Tikrit Journal of Pharmaceutical Sciences 2005,1(2):23-27

Induction of brain lesions experimentally by inoculation of Staphylococcus aureus subcutaneously in rabbits (A histological study)

***Ayad,H.I. ,and **Zainab,N.Al-Saadi**
*Dept. of Anatomy, Tikrit, College of medicine
** Dep. Of Biology, Tikrit College of Education for women

Received 12/4/2005:Accepted 3/9/2005

Abstract

Six adult rabbits of both sexes were used tostudy the effect of *Staphylococcus aureus* on the brain tissue. Animals were classified into two groups. Group(A) was the control and Group(B) was the treated. Group (A) was inoculated with 0.8ml saline solution subcutaneously, and group (B) was inoculated subcutaneously with 0.8ml suspension of *Staph.aureus* .The inculation process was repeated again with group (B) after 15 day. After (15) days from the second inoculation, the all animals of both groups were sacrified. A brain specimens from the frontel, parietal and temporal lobes of both groups were taken, processed for histological technique, embedded in the paraffin wax, sectioned at (5) µm thickness and stained by the haematoxylin & eosin for examination by the light microscopy.

The brain tissues of group B, demonstrated the histopathological changes of the whole sites of the brain lobes, included, focal necrosis of the nerve cells, demyelination of the nerve fibers and gliosis. This effect may reflect the efficacy of the bacteria or its toxin or many both on the brain tissues.

أحداث أفات في الدماغ تجريبيا بواسطة حقن المكورات العنقودية الذهبية تحت الجلد في الأرانب (دراسة نسيجية)

أياد حميد إبراهيم **زينب نشأت السعدي**

المستخلص

أستخدمت ستة ارانب بالغة من كلا الجنسين لدراسة تأثير المكورات العنقودية الذهبية على نسيج الدماغ. قسمت الحيوانات إلى مجموعتين , مجموعة (أ) مجموعة السيطرة ومجموعة (ب) مجموعة المعالجة . حقنت مجموعة السيطرة بـ 0,8مل من المحلول الملحي الفسلجي تحت الجلد وحقنت مجموعة (ب) بمعلق المكورات العنقودية الذهبية بـ 0,8مل تحت الجلد أيضا , وأعيد الحقن بعد (15) يوم تحت الجلد أيضاً لمجموعة (ب).

بعد فترة (15) يوم من الحقن الثاني , تم قتل كل الحيوانات وأخذت مقاطع من فصوص الدماغ الجدارية,الجبهية والصدغية لكلا المجموعتين , عوملت هذه القطع بطريقة التقنية النسيجية وطمرت بالشمع وقطعت بسماكة (5) مايكروميتر , وتم صبغها بالهيماتوكسلين والأيوسين لغرض الفحص تحت المجهر الضوئي.

بين الفحص النسيجي لفصوص الدماغ في مجموعة (ب) حصول تغييرات نسيجية مرضية فيها تمثل في تنخر بؤري للخلايا العصبية , فقدان النخاعين للالياف العصبية مع اضمحلال الخلايا العصبية والساندة ,هذا التأثير قد يعكس فعالية البكتريا أو ذيفانها أو كلاهما على انسجة الدماغ .

Introduction

Staphylococcal meningitis is an inflammation or infection of the membrane covering the brain and spinal cord caused by the *Staphylococcus* bacteria (1). Meningitis caused by the *Staph.aureus* or *Staph.epidermis* usually develops as acomplication of a surgical procedure or as an infection spread by the blood from another site (2).Borrie(3) revaled that skin infections can arise from the immigration of certain organsms from the external environment through skin breaks or from organism that reach the skin through the blood as part of asustemic disease. Some infections such as brain abscess or skin lesions are caused by toxins produced by the bacteria (4) . The present study investigated the efficacy of *Staph.aureus* on the brain tissue using an experimental model for induction of brain lesion in the rabbits.

Literature Review

Ryan (5) revealed that the patient may develop a brain abscess , fluminant meningitis or infection at the other body sites, excessive bleeding and shock can develop from the bacterial invasion .

Bacteria such as *Staph.aureus* may be introduced into the cerebral substance through the pentrating injury . direct spread from sinuses or the middle ear or through embolism to brain tissue (6). Initial infection leads to local suppuration followed by loculation of pus within the surrounding wall of gliosis which is chronic abscess may from a tough capsule,multiple abscen occur particulary with metastatic spread (7) .Betty (8)demonstrated that bacterial meningitis is usually secondary to bacteramic illness although the infection may result from direct spread from an adjacent focus.

Materials and Methods

Bacterial suspension was prepared for inoculation the experimental rabbits by obtaining the colonies of *Staph. aureus* from the blood agar and placed in the nutrient broth. 0.8ml of the bacterial suspension has been aspirated and inoculated subcutaneously in the experimental rabbit (9). Six adult rabbits of both sexes,were prepared ,weighting 500-750gm, food and water were available .The rabbits were classified into two groups as indicated in the table.

Group A	2 animals	Control group
Group B	4 animals	Treated group

Group A: inoculated with 0.8ml of physiological saline.Group B: inoculated with 0.8ml of dilevent bacterial suspension.

The animals of the group B were re inoculated with the bacterial suspension after 15 days . All animals were survived for (30)day post 1st inoculation. All animals were deeply ,inhalated with ether and decapatated immediately . Brain specimens from the frontal , parietal and temporal lobes of both groups were taken after breaking down the skull gently. The specimens were rinsed with running water , then placed in 10% formalin for 48 hours and processed through graded ethyl alcohol series 70,90 and absolute , xylene .Then infillterated in paraffine wax, blocked and prepared for sectioning .Sections of (5µm) thickness were prepared , stained by H&E (10), and examined by light microscope .

Results

The animals of group (B) showed decrease in appetite . The site of inoculation become tender and swollen.

-The clinical observations showed violent spasm , lasting for a few minutes and convulsive movement of the feet , these observations occurred (7 days) after second inoculation with *Staph. aureus* and lasting until the time of sacrifing

-Histological examination of the group A.

The cerebral cortex is thrown into gyri and sulci like elsewhere in the human and other animals. The gray matter was a mixture of nerve cells and nerve fibers,neuroglia and blood vessels.

The following types of the nerve cells are present in the cerebral cortex.(1) pyramided cells (2)stellate cells(3) fusioform cells(4)horizantal cells of cajal

(5) cells of material . (Fig 1,2).
Group B (treated Group)
wide spread of demyelination with swelling of axons in the white matter (Fig 3), focal necrosis of the nerve cells, atrophy of the nerve and glial cells associated ,with few leucocytic infiltration ,vaculations or pocket like appearance of most sites of the nervous system.

Discussion

Infection of the skin by the *Staph. aureus* was well established (3).It forms abscess and boils of the skin, endocaditis of the heart and meningitis of the brain membranes(1). A rabbit model for induction of the brain lesion or meningitis was applied to confirm the concept of the effect of the *Staph. aureus* on the brain tissue by inoculation of the bacteria subcutaneously.

The structural changes demonstrated in the brain tissue provided a confirmation for the relationship between the skin infection and brain lesion. The hypothesis for the effect of the bacillus or its toxin or both is occur in the form of toxic shock syndrome (11). In the present study , the neuronal damage of the brain tissue was not associated with any inflammation of the meninges , which may reflect the transmission of the bacteria or its toxin or both via blood circulation to the brain , there is another route for infection of meninges and brain tissue via cerebrospinal fluid (6). Blood brain barrier is well protection for the brain tissue (12). We inoculation. Two doses of the bacterial suspension (15) days interval in order to assure the breaking down of this barrier for arriving of the pathogen to the brain tissue. The presence of the extensive vaculation in the brain may consider as a result for degenerative condition of the neurons and glial cells (gliosis), followed by pus or oedematous formation init, and this concept agree with (13).

References

1-Cristopher,R.W., Edward,R.W. and Bouchier,I.A. :Atextbook of principles and practice of medicine. Churchill and livingstone 16th ed.UK.1992.

2-Stuart,A.E.,Smith,A.N. AND Samuel,E.:Applied surgical pathology. Chapter 18. Blackwell Scintific.Oxford and London.1975.

3-Borrie,P.Common skin diseases .London.Lewis.Co.ltd.14th ed.1975.

4-Elek,S.D. and Conan,P.E.:The virulence of *Staphylococcus pyogenes* for man. Astudy of the problem of wound infections. Br.J.Exp. pathol .1957.38.573-86.

5 - Ryan , K .J ., FallLow , C .D. and Rayes,N.P .:Medical microbiology .An introduction to infections diseases 1994.3rd ed.260.

6-Dankuc,D.,Milosevic,D. and Salvic ,L. :Simultaneous extra cranial intra cranial otogenic complication.med.pregl.2000;53(7-8) : 409-12.

7-Faris,N.A. and Hickey,W.F.: Development and characterization of an experimental model of brain abscess in the rat.Am.J.Pathol.1992;141(6).1229-307.

8 – Betty , A .F ., Sahm , D . F . and Weissfeld, A.S. :Diagnostic microbiology .1998;Vol.1.

9-الجبوري،محيميد مد الله. علم البكتريا الطبية دار الكتب للطباعة والنشر/ جامعة الموصل.1990

10-Bancroft.J.D. and Stevens :A theory and practice of the histological techniques . 2nd ed. Churchill livingstone.1982

11-Bohach,G.A.,Fast,D.J.,Nelson,R.D. and Schlivert,P.M: Staphylococcal and Streptococcal pyrogenic exotoxins involved in toxic shock syndrome and related to the illness . CRC. Crit. Review .microbiol.1990;(17).251-72.

12 – Lipovsky ,M.M ., Tsenova,L . and Coenjaets , F .E. : Crtptococcal glucuronoxy lomannan delays translocation of leukocytes across the blood-brain barrier in an animal model of acute bacterial meningitis .J .Neuroimmunol.2000;1:(111)(1-2):10-4.

13-Blackwood,W.and corsellis ,J.A.N .:Green fields neuropathology.3rd ed.946.Edward amold.London.1976.

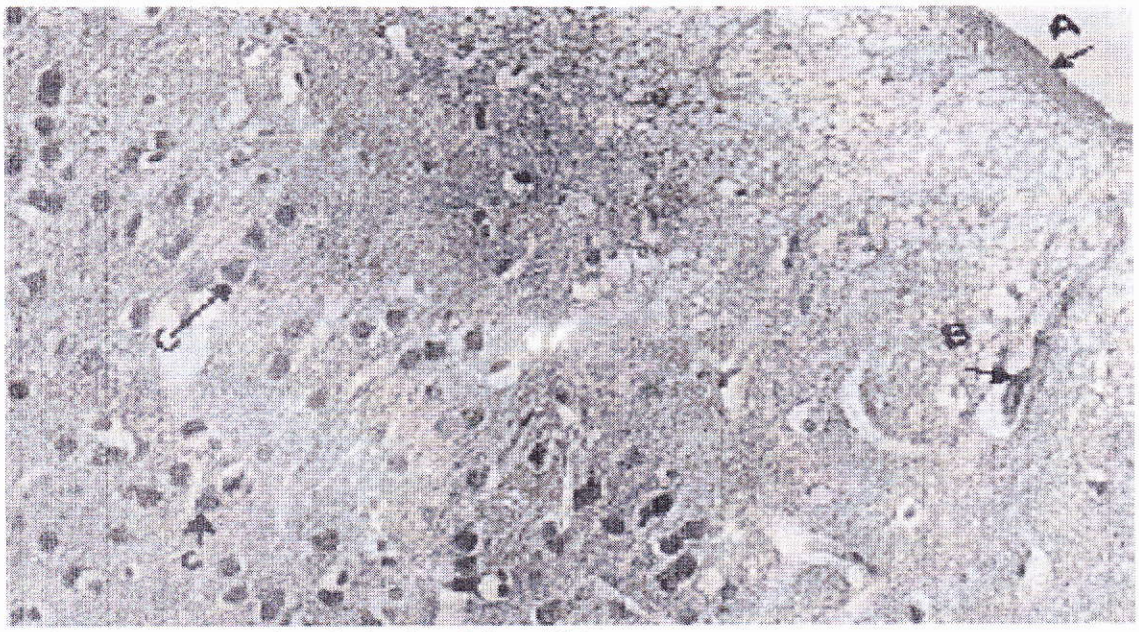

Fig .1: Demonstrating the brain of the rabbit
A-meninges
B- blood vessel
C-pyramidal cels
D-horizontal cells. (H&E.40X) .Group A.

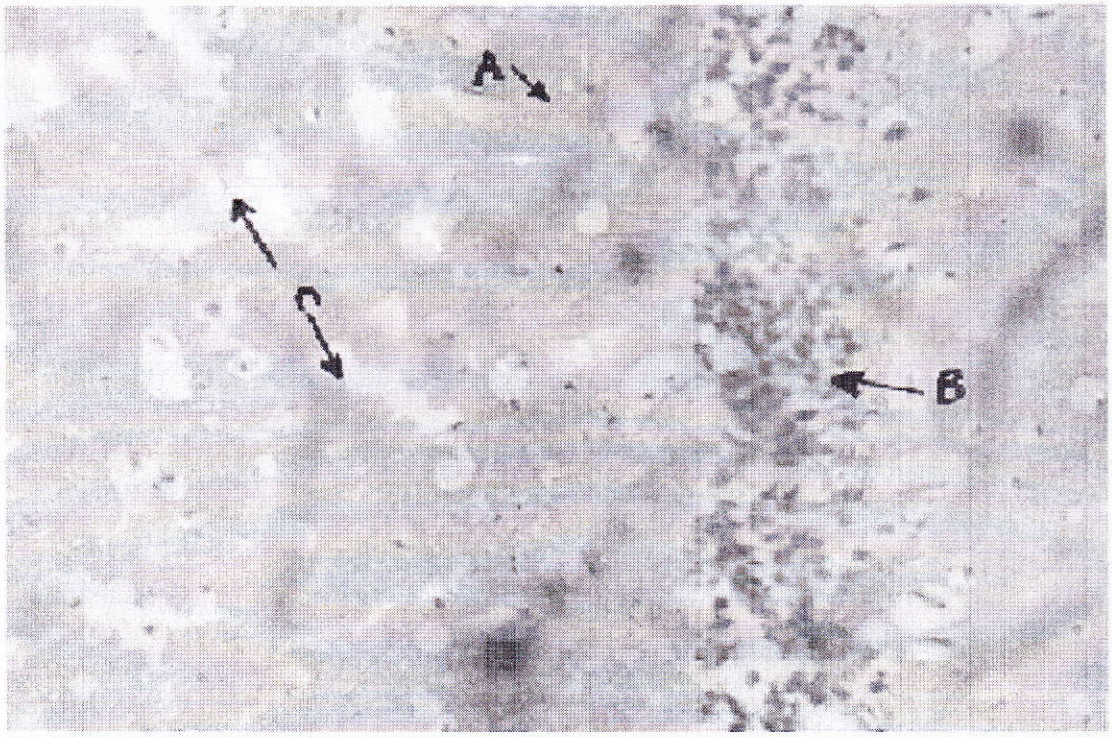

Fig.2: Demonstrate the brain of the brain tissue.
A- pyramidal cells
B- fusiform cells. (H&E.40X) Group A

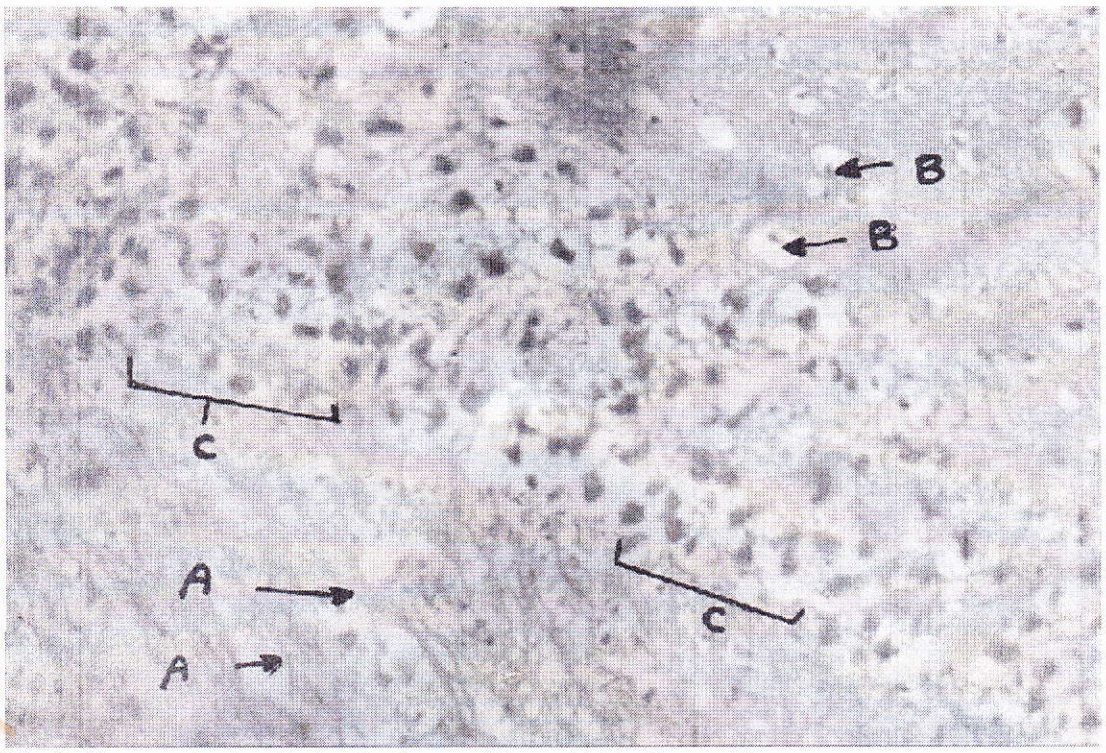

Fig.3: Demonstrates the brain tissueof the rabbit Group B.
A-Demyelination of the nerve fiber of the white matter.
B-Atrophy of the nervous of the graymatter.
C-Gliosis. (H&E.40X).

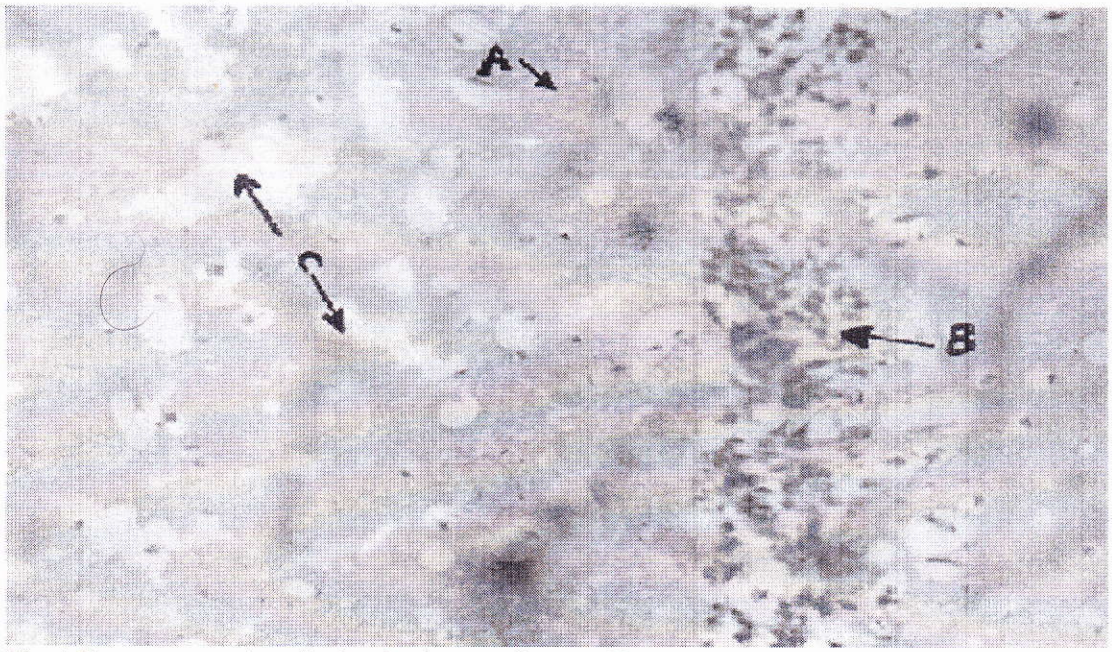

Fig.4:Demonostrating the brain tissue of group B .
A-Atrophy of the nervus .
B-Nuclation aggregation of the nurron.
C-Vaculation of the neuron. (H&E.40X).