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 organismsin 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 provious study (39).

In the present study the inoculum was prepared with Staphylococcus aureus sensitive to seftizoksim 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 seftizoksim 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 mlt) was applied once intradermally on the day of the first administration of antibiotic.

Group C (antibiotic-ascorbic acid, 60 rats): Theseanimals received antibiotic treatment following the same protocol, and 0.1 mlt 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 controlgroup, was subdivided into 5 groups and 4animals/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.

Starting day of the drug	Day of sacrifice after inoculation	Groups							
		A		B		C		D	
		DS	E	DS	E	DS	E	DS	E
The day	2	ND	0	Cr	0	Cr	2	Cr	2
before	6	ND	1	Co+Cr	1	Cr	0	Cr	5
inoculation	12	ND	0	Cr	1	Cr	0	Cr+D	3
	18	ND	0	Cr	0	Cr+D	1	Cp	1
2 nd day	6	Cr	0	Cr	0	Cr	1		
after	12	Cr	0	Cr	2	A	2		
inoculation	18	Cr(R)	0	Cr	0	R	0		
6 th day	12	R	1	Cr	1	Cr	1		
after	18	R	0	A	0	A	0		
inoculation	21	R	0	R	0	R	0	Cp	0
12 th day	18	R	1	Co+Cr	1	Cr	1		
after	21	R	0	A	1	Co+Cr	0		
inoculation	30	R	0	R	0	A	0	Cp	0
16 th day	21	R	1	Cr	1	Cr	1		
after	30	R	1	A	1	A	1		
inoculation	45	R	0	R	0	R	0	Cp	0

DS: Development stage of abscess; Co: Congestion; Cr: Cerebritis; D: Demarcation line; Cp: Capsule; R: Resorption; ND: Abscess not developed; A: Abscess; E: Intensity of oedema; 5: very severe; 4: severe ; 3: moderate ; 2: mild; 1: minimal; 0: no oedema.

Table II. Summary of microbiological findings

Starting day of the drug	Day of sacrifice after inoculation	Groups							
		A		B		C		D	
		S	C	S	C	S	C	S	C
The day	2	+	+	+	+	+	+	+	+
before	6	–	–	+	+	+	+	+	+
inoculation	12	–	–	+	–	–	–	+	+
	18	–	–	–	–	–	–	+	+
2 nd day	6	–	–	+	+	+	+		
after	12	–	–	+	+	+	–		
inoculation	18	–	–	–	–	–	–		
6 th day	12	–	–	+	+	+	+		
after	18	–	–	+	+	+	+		
inoculation	21	–	–	–	–	–	–	+	+
12 th day	18	–	–	+	+	+	+		
after	21	–	–	+	–	+	+		
inoculation	30	–	–	–	–	–	–	+	+
16 th day	21	–	–	+	–	+	+		
after	30	–	–	–	–	+	+		
inoculation	45	–	–	–	–	–	–+	+	

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 facililate 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 startedat 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 can not 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 hypothesis 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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REFERENCES

1. Barsoum AH, Lewis HC, Cannillo KL. Nonoperative treatment of multiple brain abscesses. Surg Neurol 16:283-287,1981
2. Berg B, Franklin G, Cuneo R, Boldrey E, Strimling B. Nonsurgical cure of brain abscess: early diagnosis and follow-up with computerized tomography. Ann Neurol 3:474-478,1978
3. Black P, Graybill JR, Charache P. Penetration of brain abscess by systemi cally administered antibiotics. J Neurosurg 38:705-709,1973
4. BohlI, Wallenfang T, Bothe H, SchürmannK. The effect of glucocorticoids in the combined treatment of experimental brain abscess in cats. Adv Neurosurg 9:125-133, 1981
5. Britt RH, Enzmann DR, Yeager AS. Neuropathological and computerized tomographic findings in experimental brain abscess. J Neurosurg 55:590 603, 1981
6. Britt RH, Enzmann DR. Clinical stages of human brain abscesses on CT scans after contras infusion. Computerized tomographic, neuropathologi-cal, and clinical correlations. J Neurosurg 59:972-989,1983
7. Britt RH, Enzmann DR, Placone RC Jr, Obana WG, Yeager AS. Experimental anaerobic brain abscess. Computerized tomographic and neuropathological correlations. J Neurosurg 60:1148-1159, 1984
8. Britt RH. Brain abscess. In: Wilkins RH, Rengachary SS (eds). Neurosur- gery. McGraw-Hill.Baltimore.1985. pp1928-1956.
9. Burke LP, Ho SU, Kim KS, et al. Multiple brain abscesses. Surg Neurol 16:452 -454,1981
10. deLouvois J, Gortvai P, Hurley R. Antibiotic treatment of abscess of the central nervous system. Br Med J 2:985-987,1977
11. deLouvois J, Gortvai P, Hurley R. Bacteriology of abscesses of the central nervous system. A multicentre prospective study. Br Med J 2:981-984, 1977
12. deLouvois J. The bacteriolology and chemotherapy of brain abscess. J An-timicrob Chemother 4:395-413,1978
13. Enzmann DR, Britt RH, Yeager AS. Experimental brain abscess evolution: Computerized tomographic and neuropathologic correlaons. Radiology 133:113-122,1979
14. Enzmann DR, Britt RH, Placone RC Jr, Obana W, Lyons B, Yeager AS. The effect of short- term cor-ticosteroid treatment on the CT appearance of Experimental brain abs-cesses. Radiology 145:79-84,1982
15. Everett ED, Strausbaugh LJ. Antimicrobial agents and the central ner- vous system. Neurosurgery 6:691-714,1980
16. Gavey G. Current concepts of bacterial infections of the central nervous system. Bacterial meningitis and bacterial brain abscess. J Neurosurg 59:735-744,1983
17. George B, Roux F, Pillon M, Thurel C, George C. Relevance of antibiotics in treatment of brain abscesses. Report of case with eight simultaneous brain abs-cesses treated and cured medically. Acta Neurochir (Wien) 47:285-291, 1979
18. Haines SJ, Mampalam TJ, Rosenblum ML, Nagib MG. Cranial and intracranial bacterial infections. In: Youmans J (eds). Neurological Surgery. WB Sa-unders. Philadelphia.1990. pp3707-3735
19. Haley EC Jr, Costello GT, Rodeheaver GT, Winn HR, Scheld WM. Treatment of experimental brain abscess with penicillin and chloramphenicol. Ann Neurol10:99 (abstr.),1981
20. Kamin M, Biddle D. Conservative management of focal intracerebral infection. Neurology 31:103-106,1981
21. Kitamura T. Dynamic aspects of glial reactions in altered brains. Pathol Res Pract 168:301-343,1980
22. Kobrine AI, Davis DO, Rizzoli HV. Multiple abscesses of the brain. Case report. Neurosurg 54:93-97,1981
23. Levy RM, Gutin PH, Baskın DS, Pons VG. Vancomycin penetration of a brain abscess: Case report and review of the literature. Neurosurgery 18:632-635, 1986
24. Long WD, Meacham WF. Experimental method for producing brain abscess in dogs with evaluation of the effect of dexamethasone and antibiotic therapy on the pathogenesis of intracerebral abscesses. Surg Forum 19:437-438,1968
25. Mampalam TJ, Rosenblum ML. Trends in the management of bacterial bra-in abscesses: A review of 102 cases over 17 years. Neurosurgery 23:451. 1988
26. Moussa AH, Dawson BH. Computed tomography and the mortality rate in brain abscess. Surg Neurol 10:301-304,1978
27. Obana WG, Rosenblum ML. Surgery for intracranial infections.In Post KD, Friedman E, McCormick P(eds). Post-operative Complications of Neurolo-gical Surgery. New York, Thieme, in press
28. Paşaoğlu A, Şenkal Y, Kandemir B. The effect dexamethasone on experi-mental brain abscess. Hacettepe Med J 17:161-170,1984
29. Paşaoğlu A, Yıldızhan A, Kandemir B. Treatment of experimental brain abscess. Effect of combinations of hyaluronidase with antibiotics and dexamethasone. Acta Neurochir (Wien) 100: 79-83,1989
30. Quartey GRC, Johnston JA, Rozdilsky B. Decadron in the treatment of ce-rebral abscess. An experimental study. J Neurosurg 45: 301-310,1976
31. Rosenblum ML, Hoff JT, Norman D, Weins tein PR, Pitts L. Decreased mortality from brain abscess since the advent of Computerized tomography. J Neurosurg 49 :658- 668,1978
32. Rosenblum ML, Hoff JT, Norman D, Edwards MS, Berg BO. Nonoperative treatment of brain abscesses in selected high-risk patients. J Neurosurg 52:217-225, 1980
33. Rotheram EB Jr, Kessler LA. Use of computerized tomography in nonsur-gical management of brain abscess. Arch Neurol 36:25-26,1979
34. Schroeder KA, McKeever PE, Schaberg DR, Hoff JT. Effect of dexamethasone on experimental brain abscess. J Neurosurg 66:264-269,1987 35.Spirer Z, Jurgenson V, Lazewnick R, et al. Complete recovery from an ap-parent brain abscess treated without neurosurgery. The importance of early CT scanning. Clin Pediatr 21: 106-109,1982
36. Swinyard EA. Locally acting drugs. Surface acting drugs. In: Goodman LS, Gilman A(eds). The pharmacological basis of therapeutics. McMillan Pub-lishing Co. New York.1975. pp 946-959
37. Şenkal Y, Kandemir B, Paşaoğlu A. The effect of acetazolamide on brain edema induced by experimental brain abscess. TJ Research Med Sci 4:289-292,1986
38. Wallenfang T, Bohl J, Kretzschmar K. Evolution of brain abscess in cats. Formation of capsule and resolution of brain edema. Neurosurg Rew 3:101-111,1980
39. Whelan MA, Hilal SK. Computed tomography as a guide in the diagnosis and follow-up of brain abscesses. Radiology 135:663-671,1980
40. Yıldızhan A, Paşaoğlu A, Kandemir B. Effect of dexamethasone on various stages of experimental brain abscess. Acta Neurochir (Wien) 96: 141-148, 1989