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Radiolabeling of porcine, murine growth hormone and a potential antagonist G118R-mGH for biodistribution study

Nunes, A. P.[1]; Menezes, F.[1]; Almeida, L. A.[1]; Pomin, S. A.[1]; Falcão, P. L.[1]; Soares, C. R. J.[1].

[1] *crsoares@ipen.br, Centro de Biotecnologia,*
IPEN, São Paulo - SP

1. Introduction

Growth hormone (GH) is secreted by the anterior pituitary gland and is related to the regulation of several important physiological functions, such as cell proliferation, tissue growth, protein synthesis, carbohydrate and lipid metabolism, among others. Classically, the main target tissues of GH are the liver, skeletal muscle, bones and adipose tissue, which, as a whole, were believed to be responsible for the main biological effects of this hormone [1]. However, recent evidence has indicated that the central nervous system (CNS) is also a critical target tissue for many of GH's biological functions that include regulating metabolism, controlling diverse cognitive functions, modulating behaviors, neurotrophic action, among others [2].

The mechanism by which this hormone enters the CNS from the circulation has been clarified and seems to occur independently of the growth hormone receptor (GHr). Thus, the antagonistic effects of GH may represent a therapeutic strategy to reverse pathological conditions dependent on GH-GHr interaction in the CNS.

The development of the growth hormone receptor antagonist (GHA), with a glycine codon mutation (in amino acid 120), replaced by arginine (R), allows site 1 to bind to the growth hormone receptor (GHR), but prevents functional binding at site 2, preventing intracellular signaling. Thus emerged a competitive inhibitor of human growth hormone (G120R-hGH) and the development of the drug Pegvisomant, used to treat patients with acromegaly and in studies aimed at understanding the activity of GH [4].
However, this is an area not yet explored and lacking in previous studies, since in more in vivo studies it was noticed that the hGH antagonist (G120R) was ineffective in rodents [5] [3] [4], that is, it can not antagonize the action of growth promoter of GH, showed that it has an agonist effect in rodents with a possible prolactin receptor binding (PRLR) [5], which leads us to understand the importance of producing and working with homologous models of hormones and antagonists.

Studies using GH radiolabeling have been used over the years, in the main studies that sought to understand whether and how this hormone passes from circulation to various tissues, including in the CNS. The radiolabeling technique by cloramin T method developed by Greenwood & Hunter (1963)is the incorporation of radioactive iodine atoms by the molecule by electrolytic substitution and involves the iododeprotonation of aromatic rings rich in electrons of tyrosine residues [6].

Recently our research group carried out the synthesis, production and purification of murine growth hormone (mGH) and its antagonist (G118R-mGH), in order to be able to work with homologous hormone models. Thus, in the present work, we used the potential antagonist G118R-mGH to study its passage from circulation to the various tissues of Wild Type mice (WT), compared to mGH and porcine growth hormone (pGH)evaluating the ability of these proteins to remain available in tissues after a long period of time.

2. Methodology

2.1. Animals

In this study, we used C57BL/6J mice, acquired from The Jackson Laboratory (Bar Harbor, ME, USA) and knockout mice of the global GH receptor (GHRKO) of the C57BL/6J strain, donated by John Kopchick (Ohio University). The animals were kept in the vivarium of CEBIO in light/dark cycle of 12h/12h, controlled temperature of 22 1 C and feed for rodents at will. The project was approved by the CEUA of IPEN, number 20/22.

2.2. Radiolabeling with ^{131}I

The pGH, mGH and G118R-mGH were marked with $_{131}I$ for imaging study and biodistribution, by the Cloramin T method (CLT), in partnership with the Radiopharmacy Center of IPEN. In the standardization of radiolabeling, 50 μg of pGH, mGH and G118R-mGH were used in 50 μl of saline phosphate buffer solution (PBS, pH 7.4) and 37 MBq of $Na_{131}I$. To start the oxidation process, 50 μg of CLT and incubation under orbital agitation were added for 45 minutes. To stop the reaction, 100 μg of sodium metabisulfite ($Na_2S_2O_5$) was added.

2.3. Biodistribution assay in C57BL/6J mice

Saline solution with $_{131}I$-mGH or $_{131}I$-G118R-mGH was injected intraperitoneally in C57BL/6J and $_{131}I$-pGH mice, with activity of 0.12 MBq and 0.16 μg of hormone per mouse. Four animals were used, two for each protein. After 45 minutes the animals were euthanized. Blood samples were collected from the orbital plexus (75 μl) and the following organs were dissected (liver, heart, lungs, spleen, muscle (gastrocnemius), bone (femur), stomach, intestines, pancreas, kidneys, bladder and brain), and then they were weighed and the radioactive activity was measured in the gamma ray counter.
For analysis of graphs and statistics, the software Prisma (version 8.0.2, 263, GraphPad Prism Software Inc., Jolla, CA, USA) was used by analysis of variance "two way ANOVA". Data were calculated using the percentage of injected dose per gram of tissue (%ID/g). The ratio between tissue/blood was obtained by dividing tissue activity (%ID/g) by blood activity (%ID/g).

3. Results and Discussion

3.1. Radiolabeling with ^{131}I

The radiolabeling experiment of pGH, mGH and G118R-mGH with $_{131}I$ obtained high radiochemical purity, with radiolabeling yield higher than 95%. In addition, radiolabeling remained stable for thirteen hours of incubation, diluted in fetal bovine serum. The radiolabelled was immediately used after its preparation.

3.2. Biodistribution Assay - ^{131}I

The biodistribution assay showed that all proteins dispersed similarly to all organs, but there was no statistical difference between proteins in any tissue. When comparing the percentage of injected dose per μl of blood, of $_{131}I$-pGH, it is possible to observe a significant difference, and the KO animals presented a significantly higher count than the control animals (CTL), with $P = 0.0032$. On the other hand, when comparing the percentage of injected dose per μl of blood, $_{131}I$-mGH and $_{131}I$-G118R-mGH, in animals C57BL/6J, it is not possible to observe a statistically significant difference.

4. Conclusions

In the radiolabeling of pGH, mGH and G118R-mGH with ^{131}I, the methodology presented high efficiency, according to the result of SPECT chromatography, exceeding a percentage of purity above 90%.

The performance of GH in the CNS and its transport from the bloodstream to tissues are increasingly being investigated, but the responses obtained in the literature are still inconclusive. As for in vivo biodistribution assays, although they are in early stages and have the need for continuity, we can conclude that there is a difference in activity between pGH, mGH and G118R-mGH and that this is possibly related to GHR, making clear its important action on the regions.

In general, the tests developed in this work enable new conceptions and methodological applications, aiming at a better understanding of the physiological action of GH and a potential antagonist.

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