

Effect of Intracavitary Injection of Urokinase in the Experimentally Induced Early Pyogenic Liver Abscess of the Rabbit

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This study was carried out to assess the effects of intracavitary injection of urokinase in the early liver abscess (ELA) of the rabbits. ELAs were induced on 25 in 47 New Zealand rabbits, which were divided into two groups, with 15 in group A, and 10 in group B. Urokinase was injected into the ELA of group A, and normal physiologic saline into those of group B. One and a half hours after the injections, the rabbits were sacrificed and evaluated by pathologists for the degree of fibrosis of the ELA wall, and fibrinolysis in the ELA itself. Statistical analyses were performed between the two groups. The following ELA sizes for each group were obtained: Group A, 4.3×2.9 - 10.1×7.2 mm (mean 7.1×4.1 mm); Group B, 4.6×2.7 - 15.0×9.7 mm (mean 8.5×4.57 mm). Eleven (73%) in group A showed grade II fibrosis of ELA wall, and 8 (80%) in group B showed grade III fibrosis of ELA wall ($p=0.002$). On pathological analysis, 5 (46%) in group A showed grade II fibrin, and 8 (80%) in group B showed grade III fibrin, of the ELA ($p=0.09$). In conclusion, injection of urokinase, into the ELAs, can reduce the degree of fibrosis of abscess walls.

Key Words: Liver, liver abscess, urokinase

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INTRODUCTION

Since the introductions of ultrasonography and CT, many studies have demonstrated that most pyogenic liver abscess can be effectively treated with antibiotics in combination with percutaneous drainage.¹⁻⁶

There is the likelihood for easier detection of liver abscesses with the development of imaging modality, but they are difficult to treat effectively in the early stages of pyogenic liver abscess because the pus is not mature enough to be drained.

There have been several reports of fibrinolytic agents preventing abscess formation and decreasing infection.⁷⁻¹⁰ To our knowledge there is no report on the effects of urokinase in the treatment of early liver abscess (ELA), although similar clinical studies have been carried on mature liver abscesses in humans.¹¹ This study is an animal model, evaluating the effects of urokinase in early liver abscesses.

MATERIALS AND METHODS

Animals and abscess induction

The experiments were performed on 47 New Zealand white rabbits weighing between 2.5 and

3.0 kg. Abscesses induction, and ultrasonographic examinations were performed following intramuscular anesthesia.

An initial injection of ketamine was administered, intramuscularly, at a dose of 40 to 60 mg/kg of body weight and maintained with a dose of 10 to 20 mg/kg every 30 minutes. ELAs were induced in each rabbit by laparotomic injection of 1.5×10^8 *E. Coli* (HB101), diluted in 1 ml LB agar broth (Gibco BRL, Gaithersburg, MD, USA), via 26-gauge needles.

Experimental design

Four days after abscess induction, ultrasonography [Acuson 128XP10 (Mountain View, CA, USA)] was performed using a 7 MHz probe.

Of the 47 rabbits, two died two days after inoculation with *E. coli*. 20 livers showed ill-defined, or well-defined, echogenic nodules, and the remaining 25 showed well-defined hypoechoic nodules from the ultrasonography. Ill-defined echogenic nodules, from ultrasonography, were revealed as compactly aggregated inflammatory cells, with scanty amounts of necrotic debris, on the pathologic specimens. The 25 subject rabbits showing well-defined hypoechoic nodules in the liver from ultrasonography, and the ELAs, were confirmed from their laparotomic examinations. The 25 rabbits were randomly assigned into two groups, 15 in group A and 10 in a control group, group B. The 15 in group A received urokinase and the 10 in group B received normal physiological saline. The urokinase, or normal physiological saline, was administered into the ELA cavity, following the laparotomy, via 26 gauge needles. For each centimetre of maximum abscess diameter, group A received 5000 IU of urokinase¹² and group B received 0.5 cc of normal physiological saline, a corresponding amount to 5000 IU urokinase. About one, to one and half, hours, after administration of urokinase or normal physiological saline, ultrasonography was performed, and evaluated by radiologists for sonographic finding.

About one and half hours after injection of urokinase or normal physiologic saline, the rabbits were sacrificed, by an intravenous overdose of pentobarbital, and the livers resected for patholo-

gical evaluation.

The abscess size was estimated by measuring the greatest diameters, at the level of greatest extent, and at right angles to the level of greatest extent, from the same scan.

All ELAs were verified pathologically using HE staining. Two pathologists evaluated the degree of fibrosis in the abscess walls, and the amount of abscess fibrin, without information regarding the origin (group A or B) of the ELA. Two pathologists using a three-point scale assessed the degree of fibrosis. If the maximum thickness of the fibrotic wall was less than 300 micron it was defined as grade I (Fig. 1), in the range 300-500 micron as grade II (Fig. 2), and more than 500 micron as grade III (Fig. 3). Two pathologists with three-point scales (grade I-minimum, grade II-mild, grade III-maximum), evaluated the amount of fibrin of abscess by consensus.

Statistical analysis

Cochran-Mantel-Henzel tests were used to test the hypothesis of equality of fibrosis of the ELA wall, and amount of fibrin in the ELA, between the two groups.

For abscess sizes, Wilcoxon scores were used.

RESULTS

11 (73%) of the 15 cases in group A showed grade II fibrosis, and 8 (80%) of the 10 cases in group B showed grade III fibrosis, of the ELA wall, as shown in Figs. 2 & 3, respectively ($p=0.002$) (Table 1). In group A, 5 (46%) of the 11 cases showing grade II fibrosis also showed grade II fibrin of ELA, and in group B 8 (80%) of the 10 showed grade III fibrin of ELA on the pathological specimens ($p=0.09$) (Table 2). In group A, four ELAs showed vacant abscess cavities, so it was im-

Table 1. Fibrosis of Early Liver Abscess Walls ($p=0.0018$)

| | Group A (Urokinase) | Group B (Control) |
|-----------|---------------------|-------------------|
| Grade I | 2 | 0 |
| Grade II | 11 | 2 |
| Grade III | 2 | 8 |
| Total | 15 | 10 |

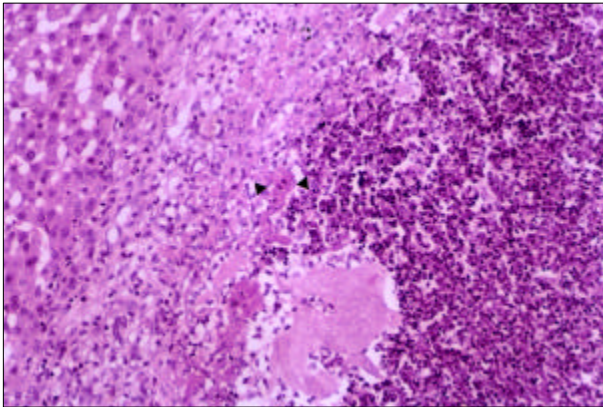


Fig. 1. Case 12. An ill-defined fibrotic wall (Grade I) can be seen at the periphery of the abscess cavity (H&E $\times 100$). Arrowhead indicates fibrotic wall.

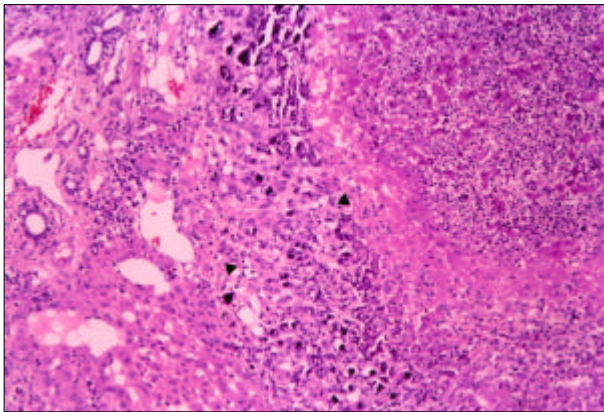


Fig. 2. Case 10. Dense neutrophilic infiltrations are apparent between the normal hepatocytes in the early liver abscess. Notes Grade II fibrotic wall at the periphery of the early liver abscess (H&E $\times 100$). Arrowhead indicates fibrotic wall.

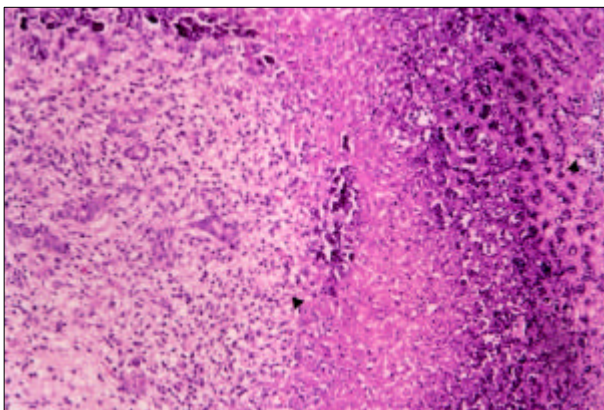


Fig. 3. Case 20. The low-power slide shows an abscess cavity surrounded by the thick fibrotic wall (Grade III). No viable hepatocyte in abscess cavity can be seen (H&E $\times 100$). Arrowhead indicates fibrotic wall.

Table 2. Amount of Fibrin of Early Liver Abscess ($p=0.947$)

| | Group A (Urokinase) | Group B (Control) |
|-----------|---------------------|-------------------|
| Grade I | 1 | 0 |
| Grade II | 5 | 2 |
| Grade III | 5 | 8 |
| Total | 11* | 10 |

*Four cases could not be evaluated because of vacant abscess cavities.

possible to assess the grade of fibrin of these ELA.

The size of the abscesses between the two groups was not statistically significant ($p=0.1917$).

The abscess sizes of each group from the ultrasonography were obtained as follows: group A, $4.3 \times 2.9 - 10.1 \times 7.2$ mm (mean 7.1×4.1 mm); group B, $4.6 \times 2.7 - 15.0 \times 9.7$ mm (mean 8.5×4.57 mm) (Table 3).

DISCUSSION

After the introduction of a pyogen, the body defenses respond to inflammatory cells that produce a number of kinins. Large protein molecules, such as fibrinogen, then leak into tissue spaces and polymerise in the fibrin. The fibrin then acts to limit the local spread of infection, but also impairs removal of bacteria, and the effect of antibiotics. The role of fibrin in abscess formation, and the potential benefit of fibrinolytic agents, has been suggested by the result of several animal reports.^{7,9,10} These reports revealed that fibrinolytic agents could decrease abscess formation and the rate of infection. Prinz et al.¹⁰ showed that heparin administration prevented the formation of intra-peritoneal abscesses. In addition to animal data, several clinical reports indicate that urokinase may improve the fibrinolytic drainage of abscesses. Vogelzang et al.¹³ supported the use of fibrinolytic agents as an adjunct to the treatment of infected haematomas. Recently, Haaga et al.¹⁴ reported that intracavitary injection of urokinase improved the drainage of abscesses, and shortened treatment times.

The effect of urokinase has duration of a few minutes, and has been used for 10 years for the drainage of multilocular abscesses. The abscess

Table 3. Size of Abscess before Administration of Urokinase and Normal Saline ($p=0.191$)

| Case | Group A (Urokinase) | Case | Group B (Control) |
|---------|-----------------------------|---------|------------------------------|
| Case 1 | 6.7×2.5 | Case 16 | 5.5×2.7 |
| Case 2 | 6.8×3.2 | Case 17 | 10.9×5.3 |
| Case 3 | 8.6×5.3 | Case 18 | 7.8×3.7 |
| Case 4 | 4.3×4.3 | Case 19 | 4.6×2.7 |
| Case 5 | 5.5×3.3 | Case 20 | 7.5×3.6 |
| Case 6 | 4.6×2.0 | Case 21 | 15.0×9.7 |
| Case 7 | 7.5×2.0 | Case 22 | 7.8×4.3 |
| Case 8 | 4.5×2.5 | Case 23 | 9.5×3.2 |
| Case 9 | 10.1×7.2 | Case 24 | 7.2×5.7 |
| Case 10 | 8.1×5.5 | Case 25 | 9.9×4.8 |
| Case 11 | 6.8×2.2 | | |
| Case 12 | 7.5×3.0 | | |
| Case 13 | 10.6×6.0 | | |
| Case 14 | 4.3×2.9 | | |
| Case 15 | 10.4×9.3 | | |
| Mean | $7.1 \times 4.1(\text{mm})$ | | $8.5 \times 4.57(\text{mm})$ |

itself is almost certainly a different entity from hepatic parenchyma, partitioned by an abscess capsule of inflammatory tissue,¹⁵ but the use of intracavitary urokinase has been well documented in many situations for the drainage of different abscesses, for example, in the spleen, empyema, etc. The initial experience of adjunctive intracavitary urokinase therapy in the spleen, and empyema, suggests it may also be beneficial in the safe drainage of percutaneous drainage of abscesses.^{15,16}

There are few examples for the use of urokinase in liver abscesses, which is a modified protocol for multiloculated empyema drainage and splenic abscess cavity.¹⁵ An injection of 5000 IU/maximum diameter did not result in any local or systemic side effects.¹²

Urokinase decreases the viscosity of purulent material, and increases the flow in all sizes of catheter.¹¹

The combination of intra-abscess urokinase and systemic antibiotics is very synergistic in graft sterilization.⁷ Percutaneous drainage is not contraindicated in the management of infected haematomas, although the drainage system must be closely observed.⁷ The drainage time may be shortened with the use of intracavitary urokinase.^{7,12,14} Intracavitary urokinase can be given safely during percutaneous drainage of an abscess,

without associated haemorrhage or changes in coagulation parameters.^{10,12}

The treatment of pyogenic abscesses usually includes both intravenous antibiotics and effective drainage.^{1,3} Surgical drainage of liver abscesses has been a controversial subject for many years, with most surgeons preferring the transperitoneal route if the abscess requires open surgery.¹ Percutaneous drainage has eliminated much of the controversy when selecting a route, whether it is a transperitoneal, transpleural or extraperitoneal approach.^{1,2,5} One report also demonstrates the introduction of a laparoscopic drain to a pyogenic liver abscesses,¹ but in all instances, the abscesses should be matured if they are to be drained easily.

It is not possible, in the early stages, to effectively drain a pyogenic liver abscess. Moreover, antibiotic therapy cannot treat early pyogenic liver abscesses, due to trapping of bacteria, in the fibrin clots, decreasing the efficacy of systemic antibiotics. We have focused on the goal of evaluating the effects of urokinase in the treatment of ELA that can not be drained effectively, or treated by systemic antibiotic therapy.

Our study revealed that intracavitary injection of urokinase reduced the grade of fibrosis of the ELA wall, which suggests this can increase the systemic effects of antibiotics. Also, the overall

amount of fibrin of the ELA was lower in group A compared to group B, although this was not statistically significant.

Clinically, our data can be used to help in the treatment of pyogenic liver abscess: Firstly, early pyogenic liver abscess, that can not be drained effectively, may be treated by intracavitary injection of urokinase in combination with systemic antibiotics.

Secondly, Rajak et al.¹⁷ reported catheter drainage of liver abscesses was more effective than needle aspiration. Although, percutaneous needle aspiration, in combination with intracavitary injection of urokinase, may become a more popular method for the treatment of early pyogenic liver abscesses, especially when the abscess to be drained is small. In conclusion injection of urokinase into ELAs can reduce the degree of fibrosis of abscess walls.

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