REPORT

Cardiac and extracardiac cytoprotective effects of GHRP6 peptide

✉ Danay Cibrián[1], Jorge Berlanga[1], Luis Guevara[2], Juan Valiente[3], Gerardo E Guillén[1], Diana García[1], Olga Sonia[4], Viviana Falcón[1], José Suárez[1]

[1]Pharmaceuticals Division, Center for Genetic Engineering and Biotechnology (CIGB) Ave. 31 e/ 158 y 190, Cubanacán, Playa, POBox 6162, CP 10600, Ciudad de La Habana, Cuba
E-mail:danay.cibrian@cigb.edu.cu
[2]Department of Cardiovascular Surgery, Hermanos Ameijeiras Hospital, Cuba
[3]Institute of Cardiology and Cardiovascular Surgery, Cuba
[4]Institute of Pharmacy and Foods, University of Havana

Introduction

The growth hormone-releasing peptide 6 (GHRP6) is a synthetic, six-aminoacid peptide, with the sequence His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2. It was originally described as an intestinal Met-enkephalin-derived agent that strongly stimulates the growth hormone release in different mammalian species, also in humans [1, 2]. The GHRP6 and its synthetic analogs are currently used for the clinical diagnosis of different forms of dwarfism [3]. Up to date, its described cytoprotective effects were related to the prevention of cardiac ischemic dysfunction [4-6] and to its hormonal axis-mediated neuroprotective actions [7]. However, current knowledge on GHRP6 and analogous peptides points towards different target structures and different intracellular signaling pathways, and their specific combination depending on the biological context where the given peptide is evaluated [8]. This is the only plausible explanation for the versatile pharmacological effects recently described for these molecules, including the present report.

The aim of the present work was to study the potential cytoprotective pharmacological effect of GHRP6 hexapeptide as an anti-necrotic and anti-apoptotic cell death agent in cardiac and extracardiac tissues subjected to different lethal insults. For this purpose, a series of versatile *in vivo* tissular damage models were implemented, induced by ischemia/reperfusion (I/R) episodes and the administration of a cytotoxic agent. In the case of I/R damage, the molecule was formerly evaluated for cytopro-tection in a provoked acute myocardial infarction (AMI) scenario in pigs, being further evaluated in a multiple organ damage (MOD) model started in rats by a prolonged I/R episode in the liver, and finally in a dilated myocardiopathy (DMC) model induced in rats by the prolonged administration of doxorubicin (DX). The main results obtained are briefly described in the following sections.

Effect of GRHP6 peptide administration to rescue ventricular mass in an AMI robust model

The effect of the GHRP6 hexapeptide was evaluated in a robust AMI model established by occlusion of the left circumflex artery in cuban Creole pigs. Reduction of infarct size was evaluated as the main parameter. Other aspects related to heart rhythm and electrical conduction disturbances, resulting from the I/R episode, were also studied. Treatment with GHRP6 reduced in almost 70% the infarction area and its mural expansion from the epicardium to the endocardium. On the contrary, treatment with GHRP6 influenced neither the heart rhythm, nor the electrical conduction in such condition. Hystopathological analyses of biopsies from adjacent zones to the infarct also showed that a higher number of myocardic fibers were preserved in treated animals, compared to untreated ones (Figure 1). A potent antioxidant effect was also detected, as part of the cardioprotective molecular mechanism [9].

Cytoprotective effects of the GHRP6 peptide in a MOD model induced by a liver I/R episode

The cytoprotective effects exerted by the GHRP6 peptide in the hepatic tissue subjected to I/R, as in others organs distant from the ischemic site (i.e., lungs, kidneys and small intestine), were evaluated [10]. Histological and biochemical results allowed us to conclude that the pharmacological preconditioning induced with the GHRP6 treatment attenuated the I/R liver damage. Besides, respiratory distress syndrome-

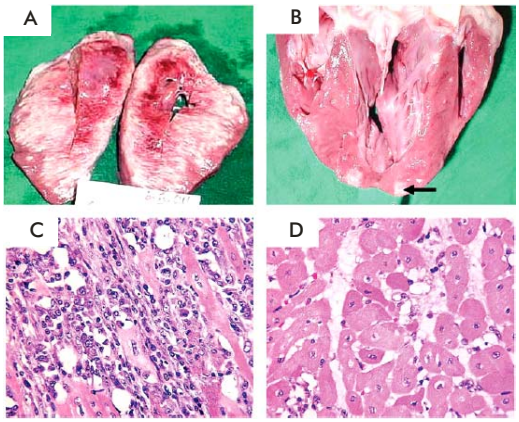

Figure 1. Effect of the GHRP6 on AMI size and severity. A,B- Macroscopic images of AMI size in animals treated with placebo (A) or GHRP6 (B). C,D- Histological images of the adjacent zone to the AMI necrotic core. Notice that cardiac fibers were mostly preserved and the inflammatory infiltration attenuated in animals from the group treated with GHRP6 (D), compared to animals treated with placebo (C).

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✉ Author of correspondence

like pulmonary changes, intestinal trans-mural infarct and acute tubular necrosis in kidneys were significantly reduced [11]. Those results indicated, for the first time, a systemic cytoprotective effect for the GHRP6, suggesting it as a potentially efficacious agent to control the inflammatory response associated to acute I/R and shock, which finally caused a MOD syndrome.

Cytoprotection induced by GHRP6 treatment was also related to the attenuated production of reactive oxygen species and preserved antioxidant defense systems (see Table 1). Histological analysis as the assessment of myeloperoxidase activity (a significant marker of inflammatory infiltration) evidenced a marked anti-inflammatory GHRP6-induced effect in the liver and other remote organs [11]. Moreover, the molecular mechanism mediating the action of GHRP6 peptide was shown to involve the phosphatidil inositol-3 kinase /protein kinase B (PI3K/PKB) and the hypoxia-induced factor 1 alpha (HIF-1α) intracellular signaling pathways, that promoting cell survival [12].

Cytoprotective effects of the GHRP6 peptide in a DMC model induced by doxorubicin administration in rats

The cardio and systemic cytoprotective effects of the GHRP6 peptide were studied in a DMC model in rats induced by DX administration. The concurrent administration of GHRP6, as part of a prolonged treatment with DX, completely prevented failure of cardiac function, evaluated as the percentage of ejection fraction by echocardiography (Figure 2A). This effect significantly increased the animals' survival. Similar results were obtained in the therapeutic administration schedule, with functional recovery of cardiac muscles to physiological levels (Figure 2B), also attenuating systemic damage and, consequently, decreasing mortality rates.

In the experimental model of DX-induced cardiac and systemic damage, GHRP6 additionally attenuated the extracardiac damage observed in the renal tubular and bronchoalveolar epithelia and in the hepatic parenchyma. Besides, GHRP6 favored the preservation of mitochondria structure and the attenuation of apoptosis in cardiac fibers. All these elements are part of the molecular mechanism of GHRP6 to preserve and restore cardiac function.

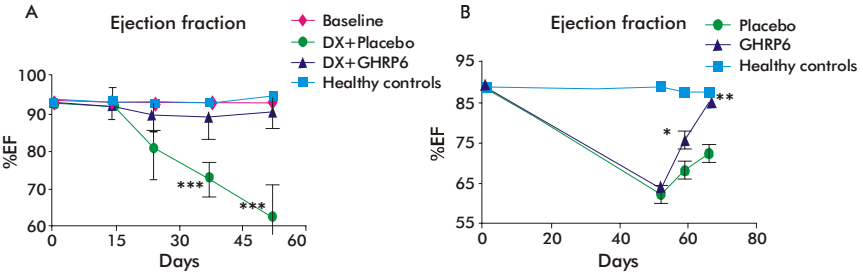

Figure 2. Echocardiography results in the DMC model. A- DMC prevention study, B- DMC reversion study. Data corresponding to percentage of ejection fraction (%EF) are represented as a mean value ± standard error of the mean for each experimental group. Asterisks (*) represent the statistically significant differences between groups treated either with placebo or GHRP6, detected by an unpaired Student´s t test.

Conclusions

Cytoprotection from damage induced by I/R episodes, or by toxicity of chemical agents, remains to be clinically addressed. Results obtained in these studies indicated that a GHRP6 treatment can potentially be used in relevant cardiovascular conditions such as AMI and DMC, which are important sources of morbidity and mortality throughout the world [13]. Our studies demonstrated that prophylactic administration of GHRP6 attenuates hypoxia damage in the liver, which is considered the main limitation for successful hepatic transplantation [14]. Additionally, GHRP6 attenuates the MOD syndrome resulting from the systemic inflammatory response during hepatic reperfusion, protecting remote organs that had not been previously tested such as: small intestine, lungs and kidneys. Moreover, GHRP6 positively modulates the redox cellular state, which is relevant for cytoprotection in both cardiac and hepatic pathological conditions. Finally, the GHRP6 favors cell survival mediated so far by the PI3K/PKB and HIF-1α pathways, apoptosis attenuation and preservation of mitochondria structure.

Table 1. Redox cellular state and inflammatory markers determinations

Biochemical parameters	Simulator group	Placebo+I/R Group	GHRP6+I/R Group
TH[a] (nmol/mg TP[b])	29.49 ± 1.24 (a)[c]	100.46 ± 6.46 (b)	51.16 ± 1.71 (c)
MDA[d] (nmol/mg TP)	0.06 ± 0.01 (a)	0.25 ± 0.03 (b)	0.12 ± 0.01 (a)
SOD[e] (U/g·min)	18261.08 ± 1260.94 (a)	9173.83 ± 645.93 (b)	18029.87± 498.28 (a)
CAT[f] (U/g·min)	16.33 ± 3.64 (a)	580.58 ± 57.39 (b)	31.50 ± 4.30 (a)
MPO-l[g] (U/g·min)	3.65 ± 0.76 (a)	75.58 ± 11.24 (b)	33.76 ± 2.32 (c)
MPO-si[h] (U/g·min)	11.27 ± 4.18 (a)	129.18 ± 37.98 (b)	55.12 ± 7.03 (c)

[a] TH-Total hydroperoxides

[b] Data reported per milligram of total proteins (TP)

[c] Data are reported as the mean value ± standard error for the mean, in each experimental group. The statistical analysis was carried out by One-way ANOVA followed by a Newman-Keul´s test. Different letters in parenthesis (a, b or c) indicate statistically significant differences between groups for $p<0.05$.

[d] MDA-Malondialdehyde

[e] SOD-Superoxide dismutase

[f] CAT-Catalase

[g] MPO-l- Liver myeloperoxidase, marker of inflammatory infiltration

[h] MPO-si- Small intestine myeloperoxidase, marker of inflammatory infiltration

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