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Sustained release and biological availability of dalarelin from the biodegradable coacervate microcapsules

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Abstract

A complex coacervation method was used to prepare microcapsules containing 74.8±1.5% of the ^{125}I labelled dalarelin incorporated in the gelatine–algin coating. Microcapsules (62±1.7%) formed, did not exceed a size of 108 μm. The high content of the small size allowed this formulation to be administered by intramuscular injection to rats. It was found that the ^{125}I labelled dalarelin in the form of microcapsules had better bioavailability and was active longer in the rat when compared with the ^{125}I labelled dalarelin solution injections. Dalarelin administered in the microcapsular form was characterised by a higher biological availability. The degree of relative biological availability was calculated as 123% for the dalarelin in the microcapsular form.

Keywords: Dalarelin; Bioavailability; Biodegradable; Coacervate; Microcapsules; Rats

1. Introduction

Dalarelin is a synthetic, superactive analogue of the gonadotrophin-releasing hormone (GnRH analogue); the hormone secreted by the hypothalamus. Dalarelin is a nonapeptide having the molecular weight 1167.3 and the following structure: des-Gly^{10}-[D-Ala^{6}]-LHRH, ethylamide. In the preliminary research bioavailability, distribution and pharmacokinetics of the peptide in question from suspensions was defined [1,2]. The perspective of widespread application of protein–peptide hormones in therapy calls for working out another form of the medicine, for example microcapsules [3]. This form was obtained with the analogus of gonadoreline–leuprolid [4]. The biodegradable co-polymer poly(D,L-lactic-co-glycolic acid) (PLGA-was used). Obtaining microcapsules with Langerhals cells isle was described as well [5]. For containing alginian with poly-L-lysine was used. The method of dust high spray-drynig for obtaining them seems an interesting solution [6].

The intention of the present study included the preparation of microcapsules in the form of a biodegradable coacervate containing dalarelin, and the determination of the pharmaceutical properties of this formulation. Parameters characterising the bioavailability of dalarelin as microcapsular biodegradable coacervate, and as reference, dalarelin solution, were determined after a single intramuscular injection in rats.

2. Materials and methods

2.1. Dalarelin

Chemical purity 98% was used in the investigation. The purity of dalarelin was determined by the HPLC method (obtained from 'Bapex' L.T.D., Riga, Latvia). The peptide was labelled with ^{125}I by a standard chloramine procedure. The free peptide was separated by the gel filtration.

2.2. Substances

The edible, B-type gelatin (120490; Gelatine Manufactures 'Zakłady Żelatyny' Puławy, Poland); sodium alginate (960480, BDH, Chemicals Ltd., Pode, England); NaI^{125} MBq, RJ-56-9 ('Opidi', Świerk, Poland).

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The substances used met the US Pharmacopea 23 requirements [7].

2.3. Animals

The adult female Wistar rats of body weight 200±20 g were kept in separate metabolic cages with free access to drinking water and standard fodder. The experiment was carried out upon the consent of the Bioethics Committee of the Silesian Medical Academy in Katowice.

2.4. The formation of a biodegradable dalarelin coacervate (in the form of microcapsules)

Dalarelin (600 ng) labelled with ^{125}I in 0.6 cm^3 0.1 N aqueous acetic acid solution was added to 2.5 cm^3 10% aqueous solution of gelatin at 326 K. The obtained mixture was homogenised for 5 min in a disintegrator. Aqueous solution of sodium alginate (2.5 cm^3 4%) warmed at 326 K were added to the mixture and the whole was homogenised again for 5 min. After the homogenisation the whole mixture was topped up with 3.5 cm^3 20% aqueous acetic acid and homogenised for 5 min once more. Water (10 cm^3) was added to the mixture. The obtained suspension was cooled until it reached a temperature below 283 K. The obtained microcapsules were filtered in a Buchner funnel, cleansed with water and isopropanol, and dried up at 293 K.

2.5. Determination of the physicochemical properties of the obtained microcapsules

The outward appearance, colour and homogeneity of the obtained preparation were determined. The content of the dalarelin in the microcapsules was determined by counting the radioactivity in the Auto Gamma Count Apparatus, manufactured by LKB. The particle size-by the optical method, and magnifying the picture by 100 ×; the water content, by heating to the temperature 377 K, until solid. The microcapsules decay time in 0.9% aqueous sodium chloride solution was determined.

2.6. Assessment of bioavailability of dalarelin in the microcapsules

The animals were divided into two groups, five animals in each group. Group A was administered dalarelin in the form of microcapsules, where particles of size less than 108 μm were suspended in 0.5 cm^3 0.9% solution of sodium chloride. Group B was given 0.5 cm^3 0.9% solution of sodium chloride. Injections were carried out intramuscularly, in a single dose of 240 ng/kg body weight. At 0.5, 1, 2, 3 and 6 h after the injection, an amount of 0.2 cm^3 blood sample was collected from the rat caudal vein into the heparinised pipettes. The radioactivity of the samples was counted in the Auto Gamma Count apparatus. The obtained results were expressed as pg of the hormone per cm^3 blood. The following parameters characterising the bioavailability of dalarelin were determined for the studied and the reference dalarelin formulation:

- C_{max}, maximum in blood hormone concentration;
- t_{maz}, the time at which the maximal concentration occurred;
- $AUC_{(0-\infty)}$, the surface area under the concentration versus time curve, which was determined according to the trapezoid method.

The determined values of the area under curve, $AUC_{(0-\infty)}$, were complemented with the residuary areas; calculated according to the formula:

$$AUC_{residuary} = C_{6\ h}/k_{el}$$

where, $C_{6\ h}$ is the last determined dalarelin concentration in blood, i.e. 6 h after the beginning of the experiment; k_{el} is the elimination velocity constant.

The elimination velocity constant was computed from the slope of the segment linking the two peptide concentration points determined for the latest times of measurement, i.e. after the 3 and 6 h of the experiment. The following formula was applied:

$$k_{el} = (\ln C_3 - \ln C_6)/(t_6 - t_3)$$

The Extent of Biological Availability of the dalarelin (EBA) parameter was calculated from the obtained data with the aid of the formula:

$$EBA = (AUC_{microcapsules}/AUC^{*}_{injections}) \times 100\%$$

where, $AUC_{microcapsules}$ is the value obtained after the administration of dalarelin in the form of microcapsules; $AUC^{*}_{injections}$ is the value obtained after the injection of dalarelin in solution [8].

The obtained data were used for the determination of the $R\Delta$ parameter by a graphical method. The $R\Delta$ parameter was defined as the ratio of prolongation ratio of the time interval of dalarelin function when administered in the microcapsular form. The parameter indicates the time ratio when more that 50% (Δ50%) of the maximal concentration of dalarelin in blood is measured. The prolongation ratio was determined from the formula [9]:

$$R\Delta = \Delta 50\%(\text{microcapsules})/\Delta 50\%(\text{solution})$$

2.7. Mathematical calculations

The results were presented as the mean values obtained from five measurements. Standard deviation

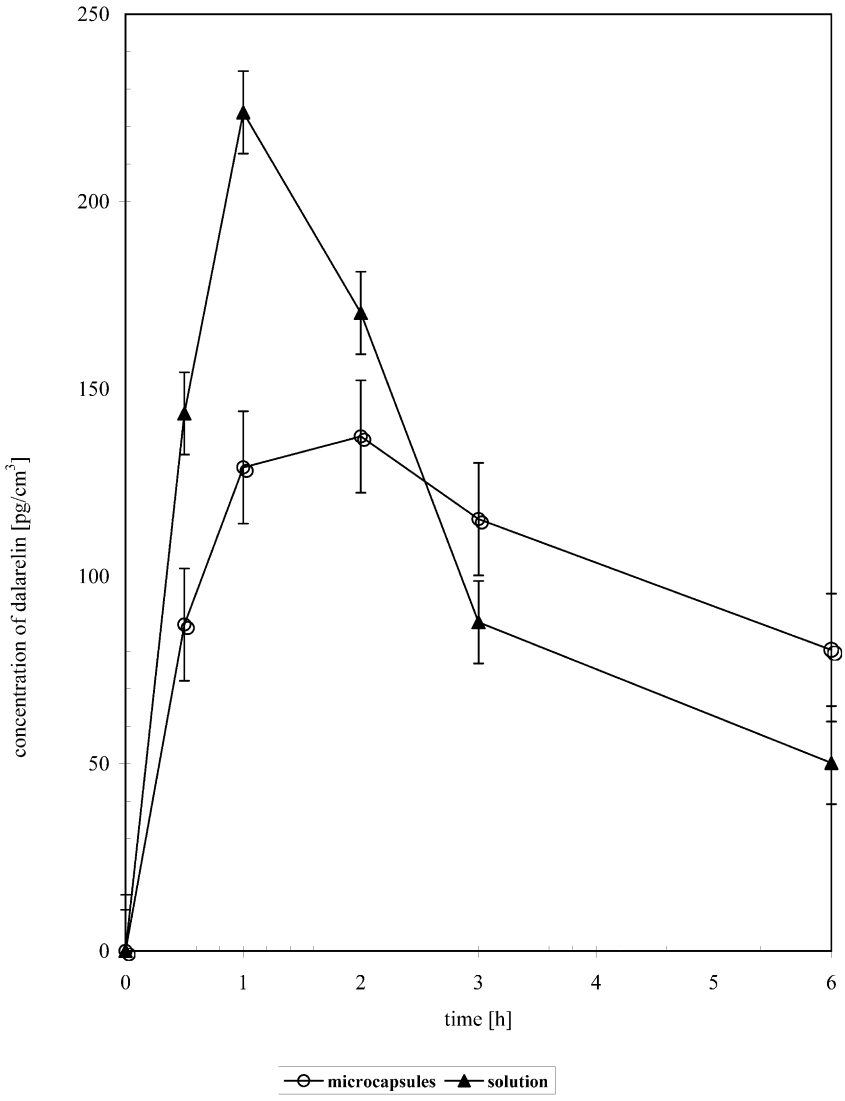

Fig. 1. The dynamics of blood dalarelin concentration changes after its administration in the form of the microcapsules and solution.

(SD) and the level of significance, P, were calculated and the t-test of Student was performed.

3. Results

3.1. The physicochemical properties of prepared microcapsules

The microcapsules containing dalarelin, with the gelatine–alginate coating, were obtained with yield of 66.4±2.1%. They were prepared by the complex coacervation method involving the pH change with the aid of acetic acid. The degree of incorporation of the peptide into the biodegradable coacervate was 74.8± 1.5%. The size distribution was estimated: 38±0.9% of microcapsules possessed particle size in the range from 108 to 600 μm. The amount of microcapsules not exceeding 108 μm particle size was 62±1.7% of the total quantity of microcapsules. The water content was 0.5–0.7% and the decay time in the 0.9% NaCI aqueous solution exceeded 3 h. The coacervate formed from sodium alginate and gelatine was suufficiently stable and did not degrade in the acidic medium. Obtaining microcapsules, the coating of which was composed of albumin, was reported as well [10–12].

3.2. The biological availability of dalarelin in microcapsules

The dalarelin concentration in blood versus time function, after administration of the two formulations of the studied drug is presented in Fig. 1.

In the case of the injection of dalarelin in solution, the maximal peptide concentration in blood attained 224± 18 pg/cm^3 in 1 h after the drug administration. When

dalarelin was administered in the form of the biodegradable coacervate microcapsules, its maximal concentration in blood attained 137 ± 21 pg/cm^3 after 2 h. The presented data suggested that dalarelin was much more slowly released from the microcapsules than the reference solution, so its activity could be prolonged. The elimination rate constant (k_{el}) for dalarelin in the form of microcapsules was 0.18 per h and for dalarelin in the form of the solution was 0.28 ± 0.033 per h.

The peptide was released from the microcapsules as a result of the slow biodegradation of the coacervate. In order to assess the retarded drug release, in the case of the microcapsular coacervate formulation, the prolongation ratio ($R\Delta$) was determined. This parameter is a ratio of the times when the concentration of the studied drug in blood exceeded 1/2 maximal concentration of the reference drug formulation. The prolongation ratio value attained 3.02. This indicated a substantial retardation of the drug release from the biodegradable coacervate. The prolongation quotient was not influenced by the dose of the administered drug and its biological availability. A comparison of the dalarelin concentrations in blood showed that the observed differences were statistically significant. The peptide concentration in blood began to drop rapidly at 2 h after the administration of the reference dalarelin solution. One of the most significant findings is the comparison of the amounts of dalarelin, which entered the blood stream after its administration as microcapsules and as the injection solution. The area ($AUC_{0-6\ h}$) for the peptide administered as an injection is 720 ± 9 pg/cm^3 per h, whereas for dalarelin microcapsules it is 657 ± 15 pg/cm^3 per h. This difference, however, is statistically insignificant, the significance level being less than 0.05. The $AUC_{(0-\infty)}$ for the solution of the peptide is 900 pg/cm^3 per h, which is less than after the administration of microcapsules, where it is 1103 pg/cm^3 per h. The degree of relative bioavailability for dalarelin in the form of microcapsules is 123%. The results show that the dalarelin administered in microcapsules could be characterised by the retarded release and by a higher biological availability than the reference dalarelin administered as the injection solution. In the investigation of the other analogue GnRH-buserelin labelled with 3H isotope, it was found that 24 h after the intravenous administration-58% of the hormone was found in the urine, and in this amount 21.6% was the non changed substance [13].

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