Pro-urokinase promotes angiogenesis but does not reduce neuronal apoptosis in infarcted cerebral tissue

Ischemic stroke is most commonly caused by vascular occlusion due to thrombosis or arterial embolism. Recently, thrombolysis has been used with increasing frequency for the treatment of acute ischemic stroke. Among the drugs used for thrombolysis, only recombinant tissue plasminogen activator is widely accepted internationally (Albers et al., 2008). In China, urokinase has been widely used for thrombolysis after acute ischemic stroke. Pro-urokinase is the precursor of urokinase. Compared with urokinase, pro-urokinase has greater ability to dissolve thrombus and is safer to use. A previous study found that the recanalization rate was significantly higher after arterio-venous thrombolysis with pro-urokinase than with recombinant tissue plasminogen activator (Fischer et al., 2005). This study compared the thrombolytic effects of pro-urokinase, recombinant tissue plasminogen activator, and urokinase in a dog model of acute cerebral embolism.

For each dog used, 10 mL of arterial blood was taken and placed at room temperature for 3 hours to allow natural consolidation. Blood clots were then pressed into a 2-mm diameter cylinder and cut into 2–3 mm lengths. After general anesthesia, dogs were fixed on an operating table and the trachea was intubated. A 3F sheathing canal was placed in the right femoral artery using a modified Seldinger method (Mars et al., 1996). The sheathing canal was advanced into the internal carotid artery guided by digital subtraction angiography. Vascular traveling to the anterior and middle cerebral arteries was observed in the anteroposterior view (Figure 1A–C). A mixture of autologous blood clots and physiological saline was injected into the internal carotid artery using a 5 mL syringe. If no embolization was observed on digital subtraction angiography after the first injection of blood clots, the process was repeated (Takano et al., 1998; Oureishi et al., 2004; Harris et al., 2007). When anterior or middle cerebral artery embolization was confirmed, the sheathing canal was withdrawn. Digital subtraction angiography was repeated every 30 minutes for 3 hours to confirm embolization (Figure 1D–F).

Stroke was successfully induced in 24 dogs. These 24 dogs were randomly divided into four groups: (1) Pro-urokinase group: 1.2 × 10^6 U/kg pro-urokinase was administered via the femoral vein. One-third of the pro-urokinase was dissolved in physiological saline and administered over 3 minutes, and the remainder was dissolved in 100 mL of physiological saline and administered over 30 minutes. (2) Recombinant tissue plasminogen activator group: 1.37 mg/kg of recombinant tissue plasminogen activator was administered via the femoral vein. One-tenth of the recombinant tissue plasminogen activator was administered over 1 minute, and the remainder was administered over 60 minutes. (3) Urokinase group: 2.15 × 10^6 U/kg urokinase was dissolved in 100 mL of physiological saline and administered by intravenous infusion over 30 minutes. Digital subtraction angiography was performed every 30 minutes for 3 hours after thrombolysis.

Based on assessment of the Thrombolysis In Myocardial Infarction flow grade, the recanalization rate was higher in the urokinase group than in the model group (Table 1, Figure 1G–U). Hematoxylin and eosin staining showed no hematoma in the infarcted area at 3 hours after thrombolysis in any of the groups, but nerve cells in the infarcted tissues showed degeneration, coagulative necrosis, vacuole-like structures, indistinct cell borders, and pyknotic or absent nuclei. In addition, the nerve cells and glial cells were obviously reduced in number or even absent. Infiltration of neutrophilic leukocytes and microglial proliferation or phagocytosis were observed in some regions. There were no obvious differences in cell apoptosis among the groups (Figure 2A–E). Hemorrhage was observed in the infarcted area in one dog from each of the pro-urokinase and urokinase groups (Figure 2F–H). Previous studies reported that patients who underwent thrombolysis over 3 hours had a high incidence of hemorrhage (Cameringo et al., 2005). Obvious hematoma was not observed in this dog model of stroke because dogs have abundant collateral cerebrovascular circulation, resulting in a limited area of infarction, and thrombolysis was performed early. The results of this study show that recanalization after thromboembolism was similar after thrombolysis with pro-urokinase and recombinant tissue plasminogen activator, and that both these drugs were more effective than urokinase (both P < 0.05). However, pro-urokinase and recombinant tissue plasminogen activator did not have any definite protective effects against neuronal injury.

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Table 1: Effectiveness of pro-urokinase, recombinant tissue plasminogen activator, and urokinase for the treatment of acute cerebral embolism

| Group                          | ACA+MCA Recanalization rate [n(%)] | ACA MCA Distribution of occluded vessels before treatment (n) |
|-------------------------------|-----------------------------------|--------------------------------------------------------|
| Pro-urokinase                 | 3                                  | 1 2                                                    |
| Recombinant tissue plasminogen activator | 3                                  | 2 1                                                    |
| Urokinase                     | 3                                  | 0 3                                                    |
| Model                         | 3                                  | 0 3                                                    |

*P < 0.05, vs. pro-urokinase group
Figure 1 Digital subtraction angiography images before and after treatment of acute cerebral embolism in dogs using pro-urokinase, recombinant tissue plasminogen activator, or urokinase.

(A–C) Observation of anterior and middle cerebral artery traveling (anteroposterior view) to confirm cerebral embolization. (D–F) Confirmation of embolism in the anterior cerebral artery (D), middle cerebral artery (E), and internal carotid artery (F) 3 hours after injection of autologous blood clots. (G–L) Pro-urokinase group. Before embolization, traveling in the middle and anterior cerebral arteries and blood flow in the left cerebral hemisphere were normal (G). After injection of blood clots, the middle cerebral artery was occluded (H). At 2 hours after thrombolysis, the middle cerebral artery was recanalized (I). Before embolization, traveling in the middle and anterior cerebral arteries and blood flow in the left cerebral hemisphere were normal (J). After injection of blood clots, the middle cerebral artery was occluded (K). At 1.5 hours after thrombolysis, the anterior and middle cerebral arteries were recanalized (L). (M–O) Recombinant tissue plasminogen activator group. Before embolization, traveling in the middle and anterior cerebral arteries and blood flow in the left cerebral hemisphere were normal (M). After injection of blood clots, the anterior cerebral artery was occluded (N). At 2 hours after thrombolysis, the anterior cerebral artery was recanalized (O). (P–R) Urokinase group. Before embolization, traveling in the middle and anterior cerebral arteries and blood flow in the left cerebral hemisphere were normal (P). After injection of blood clots, the anterior cerebral artery was occluded (Q). At 3 hours after thrombolysis, the anterior and middle cerebral arteries were not recanalized (R). (S–U) Model group. Before embolization, traveling in the middle and anterior cerebral arteries and blood flow in the right cerebral hemisphere were normal (S). After injection of blood clots, the anterior and middle cerebral arteries were occluded (T). At 3 hours after thrombolysis, the anterior and middle cerebral arteries were not recanalized (U). 1: Aortic arch; 2: common carotid artery; 3: vertebral artery; 4: internal carotid artery; 5: anterior cerebral artery; 6: middle cerebral artery.

Figure 2 Histological findings in the area of cerebral infarction at 3 hours after thrombolysis (hematoxylin and eosin staining).

Thrombus was visible in the cerebral arteries (arrows; A: × 40, B: × 100). The infarcted area included cells with vacuole-like structures (C, D: × 100), neuronal degeneration with pyknotic or absent nuclei (E, × 100), and scattered hemorrhage (F–H, arrows, × 100).