SUMMARY Thirty-one patients with acute cerebral infarction were treated with the thrombolytic agent urokinase for either a single or a double infusion period, each of ten hours. The effects of urokinase therapy administered at dosage rates of 1,200, 1,500 or 1,700 CTA urokinase units per pound of body weight per hour were followed by serial blood coagulation and other biochemical studies.

In the dosage used, urokinase produced a prompt sustained increase, 20-fold to 40-fold, of plasma thrombolytic activity with relatively minor disturbance of the blood coagulation system. Nevertheless, hemorrhagic complications occurred in several patients and distinctly favorable therapeutic effects were not observed.

Introduction

TREATMENT of the patient with acute cerebral infarction is essentially supportive, and procedures intended to increase blood supply to the infarcted area and the surrounding ischemic, but potentially salvageable, brain areas are infrequently employed. While cerebral infarction is readily produced in the experimental animal, the animal model differs in many respects from that of the human counterpart. Consequently, animal data have not provided a definitive answer as to whether increase of blood supply to the ischemic cerebral areas, some hours after the ictus, is definitive answer as to whether increase of blood supply to the infarct and contiguous cerebral tissue differs in many respects from that of the human counterpart. Consequently, animal data have not provided a definitive answer as to whether increase of blood supply to the infarct and contiguous cerebral areas might minimize tissue death and increase salvage of potentially viable cerebral tissue.

Thrombolytic agents are potentially capable of increasing blood supply to the infarct and contiguous cerebral tissue (a) by lysis of thromboemboli occluding the local blood supply, (b) by altering blood rheological properties or (c) possibly by directly influencing fibrinogen/fibrin catabolism at the infarct site. Moreover, if thrombolytic agents were administered early in the disease course, they might, by inhibiting thrombus extension, prevent potential infarct enlargement and further neurological deficit. For this reason, the plasminogen activator streptokinase, an agent inducing powerful fibrin-lysing activity in the plasma, has received limited clinical trial in patients with cerebral infarction. The trial results were not encouraging since, though angiographic study showed that thromboemboli were lysed with greater frequency in the streptokinase-treated group, the treatment was associated with a high incidence of untoward hemorrhage and the treated patients fared less well than the control group. Though it is likely that the disappointing results of this trial were partially attributable to the use of combined streptokinase/heparin therapy, there are additional reasons for believing that streptokinase is a less-than-ideal plasminogen activator for use in patients with cerebral infarction and in others where precise control of the induced blood coagulation deficit and plasma thrombolytic activity is mandatory.

Since the time of the streptokinase trial, another plasminogen activator, urokinase, has become available as an investigative drug. Urokinase appears to possess substantial advantages over streptokinase as a therapeutic agent because (1) it is non-antigenic and, consequently, its administration on a dose/body weight/hour basis produces predictable and controllable alterations in plasma thrombolytic activity, and (2) its gel phase/soluble phase plasminogen activator ratio is much higher than that of streptokinase, so that for a corresponding degree of induced plasma thrombolytic activity, alterations in the blood coagulation system are substantially less.

Consequently, the aims of the present feasibility study were (1) the investigation of three urokinase dosage regimens on blood coagulation and other biochemical findings with the aim of defining a treatment regimen which, while producing substantial enhancement of blood thrombolytic activity, would produce minimal deleterious effects on the blood coagulation system and (2) to obtain preliminary clinical assessment of treatment effects and untoward drug toxicity.

Methods

PATIENT SELECTION

Patients were admitted to the study regardless of age or sex provided: (a) that the clinical picture was that of cerebral infarction and the neurological deficit was not, in the opinion of the attending neurologist, of such extreme degree as to preclude recovery, (b) that the ictus had occurred preferably within 24 hours, but at the latest, 36 hours prior to the start of urokinase therapy (the time between best estimate of the onset of the neurological symptoms and the beginning of urokinase infusion, including the time necessary for hospital evaluation, ranged from 8 to 34 hours. Of 31 patients, this treatment lag was less than 12 hours in seven and from 12 to 24 hours in 15), (c) that blood was absent from the CSF, (d) that hypertension, defined as a sustained blood pressure in excess of 150/90 mm Hg, was either absent or could be rapidly controlled by medication (this requirement caused...
considerable difficulty and was interpreted in an elastic fashion, (e) that blood coagulation findings and the platelet count were within normal limits, (f) that complicating disease was not of gross degree, (g) that specific contraindications to urokinase therapy, recent surgical operation, a hemorrhagic diathesis, recent peptic ulcer, etc., were absent, and finally, (h) that the patient or a relative gave informed consent to the use of the investigational drug, urokinase. Eight of these patients were in our initial pilot study group where treatment was instituted after clinical evaluation and arteriography. Pretreatment arteriography was then abandoned and seven more patients were treated as part of a double-blind protocol which was later aborted because of inadequate numbers of available cases. Six control patients were infused with saline solution during this period. Sixteen more patients were treated with urokinase, admission to the study being based upon the meeting of the clinical criteria and the obtaining of informed consent for treatment.

Besides pretreatment coagulation and platelet studies, other investigations included routine chemistries, chest and skull films, admission EKG, brain scans and EEGs (Day 1 or Day 2 and Day 7), blood coagulation studies for five days and thereafter at less frequent intervals and serial stool guaiac examinations performed for four days after therapy.

NEUROLOGICAL ASSESSMENT

A standardized neurological examination suitable for numerical assessment was used with a total score of 120 points allocated to the neurologically intact individual. * "Blind" scoring of cerebral infarction patients by the attending neurologists showed that score totals, assessed by the individual neurologist who followed each patient, seldom varied by more than five points.

Neurological scores were obtained on the first, second, third or fourth, fifth and tenth days after hospital admission and were thereafter performed at approximately weekly intervals.

In general, a score of greater than 100 points documented the presence of an incomplete hemiplegic lesion; less than 100 points, a dense hemiplegia; and below 50 points, the presence of a massive neurological deficit. The patient's neurological status was interpreted as unchanged if the difference between the initial and final neurological assessment varied within ± 5 points, as either probably deteriorated or probably improved if there was variation between the scores of 5 to 15 points, and as definitely improved or deteriorated if the score varied by greater than 15 points. If the patient's initial neurological score was greater than 100 points, change in score of two-thirds that described above was used to designate changed patient status.

LABORATORY METHODS

Blood coagulation assays, plasma fibrinogen, one-stage prothrombin time, thrombin clotting time, plasma recalcification time, and assays for components of the plasminogen/plasmin enzyme system, plasma plasminogen, euglobulin lysis time and euglobulin precipitate lysis on a fibrin plate were performed by standard methods used in previous studies with urokinase. * Alpha2-macroglobulin, alpha1-antitrypsin and antithrombin III were determined immunologically by radial immunodiffusion using the Mancini method7 with antisera purchased from Behring Diagnostics, New York, and Nyegaard, Norway.

Plasma fibrinogen chromatography was performed as previously described using a semiautomated apparatus for chromatography and effluent assay. * * Percentage high molecular weight fibrinogen complexes (HMWFC), monomeric fibrinogen and derivatives of fibrinogen smaller than the parent molecule were calculated using a computer program (Wang Laboratory Users Society Program M 7-7.5, Tewksbury, Massachusetts) based on chromatographic plate theory.10 Individual moiety plasma concentrations were calculated from these data using total plasma "fibrinogen" values.

Distribution patterns in 95 clinically well subjects yielded plasma HMWFC values (mean ± SD) of 7.7 ± 6.2% (18.4 ± 21.9 mg%), monomeric fibrinogen 67 ± 10.3% and fibrinogen first derivative 25.8 ± 6.4%. Assay abnormality has been defined as a value exceeding mean + 2 SD of normal values; thus, for plasma HMWFC, the finding of values exceeding 20% or 71 mg% has been regarded as pathological.

Blood viscosity at low shear rates, 10 to 0.15 seconds14, was determined using a modified Benis coiled capillary viscometer.11

Standard statistical methods have been used12 and observational dispersion has been expressed as the standard error of the mean unless otherwise stated.

<p>| TABLE 1 Biochemical Findings Prior to and During Urokinase Infusion |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Pre-</th>
<th>30'</th>
<th>Day 1</th>
<th>2H</th>
<th>4H</th>
<th>6-8H</th>
</tr>
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<tbody>
<tr>
<td>Fibrinogen (mg %)</td>
<td>360 ± 23</td>
<td>318 ± 26</td>
<td>320 ± 35</td>
<td>248 ± 23</td>
<td>226 ± 17</td>
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</tr>
<tr>
<td>One-stage prothrombin (seconds)</td>
<td>11.7 ± 0.5</td>
<td>12.9 ± 0.4</td>
<td>13.6 ± 0.5</td>
<td>14.9 ± 0.7</td>
<td>15.6 ± 1</td>
<td></td>
</tr>
<tr>
<td>TCT (seconds)</td>
<td>11 ± 0.3</td>
<td>12.7 ± 1</td>
<td>12.9 ± 0.6</td>
<td>15.5 ± 0.9</td>
<td>16 ± 1</td>
<td></td>
</tr>
<tr>
<td>Plasma recalcification</td>
<td>121 ± 6.2</td>
<td>115 ± 23.7</td>
<td>124 ± 10</td>
<td>133 ± 14</td>
<td>141 ± 12</td>
<td></td>
</tr>
<tr>
<td>Plasma platelet count (units/ml)</td>
<td>2.25 ± 0.12</td>
<td>1.93 ± 0.1</td>
<td>2.02 ± 0.16</td>
<td>1.87 ± 0.19</td>
<td>1.64 ± 1</td>
<td></td>
</tr>
<tr>
<td>Plasminogen (units/ml)</td>
<td>297 ± 27</td>
<td>46 ± 4.6</td>
<td>53 ± 8.2</td>
<td>39 ± 6.6</td>
<td>40 ± 7</td>
<td></td>
</tr>
<tr>
<td>Fibrin plate euglobulin (mm3)</td>
<td>34 ± 8.5</td>
<td>314 ± 34.6</td>
<td>247 ± 26.8</td>
<td>312 ± 39.8</td>
<td>215 ± 27</td>
<td></td>
</tr>
</tbody>
</table>

H = hour. TCT = thrombin clotting time.
UROKINASE ADMINISTRATION

Urokinase was administered by continuous i.v. infusion, usually via a pediatric scalp-vein needle inserted into a hand or forearm vein, using a Harvard syringe pump delivering 4 ml urokinase solution per hour. Urokinase dosage was based on patient body weight, and was administered at one of three dosage rates: 1,200, 1,500 or 1,700 to 1,800 CTA units per pound of body weight per hour for a 10-hour to 12-hour period. Each infusion was preceded by administration of a urokinase loading dose, administered over a ten-minute period and equal to that calculated for a single hour’s infusion. Eight patients were treated with 1,200 CTA urokinase units per pound of body weight per hour and in five, the infusion was repeated approximately 12 hours later. Eleven patients received 1,500 CTA units per pound of body weight per hour and four of these received a second infusion. None of the 12 patients receiving 1,700 to 1,800 urokinase units per pound of body weight per hour received a second infusion. Total urokinase dosage for the first infusion, calculated on the basis described above, ranged from a low of 1.4 X 10^6 units to a high of 3.3 X 10^6 units.

The Abbott Company (Chicago, Illinois) and the Hoffmann-La Roche Company (Basel, Switzerland) donated urokinase, an I.N.D. drug. Both preparations met CTA specifications. Twenty patients received urokinase of Hoffmann-La Roche manufacture and 11 that of Abbott manufacture.

Results

PATIENT POPULATION

Twelve of the 31 patients were black and the remainder were white; 15 were female. Their mean age was 65 with a standard deviation of 12.4 years. Patient distribution by age showed two below the age of 50, six between 50 and 60, 12 between 60 and 70, eight between 70 and 80, and three more than 80. Six of the patients gave a history of prior cerebral infarction and two others gave recent histories of transient cerebral ischemic attacks (TIAs).

A history of hypertension was elicited in 13 patients of whom five also had diabetes. Two patients had diabetes without hypertension and two patients had previously experienced documented myocardial infarction.

The initial degree of neurological deficit, as assessed by the numerical scoring system, ranged from 40 to 114, with an average of 78 points. Only five of the 31 patients (16%) showed scores in excess of 100 points, compared with an overall incidence, estimated from a natural history study, of 108 of 218 patients (50%). This difference between our series and the patients admitted to the unit was significant (Chi square 19.7, P < 0.001) and indicated that the urokinase-treated patients were more severely stricken than the average patients entering our unit.

BIOCHEMICAL CHANGES PRODUCED BY UROKINASE THERAPY

The effect of urokinase infusion on the blood coagulation and plasma fibrinolytic enzyme systems is shown in table 1. Data from all three treatment schedules have been combined, for though there was a distinct trend for higher doses of urokinase to produce more intense biochemical changes, the individual treatment groups were small and differences between them were mostly not significant.

The effect of the initial ten-hour urokinase infusion (left-hand section of table 1) was to reduce average plasma fibrinogen from 360 mg% to 226 mg%, increase one-stage prothrombin time from 11.7 seconds to 15.6 seconds, the thrombin-clotting time from 11 to 16 seconds, and the plasma recalcification time from 121 to 141 seconds. These alterations in coagulation function following the initial urokinase infusion were of relatively minor degree and, as shown by the Day 2 pretreatment findings, were reverting toward normal several hours later. The effect of the second ten-hour urokinase treatment, administered only to patients receiving the two lower urokinase dosage schedules, was to reduce average plasma fibrinogen from 246 mg% to 208 mg%, increase one-stage prothrombin time from 14.6 to 15.7 seconds, thrombin clotting time from 15.1 to 15.4 seconds, and plasma recalcification time from 108 to 153 seconds. These findings are indicative of only a relatively modest degree of coagulation defect and, again, the Day 3 findings demonstrate that the coagulation parameters were returning to pretreatment values within hours after cessation of urokinase therapy.

The most abnormal coagulation values found in any patient in the three groups were: for those treated at 1,200 urokinase units per pound of body weight per hour, fibrinogen 140 mg%, one-stage prothrombin time 18.5 seconds, thrombin time 16.9 seconds, and plasma recalcification time 211 seconds; for those treated with 1,500

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pre-</th>
<th>30'</th>
<th>2H</th>
<th>4H</th>
<th>6-8H</th>
<th>Day 2</th>
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<td>Urokinase</td>
<td>20</td>
<td>36</td>
<td>32</td>
<td>40</td>
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<tr>
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<td>1.3</td>
<td>1.1</td>
<td>1.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Urokinase</td>
<td>0.9</td>
<td>1.4</td>
<td>1.3</td>
<td>1.5</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Urokinase</td>
<td>10</td>
<td>11</td>
<td>10</td>
<td>20</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Urokinase</td>
<td>0.13</td>
<td>0.15</td>
<td>0.09</td>
<td>0.09</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Urokinase</td>
<td>6.1</td>
<td>5.7</td>
<td>4.1</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Urokinase</td>
<td>18.8</td>
<td>25.4</td>
<td>35.1</td>
<td>40.3</td>
<td>55.7</td>
<td></td>
</tr>
</tbody>
</table>
units per pound of body weight per hour, 134 mg% fibrinogen, 19.2 seconds one-stage prothrombin time, 21.2 seconds thrombin time, and 183 seconds plasma recalcification time. For those treated with 1,700 units per pound of body weight per hour, the most abnormal values recorded were 120 mg% fibrinogen, 21 seconds one-stage prothrombin time, 25 seconds thrombin time, and 200 seconds plasma recalcification time.

Decrease in plasma plasminogen concentration was related to urokinase dosage. At the end of the first day's infusion, there was an average fall of 17% in those treated with 1,200 CTA units per pound of body weight per hour, 25% in those treated with 1,500 CTA units per pound of body weight per hour and 37% in those treated at the highest dose of 1,700 to 1,800 CTA units per pound of body weight per hour. These data are displayed in figure 1, where plasminogen is expressed as percentage of original concentration and plotted as a function of urokinase dosage and time. Figure 1 shows that for each sampling time (30 minutes, two hours, four hours, six hours and eight hours) depression of plasma plasminogen concentration was a function of dosage. Statistical testing between the dosage schedules confirmed that the degree of plasminogen fall was greater with higher urokinase dosage, P < 0.01. Figure 1 shows that a similar response to that seen at the time of the Day 1 infusion was also evident at the time of the Day 2 infusion with regard to the 1,200 and 1,500 CTA units per pound of body weight per hour dosages. None of the patients receiving the highest urokinase dosage, 1,700 to 1,800 urokinase units per pound of body weight per hour, were retreated on Day 2. The lowest plasma plasminogen concentration found was 0.8 unit per milliliter, compared to a normal value of 2 to 2.5 units per milliliter.

Urokinase therapy resulted in shortening of the euglobulin lysis time from a normal of two to four hours to between 20 and 70 minutes with an average value of approximately 45 minutes. Euglobulin activity on a fibrin plate rose from initial starting values of 34 ± 8.5 mm² to an average of 200 to 300 mm² on the fibrin plate; an activity remaining stable throughout the time of urokinase infusion and declining rapidly at the time of treatment cessation.

Table 1 lists these data on euglobulin lysis time and euglobulin activity on fibrin plate as a function of time prior to and during infusion. The data suggest that urokinase infusion increased plasma thrombolytic activity approximately tenfold. However, these assays underestimate the response of plasma fibrinolytic activity to urokinase, since the urokinase is incompletely precipitated with the euglobulin precipitate. Accordingly, four patients were also followed by the 125I-labeled clot lysis method and an additional six patients by plasma lysis on a fibrin plate. These assays suggested that the urokinase dosage used had increased average plasma thrombolytic activity 20-fold to 40-fold.

Twenty of the 31 treated patients were also studied by serial plasma fibrinogen chromatography performed at intervals similar to those used for other biochemical assays and these findings are shown in table 2. Since the number of patients in each urokinase group was relatively small, table 2 shows chromatographic findings combined for all dosage groups; data percentage for HMWFC, monomeric fibrinogen and the smaller fibrinogen derivatives are shown at the top of the table and for concentration (milligram percent) in the bottom half of the table.

Pretreatment values for both percentage and concentration of plasma HMWFC were significantly higher in the patient group than in the normal control group (13.7 ± 0.7% versus 7.7 ± 1.5%, t = 3.9, P < 0.001 and 40 ± 5.9 mg% versus 18.4 ± 5.3 mg%, t = 3.7, P < 0.001). Both plasma HMWFC proportions and concentration fell steadily during the initial ten-hour infusion reaching values of 4.2 ± 1.5% and 10.2 ± 3.7 mg% after eight hours' infusion; both these decreases from starting values are significant at the 0.1% significance level.

During the period of urokinase infusion, percentage figures for fibrinogen first derivative rose from 32.5 ± 2.8 to 43 ± 4.9%, an increase significant at the 5% level. However, fibrinogen first derivative concentration expressed as milligram percent did not show a similar increase. This latter finding was accounted for by the fact that total plasma fibrinogen underwent a 37% decrease during the initial period of urokinase infusion.

Results for the second day in the nine patients who underwent an additional urokinase treatment again showed a reduction in plasma HMWFC proportion and concentration and a rise in fibrinogen first derivative concentration. The plasma fibrinogen chromatographic results in the urokinase-treated group were compared with those observed in a conventionally treated group of patients. The urokinase-treated group showed substantially lower average proportions and concentration of plasma HMWFC than did the conventionally treated group. Average figures over the four days for the two groups were 10.3 ± 0.95% versus 15.4 ± 0.99% and 32.4 ± 2 mg% versus 53.1 ± 3.2 mg%, t values respectively 3.3 and 3.78, P < 0.001. However, between the fifth and sixth days, HMWFC proportions and concentration increased and equaled those seen in the conventionally treated group.
RHEOLOGICAL CHANGES

Tables 1 and 2 show that during the first period of urokinase infusion, plasma fibrinogen decreased an average of 29% and plasma HMWFC concentration, an average of 74%. Such alterations in plasma fibrinogen and HMWFC would be expected to produce alteration in blood rheological properties, and Figure 2 shows studies in six urokinase-treated patients who were followed by serial viscosity measurements. The upper panel shows that the hematocrit remained essentially unchanged during the infusion period; the middle panel shows that the erythrocyte sedimentation rate fell from an average of 20.5 to 15.5 mm per hour, while the bottom panel shows that blood viscosity, measured at 0.15 second⁻¹, fell from a initial average value of 29 centipoises (cp) to a final average value of 19 cp—a 35% decrease in blood viscosity at this low shear rate.
**INHIBITOR ASSAYS**

Table 3 shows serial assays for alpha,-antitrypsin, alpha2-macroglobulin and antithrombin III. The combined values for all antithrombin III assays showed a minor fluctuation throughout the infusion period and subsequently, but none of these changes reached conventional levels of statistical significance. However, calculation of antithrombin III concentrations as a percentage of the individual control values showed marked differences with different dosage regimens. The results with 1,200 CTA urokinase units per pound of body weight per hour averaged over Days 1 to 3 showed antithrombin to be 110.6 ± 3.1%, for those treated with 1,500 CTA units per pound of body weight per hour 97 ± 4.4%, and for those treated with 1,700 to 1,800 CTA units per pound of body weight per hour 95.1 ± 2.9%. The antithrombin III values for those treated with 1,200 urokinase units per pound of body weight per hour were significantly higher, at the 5% level (t = 2.3) than those receiving 1,500 CTA units and were higher still, significance at the 1% level (t = 2.98) than in those receiving 1,700 to 1,800 CTA urokinase units per hour.

Alpha2-macroglobulin levels showed a tendency (P < 0.1) to decline with each urokinase infusion. For instance, initial plus 30-minute readings averaged 26.4 ± 1.1 mm² and those obtained during the later stages of urokinase infusion averaged 22.7 ± 1.2 mm² (t = 1.7); a similar trend was evident with the second infusion. Alpha2-macroglobulin concentration remained stable for the remainder of the observation period following infusion. Alpha,-antitrypsin concentration was unchanged throughout the infusion period and thereafter.

**CLINICAL OBSERVATIONS**

Initially, it was planned to visualize the vascular lesion by angiography and repeat the examination after urokinase treatment. However, because one patient had a severe hypersensitivity reaction to the contrast medium at the time of repeat angiography and because of the arterial hemorrhage risk, this plan was abandoned. Angiographical examination was undertaken prior to urokinase treatment in seven patients. In one, no vascular lesion was demonstrated and, in two others, the examination was unsatisfactory. The other four patients all initially showed stenosis or occlusion of the middle cerebral artery.

**HEMORRHAGIC PHENOMENA**

Patients receiving urokinase usually had hematomas and bleeding at the site of venipunctures. However, bleeding from venipuncture sites was not excessive and patients showed neither generalized petechiae nor excessive bruising. In three instances, there was bleeding from arterial puncture sites secondary to recent angiographical examination. One patient bled from a femoral angiographical site just as the urokinase infusion was terminating; bleeding was easily controlled by pressure. A second had a large hematoma at the site of a brachial artery puncture; this resolved spontaneously within a week. In the third patient, carotid arteriography was traumatic and the carotid angiogram...
demonstrated that the injection had been partially subintimal. A large hematoma was evident at the neck on conclusion of the examination, and for this reason urokinase treatment was delayed 24 hours. Six hours after the start of the infusion, there was apparent increase in neck swelling and the infusion was stopped. One hour later, the patient had respiratory arrest and resuscitation was unsuccessful. The cause of death was considered to be laryngeal spasm consequent to the presence of blood in the neck.

It has been reported previously\textsuperscript{4-14} that patients receiving urokinase therapy may experience a late fall in hematocrit. The hematocrit was determined prior to therapy, at its conclusion and five to seven days later. Initial mean values were 43 $\pm$ 1.4%, after therapy 41.3 $\pm$ 1.3%, and one week later 39.5 $\pm$ 1.1%. The difference between the initial hematocrit value and the final one almost reaches the conventional 5% level of statistical significance, but the difference between the post-treatment and one-week value is not significant. Though one patient was observed in whom the hematocrit fell from 39% to 32% without apparent cause, the data indicate that in this series, urokinase did not induce a post-treatment fall in hematocrit. Post-treatment stool guaiac examinations remained negative.

**TREATMENT OUTCOME**

Sixteen percent of the urokinase-treated patients died in the first month. This mortality rate is of the same order of magnitude as was seen in a much larger but less severely stricken (P < 0.01) population of ischemic stroke patients\textsuperscript{15} treated during the period of the study. The number of urokinase-treated patients was too small to evaluate the effect of this therapy on disease outcome, but it was our clinical impression that no instance of unequivocal clinical improvement, attributable to treatment, was observed. Four urokinase-treated patients, originally diagnosed on clinical grounds as having acute cerebral infarction, later had evidence of intracerebral hemorrhage and are described in the following paper.

**Discussion**

This study demonstrates that urokinase may be administered to patients with acute cerebral infarction in dosages inducing substantial sustained increase in plasma thrombolytic activity without production of other than a relatively minor blood coagulation defect. Statistically significant differences in coagulation measurements or in plasma thrombolytic activity between the three dosage levels used were not demonstrated, though there was a trend suggesting that high urokinase dosage produced a greater degree of coagulation deficit and a greater increase in plasma thrombolytic activity.

Failure to show statistically different biochemical effects between the dosages employed was probably due to the fact that the number of patients in each urokinase dosage group was relatively small, initial coagulation and other biochemical findings were not uniform at the start of therapy and a limited range of urokinase dosage was studied. Other studies\textsuperscript{15} employing a wider range of urokinase dosage have demonstrated that the biochemical effects produced by urokinase infusion are both predictable and dose-related.

Two statistically significant differences between the effects of the lower and the higher urokinase dosage rates merit comment. The first finding was that plasminogen decrease in the urokinase-treated patients was, as expected, dose-related, but the second finding, that post-infusion antithrombin III assays were lower in those treated with high urokinase dosage, was not. This finding could be interpreted as indicating that the preparations of urokinase employed were contaminated with thromboplastic moieties. The presence of potent thromboplastic moieties in human urine, an activity sometimes referred to as pro-coagulant activity, has been demonstrated by several observers. During the development of urokinase as a therapeutic agent, the problem of preparation contamination with thromboplastic moieties caused anxiety. However, with the development of sensitive in vitro assay for such contamination,\textsuperscript{16} the removal of a majority of such contamination was accomplished. Consequently, our finding that antithrombin III concentration was significantly lower in those receiving the high urokinase dosage suggests that the original CTA standard for test of preparation contamination with thromboplastic moieties may have been of insufficient stringency.

The published specific activity of crystalline urokinase is 105,000 CTA units per milligram,\textsuperscript{17} but this figure has now been revised to approximately 150,000 CTA urokinase units per milligram protein.\textsuperscript{18} The original CTA specifications, devised some ten years ago, called for a minimal specific activity of 35,000 CTA units urokinase per milligram protein, together with certain other requirements. These specifications mainly have been exceeded by preparations supplied for clinical trial, but the present data suggest the need for their further upgrading.

A major uncertainty in a feasibility study of the present nature concerns selection of urokinase dosage and treatment duration. The number of patients treated with urokinase was so small that neither assessment of overall treatment efficacy nor response between urokinase dosages used could be made. Moreover, the generally severe degree of neurological deficit in our patients on hospitalization essentially precluded observation of treatment efficacy in preventing deterioration during the hospital course. This was unfortunate, since prevention of deterioration in patients who enter the hospital with a relatively mild degree of neurological deficit and who subsequently deteriorate (approximately 10% to 20% of total admissions) remains a promising area for therapeutic trial.

This study suggests that if further trials of urokinase therapy in cerebral infarction are undertaken, the use of 1,200 CTA units per pound of body weight per hour with an appropriate loading dose for a 10-hour to 20-hour period could be used without other than minimal monitoring of coagulation function. Such dosage produces a substantial increase of plasma thrombolytic activity without danger of producing a significant degree of blood coagulation deficit. Higher urokinase dosage, for instance 1,500 CTA units per pound of body weight, could probably be used with only minor increase in hazard. Both urokinase regimens reduce

\textsuperscript{14}

\textsuperscript{15}

\textsuperscript{16}

\textsuperscript{17}

\textsuperscript{18}
plasma HMWFC from the pathologically raised level seen in the conventionally treated patients with cerebral infarction to within essentially the limits of normal. The treatment substantially reduces pathologically raised blood viscosity during the first four critical days after the ictus, a time when serious deterioration may develop in cerebral infarction patients whose original degree of neurological deficit was mild.\[15\]

The following paper demonstrates that in spite of the relatively slight impairment of coagulation mechanisms produced by urokinase in the dosage range we used, there is a significant risk of cerebral hemorrhage complication in this patient group. We are convinced that these cases cannot entirely represent misdiagnosed and inappropriately treated patients with primary cerebral hemorrhage. Our early experience with pretreatment carotid arteriography persuaded us that this procedure carries an unacceptable risk. However, we believe that the advantage of early definitive diagnosis of occlusive disease may be worth the risk of local complications the cerebral vasculature exposed to reflow arterial pressure. The discouraging fact that none of our patients showed dramatic early improvement may indicate not that urokinase is an inappropriate therapeutic approach, but rather that ischemic brain necrosis was already irreversible in our patient population. We suspect that the cerebral hemorrhagic complication risk is not due to systemic affection of coagulation mechanisms, but rather to ischemic damage of the cerebral vasculature exposed to reflow arterial pressure. The risk, therefore, would be minimized only by treating patients earlier, in the first few hours after the ictus.

We are hopeful that the concept of clot dissolution for treatment of occlusive cerebrovascular disease will not be abandoned and further, that our experience may facilitate the development of a more effective therapeutic testing protocol. Selected patients and randomly chosen controls should be clearly incomplete potential stroke-in-evolution. Treatment should be considered only in those patients who reach the hospital and have completed diagnostic evaluations within a very few hours of the ictus. The latter requirement necessitates a highly organized team operation to provide immediate identification of candidate patients followed by emergency radiographical and laboratory studies. The possibility remains that such patients may benefit significantly from urokinase treatment.

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