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Management of delayed edema formation after fibrinolytic therapy for intracerebral hematomas: preliminary experimental data

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Summary

Objective. Fibrinolytic therapy for spontaneous intracerebral hemorrhage using recombinant tissue plasminogen activator (rtPA) is considered a viable alternative to microsurgical hematoma removal. However, experimental data suggest that rtPA is neurotoxic and evokes a late perihematomal edema. We present preliminary data focusing on the avoidance of late edema formation after lysis of an intracerebral hematoma in a porcine model.

Methods. Twenty pigs underwent placement of a frontal intracerebral hematoma with a minimum volume of 1 mL. Half of the pigs were subjected to rtPA clot lysis and MK-801 injection for blockage of the NMDA receptor-mediated rtPA-enhanced excitotoxic pathway. The remaining 10 pigs received desmoteplase (DSPA) for clot lysis, which is known to be a less neurotoxic fibrinolytic agent than rtPA. MRI on the day of surgery and on postoperative days 4 and 10 was used to assess hematoma and edema volumes.

Results. Late edema formation could be prevented in both the MK-801/rtPA and DSPA pigs.

Conclusion. The benefits of fibrinolytic therapy for intracerebral hematomas appear to be counterbalanced by late edema formation. MK-801 infusion as an adjunct to rtPA lysis, or the use of DSPA instead of rtPA, prevents late edema and therefore has the potential to further improve results after clot lysis.

Keywords: Intracerebral hemorrhage; fibrinolysis; tissue plasminogen activator; desmoteplase; excitotoxicity.

Introduction

With mortality rates between 27 and 70%, spontaneous supratentorial intracerebral hemorrhage (ICH) carries the worst prognosis of the 3 types of stroke. Several randomized prospective trials have shown that craniotomy and microsurgical clot removal fail to improve the poor prognosis [4, 12, 13, 27]. It had been assumed that surgical trauma contributed to the dismal results, which led to the development of minimally-invasive techniques, especially for frame-based and frameless stereotaxy, hematoma puncture, and subsequent fibrinolytic therapy with recombinant tissue plasminogen activator (rtPA) [9, 17, 19, 21]. However, recent data suggest that rtPA might be neurotoxic in the presence of an intracerebral hematoma. In our own experiments, fibrinolytic therapy in experimental ICH resulted in delayed perifocal edema [18]. Delayed edema is hypothesized to be caused by promotion of excitotoxic pathways initiated by perihematomal ischemia, or a reduction in the activity of protease nexin-1 (PN-1) and plasminogen-activator inhibitor-1 (PAI-1), which inhibit both rtPA and edema-evoking thrombin. The purpose of our investigation was to elucidate the pathways that might be responsible for the delayed edema and to propose potential therapeutic alternatives.

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Materials and methods

Animal preparation

The protocol for pig intracranial hemorrhage has been described in detail [18]. Briefly, 20 male pigs weighing 30–35 kg were sedated with a 1:6 mixture of atropine (0.3–0.5 mg/kg) and azaperone (7–10 mg/kg). The animals were further anesthetized with pentobarbital (15–20 mg/kg) administered via a line in an ear vein. After endotracheal intubation, the pigs were mechanically ventilated and vital parameters kept at physiological levels. The hematoma was induced under intracranial pressure control by injecting autologous venous blood into a preformed right frontal cavity created by balloon dilatation.

After hematoma induction, magnetic resonance imaging (MRI) scans were performed on a 1.5T system on all animals. T2-weighted fluid attenuated inversion recovery (FLAIR) turbo spin-echo images were used to quantitate edema and $T2^*$-weighted gradient echo images were acquired for hematoma measurement. Pigs with a hematoma size of less

than 1 cm^3 on MRI were excluded. The scans were repeated on days 4 and 10. Our previous studies have shown that changes in FLAIR intensity correlate with areas of perihematomal edema and inflammatory infiltration [20].

Experimental groups

Ten animals underwent fibrinolytic therapy of the hematoma with rtPA (Actilyse, Thomae GmbH, Biberach, Germany) administered via the Rickham reservoir directly following initial MR imaging. The amount of rtPA given in milligrams was equal to the maximum diameter of the hematoma measured in centimeters on T2*-weighted gradient echo sequences [19]. In the remaining 10 pigs, desmoteplase (DSPA) (Paion, Aachen, Germany), in a dosage equivalent to the lytic properties of rtPA, was used as the fibrinolytic agent.

In the 10 rtPA pigs, the NMDA receptor-antagonist MK-801 (M107, Sigma-Aldrich, Germany), which inhibits the excitotoxic pathway, was administered intravenously at a dosage of 0.3 mg/kg body weight immediately after hematoma induction (before the first MRI) and then again at 24 h and 72 h postoperatively.

Statistical analysis

All values are presented as mean ± standard deviation. For comparison of hematoma and edema volume changes during the study period, the 1-tailed paired *t*-test was used. Differences were considered significant at probability values of less than 0.05.

Results

MK-801/rtPA pigs

The mean hematoma size in the 10 animals measured $1.29 \pm 0.26\,cm^3$ and decreased to $0.57 \pm 0.34\,cm^3$ on day 4 ($p < 0.001$) and to $0.37 \pm 0.25\,cm^3$ on day 10 ($p < 0.001$), respectively. The mean edema volume on FLAIR images measured $0.73 \pm 0.54\,cm^3$ directly after surgery and increased to $1.29 \pm 0.76\,cm^3$ on day 4 ($p < 0.08$) and $1.29 \pm 1.76\,cm^3$ on day 10 ($p < 0.35$).

DSPA pigs

The mean hematoma size in the 10 animals was $1.21 \pm 0.34\,cm^3$. DSPA lysis reduced the hematoma volume to $0.55 \pm 0.31\,cm^3$ on day 4 ($p < 0.001$) and to $0.30 \pm 0.15\,cm^3$ on day 10 ($p < 0.001$). The mean edema volume on the day of surgery was $0.63 \pm 0.28\,cm^3$ and increased to $1.44 \pm 0.52\,cm^3$ on day 4 ($p < 0.001$), but dropped again to $0.94 \pm 1.4\,cm^3$ on day 10 ($p < 0.24$).

Comparison of MK-801/rtPA and DSPA pigs

The initial hematoma volume in both groups of pigs was comparable. Furthermore, the fibrinolytic effect of rtPA and DSPA was equivalent, with almost similar reductions in the initial hematoma volume on postoperative days 4 and 10. The edema volume in both the MK-801 and DSPA groups increased until day 4. Then, the edema volume remained stable in the MK-801 group but dropped in the DSPA group, as seen on day 10.

Discussion

The optimal therapy for spontaneous intracerebral hemorrhage has yet to be determined. The few prospective randomized trials comparing microsurgical clot removal with conventional medical therapy failed to support surgery. Even when surgery is performed, mortality rates are as high as 40–67% [4, 12]. Frame-based stereotactic or neuronavigationally-guided hematoma puncture and subsequent clot lysis with tPA has been proposed as a therapeutic alternative. In several prospective clinical series, promising results have been published, with mortality rates between 10 and 25% [9, 17, 19]. These good results have been attributed to the less-invasive nature of the procedure, diminishing operative trauma to the brain overlying the hematoma.

Studies on fibrinolytic therapy in experimental intracerebral hematoma are rare. Wagner and coworkers demonstrated that fibrinolysis with rtPA and subsequent aspiration of lysed clot reduces the amount of the perifocal edema in a pig model on postoperative day 1 [24]. Using a similar porcine model, our studies confirm the positive effect of clot lysis on diminishing early edema, and demonstrate increased hemorrhage site edema on day 10. This delayed edema was significantly larger than that seen in an untreated control group [18].

rtPA neurotoxicity

Two hypothetical pathways may account for the increase in delayed edema. 1) Experimental studies of focal cerebral ischemia using tPA knockout and wild-type mice showed that tPA induces neuronal death by enhancing the excitotoxic pathway [25]. Similar findings were reported in mice experiments using unilateral intrahippocampal injection of kainic acid [22, 23]. The NMDA receptor plays a dominant role in this excitotoxic pathway. tPA amplifies the NMDA-induced increase in intracellular calcium concentration and potentially provokes cell death by cleavage of a fragment from the NR1 subunit of the NMDA receptor [11, 15]. 2) Blood degradation products, especially thrombin, are well known factors contributing to the development of perihematomal edema [3, 6, 7, 14, 26]. Thrombin is inhibited by PN-1 and PAI-1. Figueroa and coworkers showed that addition of rtPA attenuates the inhibition of thrombin by competing for PN-1 and PAI-1 [2]. It seems possible that

the relative increase in thrombin concentration causes delayed perihematomal edema.

Anti-neurotoxic options

Prior to our current study there was little evidence that rtPA mediated up regulation of excitotoxic pathways or that attenuation of the PN-1/PAI-1-induced inhibition of thrombin contributed to the development of delayed edema in successfully lysed experimental hematomas. Our recent experiments clearly indicate that at least the first pathway is involved in late edema formation. MK-801 is a non-competitive blocker of the NMDA receptor and, therefore, of the excitotoxic pathway, as proven by reduction of the infarct size in experiments on focal ischemia. Similarly, NMDA receptor blockade attenuates the neurotoxic effect of external tPA with inhibition of calcium overload [1, 5]. In our previous series [18], the mean edema volume on day 10 was $3.33\,cm^3 \pm 3.2\,cm^3$ in rtPA pigs, but only $1.29 \pm 1.76\,cm^3$ in rtPA and MK-801 treated pigs, despite comparable initial hematoma volumes and fibrinolytic effects. This significant reduction in edema volume by MK-801 suggests that activation of the excitotoxic pathway by exogenous tPA plays a substantial role in delayed edema formation.

DSPA is an alternative lytic agent derived from the saliva of the blood-feeding vampire bat. In 2 models of neurodegeneration, DSPA was found not to promote kainite- or NMDA-mediated neurotoxicity [8, 10, 16]. Our study demonstrates once again that DSPA as a fibrinolytic agent is not neurotoxic, even if given in an extravasated fashion. Delayed edema volume in the DSPA pigs, at $0.94\,cm^3$, was lower than that on day 4, while in rtPA pigs the highest edema volume was observed on day 10.

We have preliminary evidence that PN-1/PAI-1-induced inhibition of thrombin additionally contributes to the development of delayed edema in successfully lysed experimental hematomas; PAI-1 injection after induction of the hematoma and rtPA clot lysis reduces delayed edema formation while only mildly reducing the lytic potential of rtPA (Samadani and Rohde, unpublished data).

Conclusion

Our work demonstrates that MK-801 may be a useful adjunct to diminish delayed edema after rtPA clot lysis. Additionally, DSPA fibrinolysis, when used in lieu of rtPA, may have a similar effect. These agents have the potential to further improve outcomes after stereotactic hematoma puncture and fibrinolytic therapy by blockage or avoidance of the neurotoxic properties of the injected rtPA. These data are preliminary and further investigations are necessary, as the effects of MK-801 and DSPA have not been compared with a direct, but with a previous control group. PAI-1 injection after clot lysis with rtPA offers a third modality for controlling delayed edema formation.

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