Thus, it remains a need to develop new formulations that limit the side effects of plasminogen activator while improving its efficacy on thrombolysis.

SUMMARY ~~OF THE INVENTION~~

The inventors surprisingly showed that sustained release of plasminogen activator in the core of a haematoma allows a better thrombolytic efficacy (reduction of the clot weight) than repeated injections of the same quantity of the gold standard tissue plasminogen activator (rtPA).

Thus, they investigated a methodology to prepare plasminogen activator compositions from high concentration stock solution to allow slow release over a few hours in order to reduce the number of injection as well as the time needed to achieve a substantial reduction of the haematoma.

To do so, a new brain-compatible formulation has been developed with plasminogen activator nanoparticles and thermoreversible polymer in order to allow high concentration loading of plasminogen activator and a release time of less than 24 hours while maintaining plasminogen activator efficacy.

In one aspect, ~~T~~the present disclosure relates to a composition comprising a thermoreversible polymer and a nanoparticle comprising a plasminogen activator. In another aspect, the present disclosure relates to use of the composition as described herein in the manufacture of a medicament for treating thrombotic or haemorrhagic disease.

In a particular ~~embodiment~~example, said nanoparticle comprises a poloxamer, preferably selected from the group consisting of: 188, 338 and 237, more preferably poloxamer 188.

In another particular ~~embodiment~~example, said thermoreversible polymer is a poloxamer, preferably a poloxamer 407. Said poloxamer 407 is preferably at a concentration between 15 to 25% (w/v), more preferably between 17 and 23% (w/v), again more preferably 17% (w/v).

In a particular ~~embodiment~~example, said plasminogen activator is selected from the group consisting of Alteplase, Tenecteplase, Pamiteplase, Monteplase, lanoteplase, reteplase, desmoteplase, urokinase or streptokinase. In another particular embodiment, said plasminogen activator is a mutated plasminogen activator, preferably W253R/R275S mutant plasminogen activator, more preferably W253R/R275S mutant plasminogen activator consisting of the sequence SEQ ID NO: 1.

In another ~~aspect~~example, the present disclosure relates to said composition for use as medicament, preferably for treating thrombotic or haemorrhagic disease. Said thrombotic or haemorrhagic disease is selected from the group consisting of: thrombotic or embolic ischemia, artery or vein occlusions, deep haematoma, cerebral haemorrhages or haematoma and ocular haemorrhages or haematoma, preferably from intra-parenchymatous haemorrhages or haematoma, intra-ventricular haemorrhages or haematoma, cerebral subarachnoid haemorrhages or haematoma, age related macular degeneration, central retinal occlusion, vitreous haemorrhages, any deep haematoma traumatic or not, any post-surgical haematoma including cerebral haematoma or following intervention for cancer, more preferably cerebral haemorrhages or haematoma such as intra-parenchymatous, intra-ventricular and subarachnoid haemorrhages or haematoma.

In a further aspect, the present disclosure also relates to a method of preparing a composition comprising a thermoreversible polymer and a plasminogen activator-poloxamer nanoparticle, said method comprising the steps of: i) preparing an aqueous solution comprising a plasminogen activator and a poloxamer, ii) contacting the obtained solution with a protein precipitation solvent in a sufficient amount to precipitate plasminogen activator in combination with a poloxamer to form a plasminogen activator-poloxamer nanoparticle, and iii) adding said nanoparticle in a thermoreversible polymer.

In a last aspect, the present disclosure relates to a nanoparticle comprising a plasminogen activator in combination with a poloxamer, preferably poloxamer 188.

FIGURE LEGENDS

Figure 1: Amidolytic and fibrinolytic characterization of OptPA vs rtPA. A) Mean amidolytic activity of rtPA and OptPA (W253R/R275S tPA) toward spectrozyme® rtPA chromogenic substrate, measured in the range of concentration from 0 to 200 ng per well and in absence of fibrin (n=4). B) Detail of the amidolytic activity of OptPA and rtPA at the different concentrations tested. Amidolytic activity is expressed as the maximal velocity (optical density per second per µg) (10 concentrations tested, n=4 per concentration); C) Maximal turbidity assessed by optical density at 405 nanometers for each concentration of OptPA and rtPA tested (n=5); D) Fibrinolytic activity measured as the time to reach 50 per cent lysis with OptPA or rtPA in a whole plasma clot lysis assay (pool of human plasma,

CLAIM

1. A composition comprising a thermoreversible polymer and a nanoparticle comprising a plasminogen activator.

2. The composition of claim 1 wherein said nanoparticle comprises a poloxamer.

3. The composition of claim 2 wherein said poloxamer is selected from the group consisting of: poloxamer 188, 338 and 237.

4. The composition of claim 3 wherein said poloxamer is poloxamer 188.

5. The composition according to any one of claims 1 to 4 wherein said thermoreversible polymer is a poloxamer.

6. The composition of claim 5 wherein said thermoreversible poloxamer is poloxamer 407.

7. The composition of claim 6 wherein said poloxamer 407 is at a concentration between 15 to 25% (w/v), preferably between 17 and 23% (w/v), preferably 17% (w/v).

8. The composition according to any one of claims 1 to 7 wherein said plasminogen activator is selected from the group consisting of: ~~rtPA, -~~alteplase, tenecteplase, pamiteplase, monteplase, lanoteplase, reteplase, desmoteplase, urokinase, and streptokinase.

9. The composition according to any one of claims 1 to 7 wherein said plasminogen activator is a double mutant W253R and R275S tPA, preferably consisting of the sequence SEQ ID NO: 1.

~~10. The composition according to any one of claims 1 to 9 for use as a medicament.~~

~~11.~~10. Use of ~~T~~the composition according to any one of claims 1 to 9 in the manufacture of a medicament ~~for use~~ for treating thrombotic or haemorrhagic disease.

~~12.~~11. The ~~composition for~~ use of claim ~~11~~10, wherein the ~~for treating a~~ thrombotic or haemorrhagic disease is selected from the group consisting of: thrombotic or embolic ischemia, artery or vein occlusions, deep haematoma, cerebral haemorrhages or haematoma and ocular haemorrhages or haematoma, preferably from intra-parenchymatous haemorrhages or haematoma, intra-ventricular haemorrhages or haematoma, subarachnoid haemorrhages or haematoma, age related macular degeneration, central retinal occlusion, and vitreous haemorrhages, any deep haematoma traumatic or not, and any post-surgical haematoma including intracerebral or following intervention for cancer.

~~13.~~12. The ~~composition for~~ use of claim ~~12~~ 11 for treating cerebral haemorrhages or haematoma such as intra-parenchymatous haemorrhages or haematoma, intra-ventricular haemorrhages or haematoma ~~and~~ or subarachnoid haemorrhages or haematoma.

~~14.~~13. A method for preparing a composition comprising a thermoreversible polymer and a plasminogen activator-poloxamer nanoparticle said method comprising the steps of:

i) Preparing an aqueous solution comprising a plasminogen activator and a poloxamer,

ii) Contacting the obtained solution with a protein precipitation solvent in a sufficient amount to precipitate plasminogen activator in combination with a poloxamer to form a plasminogen activator-poloxamer nanoparticle, and

iii) Adding said nanoparticles in a thermoreversible polymer.

~~15.~~14. A nanoparticle comprising a plasminogen activator precipitated in combination with a poloxamer, preferably poloxamer 188.