ASSESSMENT OF DOSE EFFECT AND THERAPEUTIC TIME WINDOW IN PRECLINICAL STUDIES OF EGF AND GHRP-6 CO-ADMINISTRATION FOR STROKE THERAPY

Authors: **Nelvys Subirós**, Héctor Pérez-Saad, Jorge Berlanga, Yaima Rodríguez, Sasha Sánchez, Lizet Aldana, Gerardo García-Illera, Diana García-del-Barco

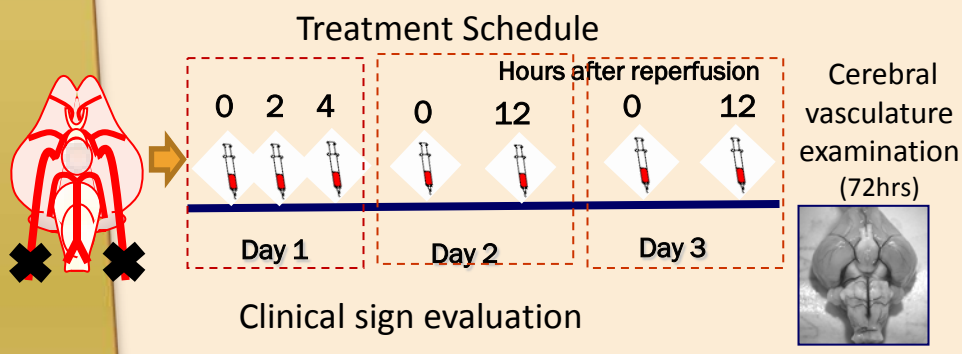
Neuroprotection
Varadero, 2015

Introduction

Stroke continues to be a leading cause of mortality and morbidity worldwide and novel therapeutic options for ischemic stroke are urgently needed. In this context, drug combination therapies seem to be a viable approach which has not been fully explored in preclinical studies.
Objectives: In this work, we assessed the dose response relationship and therapeutic time window, in a global brain ischemia model, of a combined therapeutic approach of Epidermal Growth Factor (EGF) and Growth Hormone releasing Peptide-6 (GHRP-6).

Methods

Mongolian gerbils underwent 15 minutes occlusion of both common carotid arteries. Four different doses of EGF, GHRP-6 and combined agents were intraperitoneally administered immediately after the onset of reperfusion. EGF and GHRP-6 were administered independently and in combination. For GHRP-6 alone, the doses of 200, 600, 1000 and 1800 µg/kg were used. For EGF alone the doses of 50, 100, 200 and 600 µg/kg were examined. Four combinations of doses were studied: 25 µg/kg + 600 µg/kg, 50 µg/kg + 600 µg/kg; 100 µg/kg + 600 µg/kg, and 100 + 400 µg/kg of EGF and GHRP-6 respectively (n=20 for each experimental group, except for the sham operated group with n=10).
Animals were evaluated daily for neurological deficits. The neurological score assigned to each animal was based on the evaluation of palpebral ptosis, grip strength and flexor reflex, body posture, and gait pattern (including speed and circling).
Infarct volume was assessed by 0.5% TTC staining of 2mm-thick coronal slices of , at three days post-occlusion.



To assess the time window of effectiveness, the onset of treatment for each group (n = 20/group) was delayed to 0, 2, 4, 6, 8 and 24 hours following the onset of reperfusion.
The therapeutic time window of effectiveness was assessed for the combination dosages of 600µg/kg of GHRP-6 and 100µg/kg of EGF.

Results

In this study, no effect on the established endpoints could be detected for the components given independently. However, a dose-effect relationship was shown for both functional (Fig. 1) and pathological (Fig. 2) endpoints when EGF and GHRP-6 were given as a combined therapy. A therapeutic window of 4 hours was demonstrated.

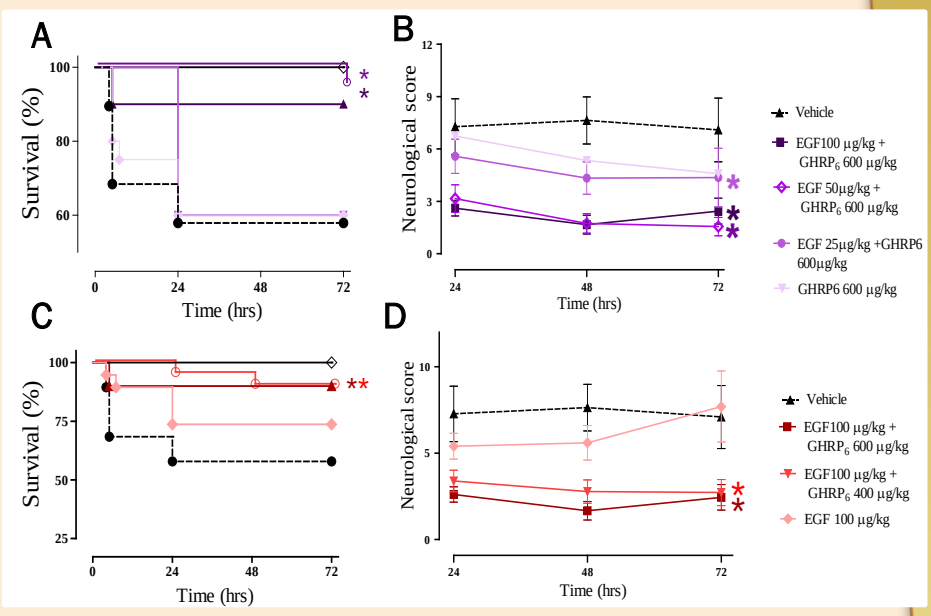


Fig. 1 Survival and neurological score of combined dosages of EGF and GHRP-6, varying the EGF dose (A,B), or the GHRP-6 dose (C,D). *: p<0.05 compared to the vehicle treated group.

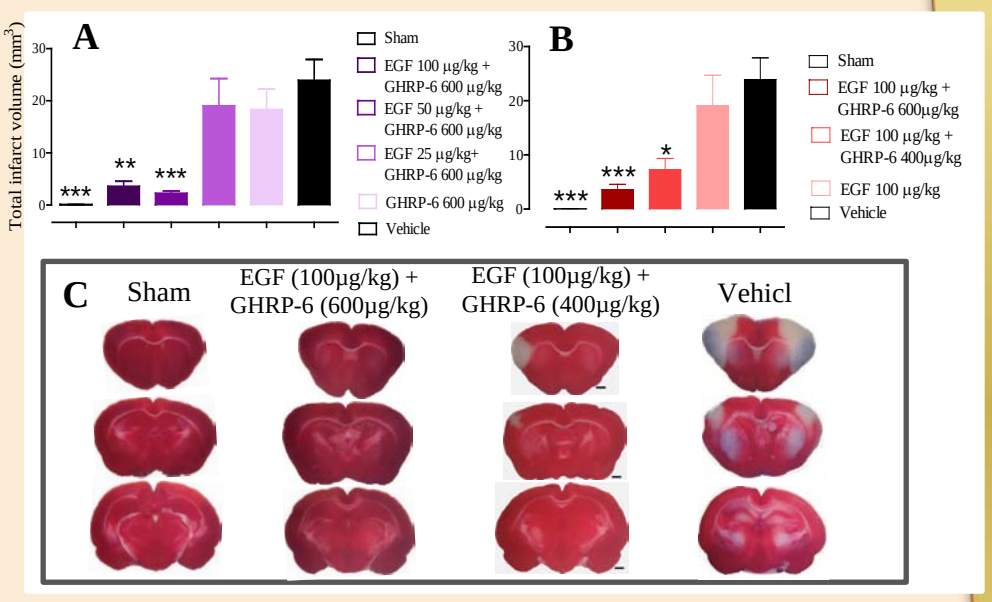


Fig. 2 Infarct volume of combined dosages of EGF (A) and GHRP-6 (B). (C) Representative TTC images *: p<0.05 compared to the vehicle treated group.

Conclusions

These results are considered as an additional evidence supporting a combined therapeutic approach and justify further development of this preclinical research.

CENTER FOR GENETIC ENGINEERING AND BIOTECHNOLOGY
Havana, Cuba http://www.cigb.edu.cu http://biomed.cigb.edu.cu