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Short Communication

Bioavailability of dalarelin — a superactive GnRH analogue — in rats

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Abstract

^{125}I-marked dalarelin (a modified analogue of GnRH) or GnRH (gonadotropin-releasing hormone) were administered to the tested rats in single doses of 127 ng/kg by subcutaneous injections. Dalarelin and GnRH were absorbed from the injected doses in 0.64 and 0.49%, respectively. Only one remarkable maximal concentration of these hormones was noticed in rats' blood 30 min after the administration. Dalarelin maximal concentration was 261.5 pg/cm^3 and was 93.43% higher than the maximal concentration of GnRH. Dalarelin bioavailability was 1651.89 pg/cm^3, whereas GnRH bioavailability was 718 pg/cm^3 h. The bioavailability level of dalarelin was 230% compared with that for GnRH, which was accepted as a pattern of bioavailability.

Keywords: Dalarelin; GnRH analogue; Bioavailabilty

1. Introduction

GnRH (gonadotropin-releasing hormone) is the basic regulator of the hypothalamus. It stimulates the secretion of gonadotropin hormones, i.e. folitropin (FSH) and lutropin (LH), and it is a dekapeptide of the following structure: piro–Glu–His–Trp–Ser–Tyr–Gly^6–Leu^7–Arg–Pro^9–Gly^{10}–NH_2 with a molecular weight of 1182.3 Da [1]. It is sensitive to the activity of brain peptidase. Peptidase splits the bond at the positions Gly^6–Leu^7 and Pro^9–Gly^{10} and causes the loss of its biological activity [4]. For this reason, we modified the sequence of GnRH amino acid residue at positions 6 and 10. This made it possible to obtain analogues of different biological activity strengths [2]. It was shown that the in vitro modified C-terminal amino acid residue of GnRH analogues was more resistant to enzyme activity than the N-terminal amino acid residue [3].

One of the newest GnRH analogues is dalarelin, a superactive analogue which is still under investigation in clinical trials. Dalarelin is a nonapeptide of the following structure: D-Ala^6–des–Gly^{10}–NHEt–GnRH and a molecular weight of 1167.3 Da [4].

The aim of this work was to determine the bioavailability of ^{125}I-marked dalarelin, which was administered to rats in a single dose by subcutaneous injections. Bioavailability of dalarelin, a superactive gonadoreline analogue, was compared with the bioavailability of GnRH.

2. Materials and methods

2.1. Substances

Dalarelin — chemical purity: 98%, manufactured by 'Bapex' Ltd., Riga (Latvia); GnRH — chemical purity: 98.3%, manufactured by the Department of Organic Chemistry, Chemical Institute, University of Opole (Poland).

The chemical purity of the peptides was determined by HPLC and ^{125}I was marked in accordance with the method described in Ref. [5].

2.2. Animals

Adult female Wistar rats of average body weight 200 ± 20 g were used. In the course of experimental procedures the animals were fed a standard pellet diet

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and had free access to water. They were treated in a humane way; the experiment was approved by the Bioethics Committee of the Silesian Medical Academy, Katowice, Poland.

2.3. Experimental procedure

All the animals were divided into two groups. Each group consisted of five rats. Group A rats were administered dalarelin, whereas Group B rats were administered GnRH. The hormones were administered in single doses of 127 ng/kg by subcutaneous injections.

A 0.2 cm^3 sample of blood was taken from the rat's tail vein 0.5, 1, 2 and 4 h after administration of dalarelin or GnRH. The blood was collected in heparinised pipettes. The radioactivity of the samples was measured in an Auto Gamma Count, LKB. The results were given in pg hormone/cm^3 of blood. The following parameters were taken into consideration to determine the bioavailability of peptides: C_{max}, the maximal concentration of hormone in blood; t_{max}, the time after which the maximal concentration occurred; AUC, area of changes under the concentration–time curves. The trapezium rule was used for our calculations [6].

The relative biological availability degree (EBA) for dalarelin [D] in relation to the accepted as a pattern gonadorelin (GnRH) was calculated.

2.4. Mathematical calculations

The results are the mean value of the results calculated for five rats. The standard deviation for each sample was calculated and Student's t-test was used to determine the significance level. The threshold of significance was $P < 0.05$.

3. Results

Fig. 1 shows the changes observed for hormone concentrations in blood in relation to the period of time passed after administration in a single dose by subcutaneous injection.

A total of 0.64% of dalarelin and 0.49% of GnRH was absorbed from the single dose of 127 ng of ^{125}I-marked hormone. The results obtained for dalarelin are comparable to the results obtained for the GnRH analogue burserelin [7].

After administration of GnRH and dalarelin, only one change in their maximal concentrations could be noticed. The maximal dalarelin concentration was 261.5 pg/$cm^3 \pm 0.96$ and is 93.42% higher than the maximal concentration of GnRH, which was 135.2

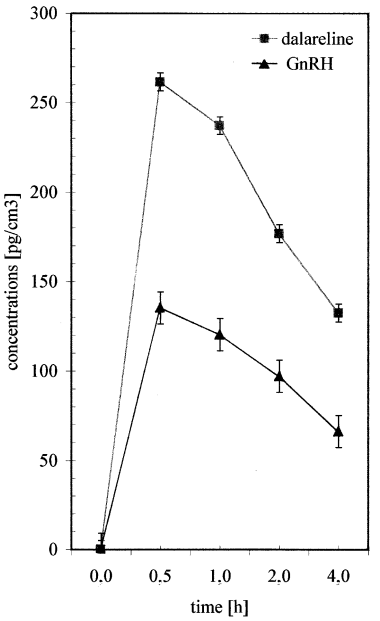

Fig. 1. GnRH and dalarelin concentration in the blood in relation to time.

pg/$cm^3 \pm 1.32$ ($P < 0.001$). The maximal concentration of the tested peptide hormones in rats' blood was achieved 30 min after their administration by subcutaneous injection. Dalarelin is characterised by the modified amino acid composition and, similar to buserelin, shows prolonged activity compared to GnRH. The dalarelin concentration in the blood samples was 132.42 pg/$cm^3 \pm 0.05$ 4 h after the administration, and GnRH concentration was 66.20 pg/$cm^3 \pm 0.10$ ($P < 0.01$). We also observed that the dalarelin concentration in the blood was twice as high as the GnRH concentration 4 h after administration by subcutaneous injection. The prolonged activity of buserelin was proved by pharmacokinetic studies that involved the use of radioisotopes [8].

Mean values of the areas under the curves representing the ratio of concentration:time, which characterise drug bioavailability were as follows: 1651.89 ± 0.421 and 718 ± 0.092 pg/cm^3 h ($P < 0.001$) for dalarelin and GnRH, respectively.

The degree of relative dalarelin bioavailability was 230% in relation to the pattern for GnRH. The results obtained in these studies show that dalarelin, a superactive analogue, has higher bioavailability than GnRH. It has been proved that the bioavailability of GnRH and its superactive analogue buserelin can be increased if they are administered in the form of a prolonged activity suspension containing Zn^{2+} [7,9]. It is probable that Zn^{2+} ions cause the lowering of hormone inactivation due to cathepsines.

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