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CALORIMETRIC INVESTIGATION OF THE INTERACTION BETWEEN A MACROMOLECULAR PRODRUG OF DIFLUNISAL AND HUMAN PLATELETS

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Abstract

The thermal effect due to the interaction between human platelets and α,β poly(N-hydroxyethyl)-DL-aspartamide (PHEA) or the PHEA-Diflunisal conjugate was measured by the calorimetric technique at 25°C. The experimental data confirm that PHEA is a biocompatible macromolecule and that its conjugate influences the physiological activity of human platelets.

Keywords: calorimetry, macromolecular prodrug

Introduction

Macromolecular prodrugs are of considerable current interest because they allow significantly to modify the distribution of the drug in the body, to achieve a prolonged drug action and to decrease the incidence of side effects [1–3].

Among the polymers able to act as drug carriers, a new synthetic polymer, the α,β poly(N-hydroxyethyl)-DL-aspartamide (PHEA),seems to possess the required properties of a suitable drug carrier, i.e. it is highly water-soluble, non-toxic, non-antigenic, non-teratogenic [4]. Moreover PHEA macromolecules are characterized by several side hydroxylic groups which permits linking, via chemical bonds, to many drug molecules [3, 5].

On the other hand, the nonsteroidal anti-inflammatory drugs (NSAIDs) currently represent a large section of agents for relieving pain and controlling inflammation in rheumatic and arthritic diseases. Their use contributes to improve the quality of life for million of patient. Unfortunatelly, these drugs have same grave disadvantages as a pronounced ulcerogenic activity and a relatively short plasma half-live, resulting in a short activity duration [6, 7]. All

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these considerations suggest that the administration of macromolecular prodrugs of these therapeutic agents represents a tempting way to reduce their side effect and to improve the compliance of patients. In order to test this hypothesis, we have recently synthesized a macromolecular prodrug constituted by PHEA and Diflunisal. Diflunisal is a salicilate derivative with analgesic and antiinflammatory activity commonly used for the control of phlogistic and postoperative pain and in the treatment of the osteoarthritic diseases [8, 9].

The first experiments performed in order to test same PHEA-Diflunisal properties showed that this conjugate has many of the features required to make a therapeutical application possible both parenteral and peroral via. In fact, instead of Diflunisal, it is highly water-soluble, it does not induce platelet aggregation and it is able to release all the linked drug by enzymatic cleavage within 24 h. Moreover it allows a delayed release of the drug in the active form, at *pH* 1.1, in simulated gastric juice [10].

With the aim to increase our knowledges on the properties of PHEA-Diflunisal conjugate we have performed a calorimetric study on the interaction between the PHEA–Diflunisal conjugate and human platelets. Human platelets have been chosen as probes of the NSAIDs activity because the mechanism of action of Diflunisal, as all other NSAIDs, is the inactivation of the cyclooxygenase and the consequent inhibition of the synthesis of prostaglandins, which are mediators of the inflammatory process [11–13]. Since human plateletes are unable to restore the inactivated cyclooxygenase, it follows that they can be used as suitable calorimetric probes of the NSAIDs activity [11, 14].

Experimental

Materials

Preparation and purification of PHEA and PHEA–Diflunisal conjugate have been described elsewhere [10, 15].

Platelets were obtained using a standard method reported in literature [16]. Practically, blood obtained from volunteer healty blood donors, was collected in Baxter bags containing 63 ml of a standard anticlotting solution CDP (aqueous solution containing sodium citrate at 2.63%, monohydrate glucose at 2.55%, monohydrate citric acid at 0.327%, sodium phosphate monobasic at 0.251%). Then, the venous blood was centrifugated at 2000 rpm for 3 min and the supernatant was further centrifugated at 5000 rpm for 5 min. After removal of the supernatant of the second centrifugation, the freshly prepared platelet rich plasma (PRP) was used for the calorimetric experiments.

Apparatus

Calorimetric measurements were carried out at 25°C with a LKB Thermal Activity Monitor (TAM) equipped with a mix-flow cylinder (LKB-204)

and a perfusion cell (LKB 2277-402), using the following procedure. A known amount (about 1.5 g) of sample (see below) was placed into the sample compartment of the perfusion cell. Then, the cell was introduced into the calorimeter and after a period quite long to allow thermal equilibration, a small amount (200 μl) of PRP was added in the sample compartment by an injection cannula connected to a Hamilton syringe. The calorimetric signal, appropriately amplified, was recorded by a Digicass 200 Laumann recorder.

The investigated samples were:

(a) sterile physiological solution
(b) PHEA in sterile physiological solution at 1.61% w/w
(c) PHEA–Diflunisal adduct in sterile physiological solution at 1.91% w/w
(d) Diflunisal in sterile physiological solution at 0.30% w/w.

Concentrations were chosen so that (b) and (c) samples contain about the same amount of PHEA and (c) and (d) samples contain about the same amount of drug.

Results and discussion

Figure 1 shows the calorimetric signal (P, μW) as a function of time occurring when 200 μl of PRP are added to 1.5 g of (a), (b), (c) and (d) samples (see Experimental).

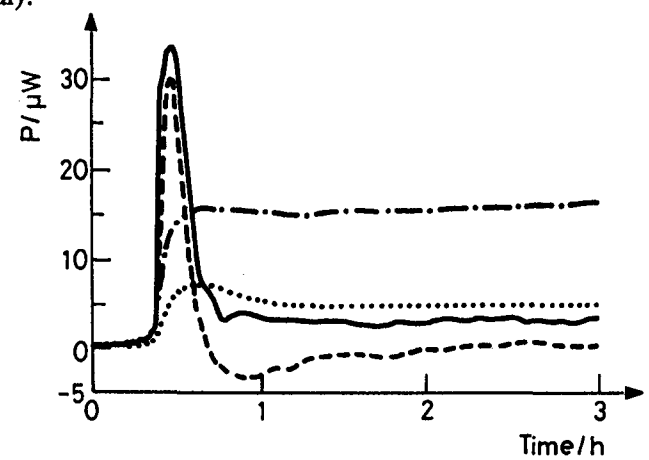

Fig. 1 Calorimetric signal (P, μW) as a function of time obtained when PRP is added to: pure sterile physiological solution (a) (·····); PHEA in sterile physiological solution (1.61% w/w) (b) (——); PHEA–Diflunisal conjugate in sterile physiological solution (1.91% w/w) (c) (·—·—); Diflunisal in sterile physiological solution (0.30% w/w) (d) (— — —)

First of all, it must be pointed out that, quite reproducible power-time profiles were obtained when each experiment was replicated (for simplicity, the

replicates are not shown in Fig. 1). By inspection of Fig. 1 [curve (a)], it can be noted that the human platelets added to sterile physiological solution give a small exothermic signal which does not appreciably change at least during the period of the experiment (about 3 h). It seems reasonable to attribute this heat production to the normal physiological activity of platelets. Quite different thermal effects are observed when PRP is added to physiological solution containing PHEA, PHEA–Diflunisal or Diflunisal [curves (b), (c), (d)]. In particular, PHEA and Diflunisal show an initial exothermic spike followed by a flat thermal effect whereas for PHEA–Diflunisal conjugate, a nearly constant trend is observed.

As to concern the initial exothermic spike observed when PHEA interacts with human platelets, it seems reasonable to attribute this thermal effect to the fast adsorption of the PHEA to the platelets membrane. In this adsorption process, the electrostatic forces could be notable because the platelets have on the surface negative charges due to the sialic acid groups of the glycoproteins [14, 17] and PHEA has a positive charge density [18–21].

For the smaller Diflunisal molecule, the initial thermal effect could be reasonable ascribed to an absorption process on the cellular surface followed by a fast transfer inside the platelets allowing a direct interaction with their internal components.

The absence of an initial exothermic spike for PHEA–Diflunisal conjugate can be explained in terms of a lower affinity of this polymer toward the human platelets. This because Diflunisal is essentially a hydrofobic molecule and then its attachment to the PHEA will give a less hydrophilic macromolecule with a lower positive charge density.

As to concern the long period thermal effect, we can observe that similar trends are obtained when PRP is added to physiological solution or PHEA in physiological solution. This experimental evidence suggests that, after the absorption process, PHEA does not involve significant changes in the normal physiological activity of platelets. Quite different long period thermal effects are observed when Diflunisal or PHEA–Diflunisal conjugate interact with platelets. Considering that these molecules obviously involve the occurrence of different biochemical processes when are inside the platelets, the observed trends could be both taken as an indication of an effective inactivation of the cyclooxygenase due to Diflunisal or PHEA–Diflunisal conjugate [13].

In conclusion our calorimetric investigation confirm that PHEA is a biocompatible macromolecule [4] and suggests that the PHEA–Diflunisal adduct influences the physiological activity of human platelets. In particular, our data support the hypothesis that the PHEA–Diflunisal adduct after an endocytic process and an enzymatic hydrolysis (of the drug-polymer bonds) releases free Diflunisal molecules inside the cells.

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Zusammenfassung — Mittels einer kalorimetrischen Methode wurde der thermische Effekt im Zusammenhang mit der Wechselwirkung zwischen Thrombozyten des Menschen und α,β-Poly(N-hydroxyethyl)-DL-Aspartamid (PHEA) oder dem PHEA-Diflunisal-Konjugat gemessen. Die experimentellen Daten zeigen, daß PHEA ein biokompatibles Makromolekül ist und daß sein Konjugat die physiologische Aktivität der Thrombozyten beeinflußt.