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Expression of reteplase by a non-viral insect cell expression system

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Background and Aims: Thrombolysis is the first choice of therapy for acute myocardial infarction (AMI) and reteplase is a thrombolytic agent often used for the treatment of acute myocardial infarction. In the present study we evaluated the expression of reteplase by a non-viral insect cell expression system.
Methods: Coding sequence of the reteplase was cloned into the pMIB/V5-His plasmid. The recombinant plasmid was used to transfect Sf9 insect cells. Stable cell lines were produced at the presence of 80µg/ml Blasticidin S HCl. Finally, the stable cells were screened for the expression of the reteplase protein by dot-blot and Western blot analysis using HRP conjugated anti His-tag and anti-V5 tag antibodies.
Results: DNA sequencing confirmed the fidelity of the cloned sequence. Screening of the transfected cells using Blasticidin S HCl resulted in some stable clones. Some producing cell lines were selected following dot-blot analysis of the concentrated culture medium of the stable cells. The producer stable cells were propagated into larger cell culture monolayer. Western blot analysis confirmed the expression of the reteplase protein by showing bands of about 45 kD.
Conclusions: These results confirmed the ability of the non-viral insect cell expression system to produce reteplase. Therefore, the obtained stable cell lines could be used for further expression of the recombinant protein in order to evaluate its biological activity and also for large scale protein production.

Keywords: Reteplase; Thrombolysis; Insect cell expression system; pMIB/V5-His