Lysis of intracranial hematomas with urokinase in a rabbit model

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Urokinase (UK), a potent thrombolytic agent, was tested in a rabbit model for safety and efficacy in lysing intracranial hematomas. Intracerebral-intraventricular (IC-IV) hematomas were created by stereotaxically injecting 0.2 ml of clotted human blood into the frontal lobe and lateral ventricle of a total of 57 anesthetized adult New Zealand White rabbits (weighing 1.6 to 2.5 kg). Control animals received 0.2 ml of normal saline injected into the clot, and the experimental group received an equal volume of UK solution (50,000 units/ml) immediately after the clot injection. Some animals were sacrificed at 3 hours and others at 24 hours postinjection. At 3 hours, clot lysis had been achieved in nine (90%) of 10 UK-treated animals as compared to one (14%) of seven controls. By 24 hours, clots had been lysed in 10 (83%) of 12 UK-treated animals and in two (33%) of six controls. Overall, clot lysis was demonstrated in 19 (86%) of the 22 UK-treated animals and in only three (23%) of the 13 controls (p < 0.001). There was no significant difference in results between these animals and a further set of 22 rabbits that were treated with UK or saline 24 hours after clot injection. There was no histological evidence of damage or inflammation noted on careful light microscopic examination of three to five sections from each brain, although findings consistent with encephalitozoonosis, an incidental protozoan infestation, were encountered in four animals. These studies suggest that UK may be safely and effectively employed for the lysis of intracranial hematomas in this animal model, and that a delay in therapy of up to 24 hours does not significantly compromise its efficacy.

KEY WORDS • intracranial hematoma • intraventricular hemorrhage • urokinase • rabbit • experimental model

Large deep-seated intracerebral hemorrhages are generally associated with high morbidity and mortality rates. Several authors have reported their experience with the stereotaxic evacuation of these lesions using standard4,10,17,22,34 or special1,16 needles. These authors have suggested that these approaches may be preferable to conventional surgical evacuation because of limited invasion of the overlying normal brain. Nevertheless, the adequate evacuation of clotted blood remains a problem. There is an apparent need for a thrombolytic agent that could be used intracranially to facilitate more complete drainage of intracerebral and intraventricular hematomas. A considerable body of data has accumulated on the intravascular use of urokinase (UK) and streptokinase.3,36 That these agents can successfully lyse clots is no longer in question. However, neither agent has been systematically studied in an animal model in order to establish its efficacy and, more importantly, its safety, when used intracranially outside the vascular system. We have attempted to establish the efficacy and safety of UK in a rabbit intracerebral-intraventricular (IC-IV) hematoma model.

It must be emphasized at the outset that there is no perfect animal model for the study of intracerebral hematomas. Although we developed this model specifically for purposes of this study, it also has its limitations in terms of comparability with the human condition. Perhaps its principal limitation is its inability to quantify the potential risk of rebleeding secondary to clot lysis. In the final analysis, perhaps human trials alone will be able to settle this issue. However, studies
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such as this may help to define the effect of thrombolytically effective doses of these agents on the central nervous system.

Materials and Methods

Animal Preparations

Adult New Zealand White rabbits (each weighing between 1.6 and 2.5 kg) were anesthetized with intravenous pentobarbital (30 mg) followed by inhalation of ether and were then placed in a stereotaxic frame with a rabbit head-holder.* Banked human whole blood (50 ml) was treated with human thrombin (200 units) to initiate clotting. Fifteen minutes later the clot was separated from the plasma by centrifugation at 4000 G for 10 minutes. Frontal IC-IV hematomas were produced soon thereafter by stereotaxic injection of the clotted blood as follows. A parasagittal incision 2 cm in length was made, and the angulation of the head in the frame was standardized by ensuring that the bregma was 1.5 mm higher than the lambda. A No. 20 needle hole was created along the coronal suture 3 mm lateral to the midline. Through this hole, 0.2 ml of clotted blood was injected stereotaxically by means of a tuberculin syringe via a No. 22 needle at a depth of 5 mm from the cranial surface. The needle was left in situ to prevent extrusion of the injected blood clot.

Control animals received an injection of 0.2 ml normal saline into the clot, whereas the experimental group received an equal volume of UK solution (50,000 units/ml; molecular weight 35,000). The needle was allowed to remain in place for a further period of 5 minutes and was then withdrawn. No attempt was made to aspirate the clot or the lysed material. The incision was closed with skin staples and the animal was allowed to wake up. Animals were then sacrificed with intravenous pentobarbital at either 3 hours or 24 hours post-injection. A total of 35 animals were thus studied. In a further group of 22 animals, UK or saline administration was delayed until 24 hours after clot injection.

Pathological Analysis

The rabbit brains were removed and fixed in 10% neutral buffered formalin for 2 weeks prior to sectioning. Serial coronal sections of the fixed brains were made at 3-mm intervals for a total of three to five sections per brain, and the gross presence or absence of an IC-IV hematoma was noted. Brains in which a hematoma was clearly still present were graded as positive, and those in which the hematoma had been lysed were graded as negative. Photographic documentation was obtained in every sectioned brain (Fig. 1).

Paraffin sections were made from each 3-mm brain slice in every animal and were stained with hematoxylin and eosin. These sections were studied by a neuropathologist (D.A.K.) with the purpose of assessing the histological effects of UK on the brain.

Results

Clot Lysis

A total of 35 animals with immediate therapy were studied (Table 1). Seventeen were sacrificed at 3 hours and 18 at 24 hours postinjection. Of the 10 UK-treated animals that were sacrificed 3 hours after clot injection, nine (90%) demonstrated clot lysis. In contrast, only one (14%) of the seven control animals exhibited clot lysis (p < 0.01). In the animals sacrificed 24 hours after injection, 10 (83%) of 12 UK-treated animals demonstrated clot lysis as compared to two (33%) of the six controls (difference not significant). Comparing the overall figures, clot lysis was achieved in 19 (86%) of

* Stereotaxic frame manufactured by David Kopf Instruments, Tujunga, California.
TABLE 1
Clot lysis in control and urokinase (UK)-treated animals at 3 and 24 hours

<table>
<thead>
<tr>
<th>Time Post-Injection</th>
<th>Control Group Lysis</th>
<th>UK-Treated Group Lysis</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
</tr>
<tr>
<td>3 hrs</td>
<td>7</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>24 hrs</td>
<td>6</td>
<td>33</td>
<td>12</td>
</tr>
<tr>
<td>totals</td>
<td>13</td>
<td>23</td>
<td>22</td>
</tr>
</tbody>
</table>

* Fisher's exact test. NS = not significant.

TABLE 2
Effect on clot lysis at 24 hours of 24-hour delay in UK treatment*

<table>
<thead>
<tr>
<th>Timing of Therapy</th>
<th>Control Group Lysis</th>
<th>UK-Treated Group Lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Percent</td>
</tr>
<tr>
<td>immediate</td>
<td>6</td>
<td>33</td>
</tr>
<tr>
<td>delayed 24 hrs</td>
<td>11</td>
<td>9</td>
</tr>
</tbody>
</table>

* There is no significant difference between the immediate-therapy and the delayed-therapy groups in terms of the efficacy of clot lysis with urokinase (UK).

the 22 UK-treated animals and in only three (23%) of the 13 controls (p < 0.001).

Table 2 shows the results in the group of 22 animals in which therapy was delayed for 24 hours after clot injection, and the animals were sacrificed a further 24 hours later. No significant compromise of the lytic efficacy of UK was noted with the delayed treatment.

Histological Studies

The results of the histological studies were unremarkable in 53 of the 57 brains studied. No abnormalities were noted in the ependyma, parenchyma, or pia-arachnoid, except those related to the injection of the clot. Particular attention was paid to the ependymal lining and the subjacent regions of the UK-treated animals. These areas were not remarkable (Fig. 2). In four animals, however, there were scattered leptomeningeal and, more commonly, intraparenchymal foci of mononuclear cell infiltrates with perivascular cuffing associated with multiple small granulomatous foci (Fig. 3A). No polymorphonuclear cells or eosinophils were seen. In one instance, a particularly cellular area contained apparently intracellular round or ovoid structures with a surrounding clear zone, measuring approximately 3 μ in greatest dimension (Fig. 3B). All of these animals were in the 24-hour UK-treated group; however, these histological abnormalities were typical of encephalitozoonosis, an intracellular infestation caused by the protozoan Encephalitozoon cuniculi that has a high prevalence in certain untested animal colonies.7,18 This diagnosis could not be tested serologically since it was made long after the animals had been sacrificed. However, the slides were reviewed independently by two veterinary pathologists (Drs. F. M. Garner and J. H. Vickers) who confirmed the diagnosis.

Behavioral Observations

The animals were allowed to wake up after the experimental intervention and were allowed to feed ad libitum. A few animals (approximately 25%) demonstrated a mild to moderate hemiparesis contralateral to the hematoma, which tended to become less noticeable by 24 hours. The animals were not alive long enough in this study to assess long-term neurological sequelae. There did not appear to be a higher incidence of hemiparesis in either group. The 3-hour animals had not always completely recovered from the effects of the initial anesthesia when they were sacrificed. The 24-hour animals, however, were awake and alert; except...
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For the hemiparesis mentioned above, they appeared to have tolerated the clot injection and therapy with no apparent ill effects.

Discussion

Intraparenchymal brain hemorrhages constitute somewhere between 6% and 16% of the approximate 297,000 initial cerebrovascular accidents that occur annually in the United States. 2733 Typical figures relating to the distribution of these hemorrhages indicate that 53% are putaminal, 13% thalamic, 5% pontine, and 10% cerebellar; 19% are found elsewhere. 14 Thus, most of these lesions are deep-seated and virtually none occur in the surface gray matter. Although conventional dogma holds that hypertension is the etiological basis for most of these bleeds, this has been disputed. 23

The outcome in a given case of intracerebral hemorrhage is influenced by several variables, notably the size and location of the hematoma and the patient's age and clinical status on admission. Thus, mortality rates can approach 100% in certain categories of patients, 1221 fostering a sense of therapeutic nihilism. Whether surgical or conservative treatment of these lesions is preferable has been a long-standing controversy. 3,6,8,9,11,19,20,24,29,32 There is an understandable reluctance among many neurosurgeons to approach large deep-seated hemmorhages with open surgery. Stereotaxic aspiration of these lesions has been proposed by several authors. 4,10,17,34 However, this technique allows for only partial removal of the hematoma, even with the use of special needles. 16 An agent that safely lysed the remaining clot could facilitate evacuation of the mass, and possibly improve outcome. This study was designed to evaluate the intracranial use of such an agent in an animal model.

Blood normally contains a fibrinolytic system which is capable of dissolving blood clots (Fig. 4). This system is composed of a number of enzyme precursors, activators, and inhibitors. Essentially, the inactive proenzyme, plasminogen, is converted to the active enzyme, plasmin, which in turn lyses fibrin clots. This conversion is dependent upon a number of endogenous and exogenous plasminogen activators. 3 Of the exogenous activators studied for their therapeutic potential in thromboembolic disease, the best known are urokinase (UK), which is synthesized by the kidney and normally found in human urine, and streptokinase, which is produced by beta-hemolytic streptococci. 36 Although the mechanism of plasminogen activation by these two agents differs somewhat, they both result in the production of plasmin, a nonspecific proteolytic enzyme that can digest various proteins including fibrin. Within the
circulation, the activity of plasminogen activators and plasmin itself is modulated by specific inhibitors. Pharmacological intervention with agents such as UK can overwhelm this inhibitor influence and greatly accelerate the dissolution of a clot.

Several preliminary experiments were performed in order to develop the IC-IV hematoma model described in this report. Human blood was used instead of rabbit blood in this set of experiments since our intent was to reproduce the human situation as closely as possible, and preliminary in vitro studies in our laboratory indicated that rabbit blood required approximately three times higher concentrations of UK than did human blood to achieve a comparable degree of clot lysis. Furthermore, since these were short-term experiments, immunological differences were not considered important.

The injected clots were proportionally comparable to human intracerebral hematomas in volume. The injection of larger volumes often resulted in either tracking back of the fluid or sudden respiratory arrest, presumably due to a herniation syndrome. Injecting unclotted blood into the brain with the intent of allowing it to clot in situ did not work well because of leakage along the needle track and diffuse spread of the injected material throughout the subarachnoid and ventricular systems. Forming a localized lesion that morphologically resembled the human condition and that was easy to define proved to be much easier with clotted blood.

As stated earlier, this model is certainly not without its limitations. The IC-IV hematoma is not spontaneous, and there is no underlying pathology, such as a ruptured microangioma. Therefore, the question of possible rebleeding secondary to dissolution of a hemostatic plug by UK cannot be addressed with this model. It should be pointed out, however, that bleeding problems are associated with protracted systemic and not local use of these agents. Whether or not local application of UK will result in rebleeding remains to be seen. It has been shown previously that the lytic activity of UK is proportional to its concentration. The amount of enzyme injected can therefore be modified depending on the size of the hematoma and the rapidity of clot dissolution desired. Furthermore, rinsing of the evacuated clot bed with normal saline, or even possibly with antifibrinolytic agents, may theoretically be useful in reducing the incidence of this problem, were it to arise. Although the injection of a formed clot through a No. 22 needle is likely to result in a certain degree of clot homogenization, this did not seem to affect the results of these experiments, in that the control animals demonstrated a high incidence of well formed residual hematomas. Thus, despite its limitations, we believe this to be a satisfactory model for the study of intracranial hematomas. The few other models described in the literature, besides being more expensive, complicated, and time-consuming, are unlikely to answer the questions addressed by this study any more effectively.

No histological abnormalities were detected in 53 of the 57 animals in our series; the histological findings were identical in the control and UK-treated brains except for the presence or absence of hematomas. In four animals, however, there was histological evidence of meningoecephalitis. Since these abnormalities all occurred in UK-treated animals, we considered the possible role of UK in their genesis. However, neither the nature nor the widespread distribution of the lesions suggested a relationship to UK treatment. Subsequently, an independent review of the slides by two experienced veterinary pathologists confirmed that the findings were typical of encephalitozoonosis, a protozoan infestation caused by *Encephalitozoon cuniculi* that occurs in up to 30% of untreated rabbit colonies and is usually clinically silent. Human cases of encephalitozoonosis have also been reported, although these are uncommon and are generally associated with immunosuppressed patients.

The literature contains reports from two Japanese groups on the use of UK in humans for lysis of intracranial hematomas. The more extensive of these studies was reported by Matsumoto and Hondo, who described their experience with computerized tomography (CT)-guided stereotaxic evacuation of hypertensive intracerebral hematomas in 51 cases. After defining the size and location of the hematoma with a CT scan, they directed a catheter toward its center via a burr hole with the patient under local anesthesia. The hematoma was then aspirated as completely as possible with a syringe. Thereafter, 6000 units of UK in 5 ml of saline was infused into the hematoma every 6 to 12 hours for a total of one to 18 doses, with aspiration of the lyed material prior to the next injection. Mean doses of approximately 40,000, 30,000, and 29,000 units of UK were required in the acute, subacute, and chronic groups, respectively, to eliminate the hematoma. There were reportedly no complications such as bleeding, convulsions, or infections related to these treatments.

A smaller study was reported in Japanese by Doi, *et al.* They reported stereotaxic hematoma evacuations in 12 conscious patients 7 to 37 days after the ictus. Residual hematomas were lysed with UK and drained through a silicone catheter over 3 to 4 days. All the patients were described as having had a "good functional outcome," and rebleeding due to UK therapy was not encountered.

Fig. 4. A simplified scheme of fibrinolytic pathways. UK = urokinase; SK = streptokinase.
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The only other study describing the intracranial use of UK was performed in monkeys and was reported in abstract form by Segal, et al. They created intracerebral hematomas in five monkeys by stereotactically injecting 6 cc of autologous blood into the internal capsule region. Three hours later, the control group of three animals received 0.1 ml of normal saline, and the experimental group of two animals received the same volume of saline containing 5000 units of UK. Follow-up CT scans demonstrated virtual resolution of the clot by 3 days in the two UK-treated animals, whereas control animals showed residual high-density lesions for up to 7 days after injection. The control group remained hemiplegic, while the UK-treated animals recovered partial motor function. The longer time taken to achieve the desired effect in this study could have been related to the smaller dose of UK used.

In conclusion, this study demonstrates that UK injected in situ can effectively and safely lyse intracranial hematomas without any immediately apparent adverse effects in a rabbit model. Delaying therapy for up to 24 hours did not appear to adversely influence its efficacy. Although the results of these animal studies are encouraging, caution is advised in the direct extrapolation of these findings to the human condition.

Acknowledgments

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References

30. Pauternak JF, Groothuis DR, Fischer JM, et al: Regional