each weighing approximately 250 g were used. Methods to produce cerebral abscesses in rats and analyze the neuropathological and microbiological results have been described in our previous study (39).

In the present study the inoculum was prepared with Staphylococcus aureus sensitive to seftizoksım sodium.

A total of 194 rats was divided into four groups:

Group A (antibiotic-hyaluronidase, 60 rats): These animals received 200 mg/kg/day of intramuscular seftizoksım sodium administered every 12 hours, and
5 units/kg/day of hyaluronidase injected intramuscularly at 12-hourly intervals.

Group B (antibiotic-BCG, 60 rats): Antibiotic treatment was the same as for group A. BCG (100,000 live germ/0.1 ml) was applied once intradermally on the day of the first administration of antibiotic.

Group C (antibiotic-ascorbic acid, 60 rats): These animals received antibiotic treatment following the same protocol, and 0.1 ml of ascorbic acid every 12 hours injected intramuscularly to the gluteal region commenced with the antibiotic administration.

Group D (Control, 14 rats): These received no drugs and served as controls. Two animals were sacrificed 2, 6, 12, 18, 21, 30 and 45 days following bacteria inoculation.

Each group, except the control group, was subdivided into 5 groups and 4 animals/day were sacrificed at the determined days. The days of treatment and sacrifice are shown in Table I.

**RESULTS:**

The findings were comparable between the animals of each group. Although negligible histopathological differences could be demonstrated, the microbiological findings were identical.

The main features of the histopathological and microbiological findings are summarized in tables I and II.

### Table I. Stages of development of brain abscess and summary of histopathological findings.

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<th>Starting day of the drug</th>
<th>Day of sacrifice after inoculation</th>
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**Table II. Summary of microbiological findings**

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<td>16 th day after inoculation</td>
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S: Smear; C: Culture; (+): Presence of bacteria with Gram's stain or multiplication in culture; (-): Absence of bacteria with Gram's stain or no multiplication in culture.
DISCUSSION

Brain abscess evolution both experimentally and clinically may be divided into four stages based on neuropathological and computed tomographic findings: 1) early cerebritis (1–3 days), 2) late cerebritis (4–9 days), 3) early capsule formation (10–13 days), and 4) late capsule formation (14 days or more) (4.5.9.14). These stages vary according to the immunological state of the host, the virulence of the organism, species differences, the origin of infection, and the antibiotic and/or corticosteroid therapy used. (3.4.5.7.35.40).

In spite of significant progress in imaging, newer antibiotics with greater penetration into the central nervous system, and meticulous bacteriological techniques, surgery remains the treatment of choice for brain abscess (7.18.25.27.30). There is accumulating data on patients with brain abscess successfully treated nonoperatively (1.6.8.21.22.30.31.32). However, studies demonstrate progressive enlargement of brain abscesses despite appropriate antibiotic coverage (2.12).

The therapeutic benefit of an antibiotic depends on the bactericidal concentration of the drug for a given organism as well as the concentration of the drug in the infected area. CSF concentrations of antibiotics do not always demonstrate the concentration within the abscess cavity (2.10.11.13.24). Therefore, antibiotics alone cannot be expected to eliminate bacteria at certain stages and in certain zones of the infected area (2.12.22.29.32.37.40).

The penetration of antibiotic into the infected area is of greatest concern in the capsule stages of abscess development since the formation of the collagen capsule limits the spread of infection and lessens the inflammatory response which facilitates the penetration of antibiotic into the CSF and across the blood-brain barrier (14.15.20.38.40). In a study (28) based on the hypothesis that encapsulation itself may form a barrier preventing adequate diffusion of antibiotics into the abscess centre and influence the efficacy of antibiotics in eradicating infection, hyaluronidase was used to facilitate antibiotic penetration into the cavity and the results showed that combined therapy with antibiotic and hyaluronidase before encapsulation caused an increase in the spread of cerebritis but the same combination led the abscess to resorption when started at capsule stages and prevented its development when given the day before inoculation of bacteria. The present study confirms histopathologically as well as microbiologically the former study.

The question of what is the mechanism of hyaluronidase action, which is known as a locally acting drug (34) on brain abscess is now considered. Ascorbic acid was given to simulate the local effect of hyaluronidase assuming that hyaluronidase may nonspecifically activate the immune system by causing nonspecific muscle damage at the site of injection. BCG was administered considering the activation of the immune system. Rats treated with hyaluronidase (group A) and BCG (group B) showed an increase in the spread of cerebritis when treatment was started at the cerebritis stages. In group A, white cerebritis and abscess formation did not develop when the treatment was begun the day before inoculation. In group B animals cerebritis was the main feature of this period. In group C except for widespread cerebritis during the cerebritis stage there was no apparent histological difference compared with group B. The microbiological results are also striking. Hyaluronidase - antibiotic combination stopped bacteria multiplication and eliminated the organisms completely in all stages but the very early days. The same microbiological findings were true for group B and C only at the late periods of the stages.

From our results it seems difficult to find a definite answer for the action of hyaluronidase on brain abscess. It cannot be explained solely with the suggestion of specific or nonspecific activation of the immune system or by its systemic effect since there is no mention in the literature of this action.

We can only hypothesize that hyaluronidase acts systemically in a manner similar to its local action, and probably activates the host immune system. Accordingly, hyaluronidase secures a temporary decrease in viscosity by hydrolyzing the mucopolysaccharides which are components of the substance filling the intercellular space of the capsule and provide sufficient antibiotic diffusion into the necrotic centre.

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