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Targeted drug delivery to the central nervous system via phosphonate derivatives (Anionic delivery system for testosterone)

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An anionic chemical delivery system (aCDS) has been developed and applied to deliver testosterone (T) to the central nervous system (CNS). The delivery of a target compound is achieved through the use of a specific targetor moiety which is an (acyloxy)alkyl-phosphonate-type functional group. The T-aCDS readily penetrates biological membranes by passive transport due to its increased lipophilicity and enters the target organ. Hydrolytic cleavage by esterases provides a negatively charged, hydrophilic intermediate phosphonate compound (TP^-), which is "locked in" the CNS and should provide sustained, site-specific release of the drug. *In vitro* and *in vivo* investigations in rats showed that methyl-pivaloyloxymethyl-17-testosterylphosphonate (T-aCDS) might function as an anionic chemical delivery system of testosterone. The concentration of T-aCDS decreased fairly rapidly *in vitro*. The half-lives ($t_{1/2}$) in different organs are as follows: blood 4.48 min ($r = 0.9388$), lung 5.53 min ($r = 0.9661$), liver 2.82 min ($r = 0.9498$), and brain 7.37 min ($r = 0.9972$). Simultaneously with the disappearance of T-aCDS, testosterone-phosphonate (TP^-) appeared as a main metabolite in increasing concentration. *In vivo* evaluations (tail vein 11.3 mg/kg in DMSO) found maximum T-aCDS brain levels 5–10 min after administration; they fell under the borderline of detectability (<0.1 μg/g) after 60 min. Maximum concentration of the decomposition product (TP^-) was obtained at 30 min after administration; it did not decrease significantly during the study. Even if the phosphonate derivative of the secondary, hindered hydroxyl group in this product was fairly resistant to phosphorolytic attack, the design principle can work for other compounds.

1. Introduction

The aim of the present work was to develop and investigate targeted drug delivery via phosphonate derivatives. Numerous techniques have been examined for targeted drug delivery including physical, biological, and chemical approaches [1]. Probably, chemical delivery systems [2], which share the common feature of having specific carrier moieties (targetors) that enable targeted delivery of the selected compound, offer one of the most promising ways of drug delivery.

The major novelty of the present approach is the use of an (acyloxy)alkyl-phosphonate moiety, in the present case a (pivaloyloxy)methyl-phosphonate moiety, for targeting purposes. With this type of targetor moiety, formation of an anionic intermediate is expected to provide the brain "lock in" mechanism [3].

While for carboxylic acids a wide range of derivatives have been prepared and investigated, there are only relatively few publications dealing with derivatives of phosphonates or their closely related analogues. Nevertheless, after the introduction of the concept by Farquhar et al. [4], a number of studies report the use of some (acyloxy)alkyl derivatives of organophosphates as lipophilic prodrugs [5] to enhance penetration across biological membranes [6, 7]. It was found [8] that simple alkyl ester or amide derivatives cannot be successfully used because of poor stability and/or poor activity. However, (acyloxy)alkyl esters are unique mask for the phosphonate group and indeed provide remarkable enhancements of permeation across biological membranes and of oral bioavailability.

Here we report studies on the organ-targeting properties of a novel anionic chemical delivery system (aCDS) for testosterone (T). Selection of testosterone was motivated by our earlier work on the delivery of testosterone using a redox chemical delivery system [9].

2. Investigations and results

2.1. In vitro study of T-aCDS (1)

A group of seven rats were used (control: another group of three animals). The investigations were performed in blood, brain, liver, and lung, respectively. Freshly collected whole blood was used. Tissue (brain, liver, and lung) homogenates were prepared by homogenizing freshly collected organ tissues with isotonic phosphate buffer (pH 7.4) to make 20% (w/w) homogenate. An HPLC method was developed for the determination of T-aCDS (**1**) and metabolites in biological fluids. Retention times were as follows: T-aCDS (**1**) 7.74 min, TP^- (**2**) 4.88 min, T (**3**) 9.06 min.

The Scheme shows the metabolic conversion of the anionic chemical delivery system (aCDS). Fig. 1 and 2 demonstrate the *in vitro* concentration change for T-aCDS (**1**) and the negatively charged intermediate TP^- (**2**) in different organs.

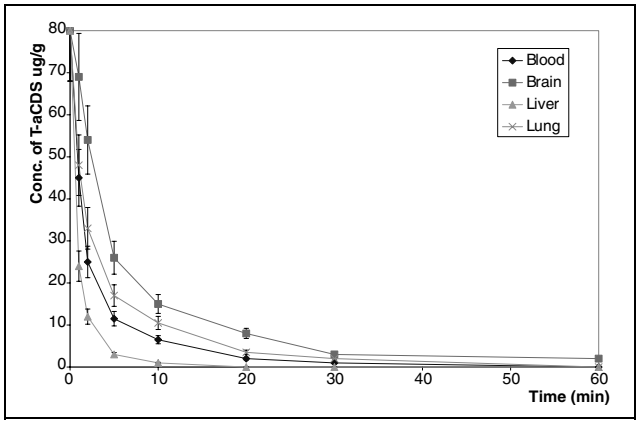
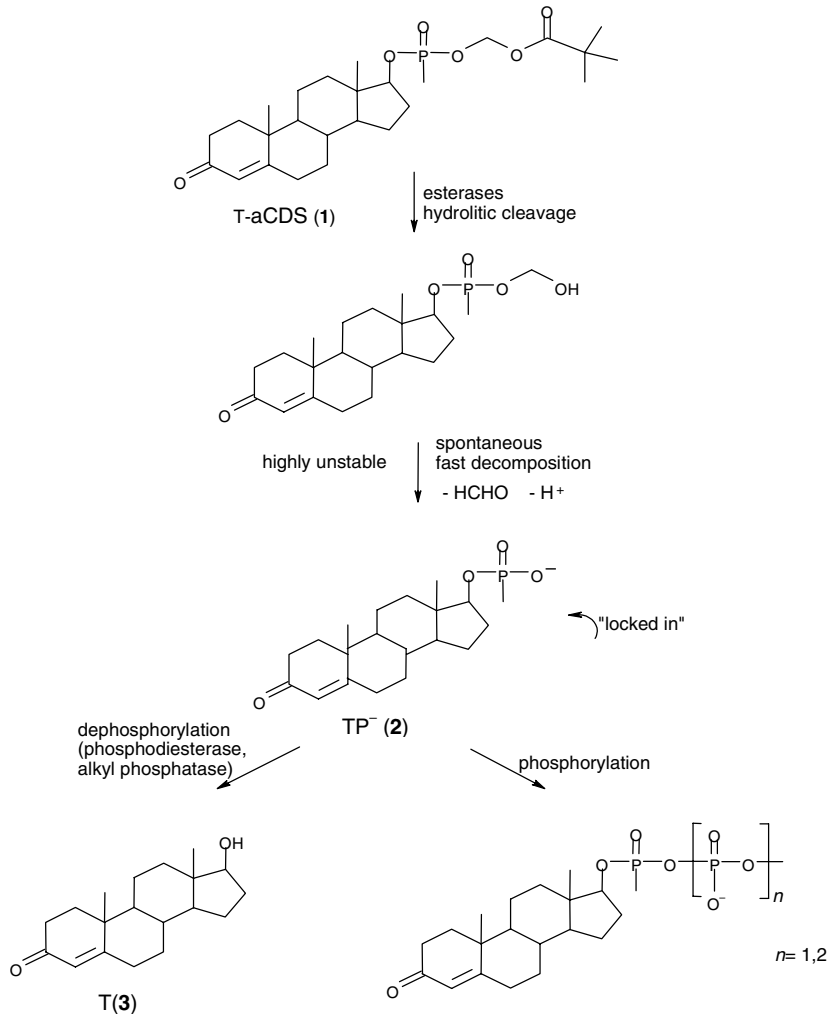


Fig. 1: *In vitro* concentration change of T-aCDS (**1**)

Scheme



2.2. In vivo study

Adult, male Sprague-Dawley rats weighing 175–200 g were used. Groups of at least five rats were used. In the control group (three rats) only the solvent was administered.

Figs. 3 and 4 show the *in vivo* concentration change of T-aCDS (**1**) and the main metabolic product TP^- (**2**) in various organs after i.v. administration of a 11.3 mg/kg T-aCDS dose in rat. Each value is the mean of five independent determinations.

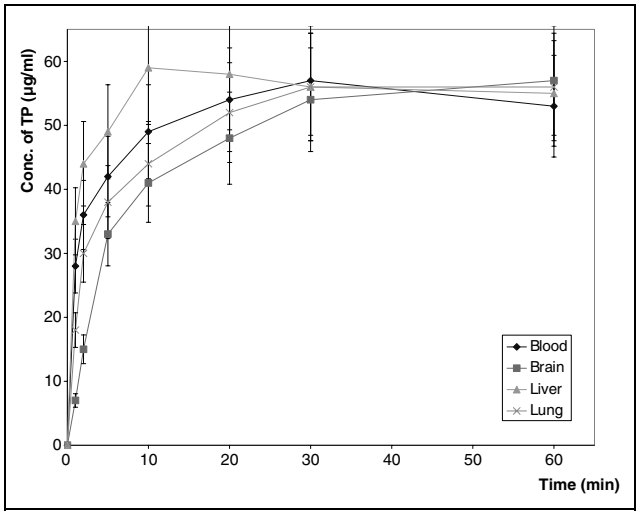


Fig. 2: *In vitro* concentration change of TP^- (**2**)

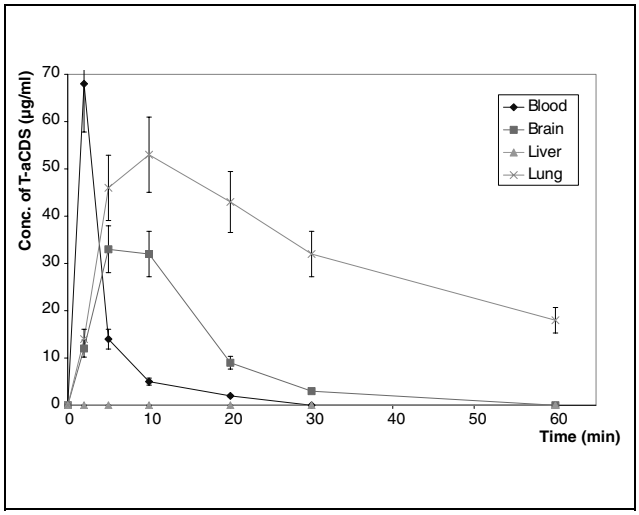


Fig. 3: *In vivo* concentration change of T-aCDS (**1**)

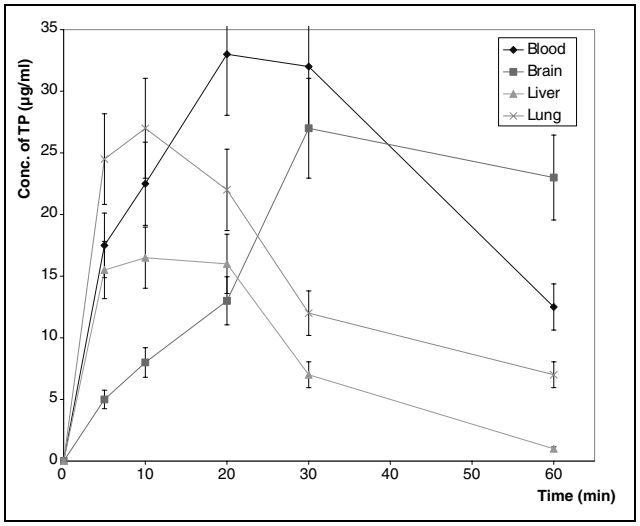


Fig. 4: *In vivo* concentration change of Tp^- (**2**)

3. Discussion

The concentration decrease of T-aCDS (**1**) is fairly rapid. The corresponding half-lives ($t_{1/2}$) are: blood 4.48 min, lung 5.53 min, liver 2.82 min, and brain 7.37 min, respectively. Simultaneously with the disappearance of T-aCDS, methyl-17-testosteryl-phosphonate (TP^-) appears as a main metabolite in increasing concentration. These profiles are consistent with the predicted metabolism of this system.

The concentration level of the starting compound (T-aCDS) was the highest in the blood, but disappeared rapidly with a $t_{1/2}$ of approximately 4 min, and by 30 min no T-aCDS was detectable. For lung and brain, we found lower maximum levels, and only the brain concentration fell bellow detection limits in 60 min. The situation was dramatically different for the liver. Due to rapid metabolism, T-aCDS could not be found in detectable concentration during the investigated time intervals.

The negatively charged intermediate (TP^-) levels were practically at their maximum at the first sampling (5 min), did not change significantly between 5 and 20 min, and then rapidly disappeared after about 60–65 min. In blood and lung, higher levels were measured.

In the brain, maximum concentration of the TP^- was obtained at 30 min, and this concentration did not decrease significantly during the study: this form is indeed "ocked in" the brain. The secondary hydroxyl group of TP^- was fairly resistant to phosphorolytic attack. Even if release of the active drug could not be detected during the investigated time interval, the design principle can work for other compounds (for example, phosphonate derivatives of primary hydroxyl groups).

4. Experimental

4.1. Analysis

A Spectra-Physics (Palo Alto, CA) HPLC system was used with UV/VIS detection operating at ambient temperature. Column: Phenomenex (Torrance, CA) Bondclone C18 (150 × 3.9 mm I.D.) with Rainin (Ridgefield, NJ) C18 guard. Mobile phase: 50% of AcCN, 50% of 0.01 M phosphate buffer (pH 7.0). Flow rate: 0.9 ml/min. Wavelength: 254 nm.

4.2. In vitro study

Stock solution (400 µl) of the investigated compound (approx. 1 mg/ml in DMSO) was added to 5 g of 20% (w/w) biological medium at 37 °C and was mixed for 10 s. The vials were put back into the water bath. At 0.5, 2, 5, 10, 20, 30, and 60 min 400 µl of samples were taken and mixed to 800 µl of acetonitrile containing 5% DMSO and 1% acetic acid. The mixture was shaken for 1 min and centrifuged for 10 min at 12000 rpm. The supernatant was removed and filtered through a Millipore filter. The solution was analyzed without any further dilution by HPLC injecting 20 µl of samples. Quantitation was done by a calibration curve.

4.3. In vivo study

The investigated compounds (T-aCDS, TP^-) were dissolved in DMSO, and the solutions were administered in the tail vein of conscious animals at a dose of 11.3 mg/kg. Animals were sacrificed by decapitation at appropriate time intervals (2, 5, 10, 20, 30, and 60 min) after the intravenous injection. Trunk blood was collected into heparinized tubes. The brain, the liver, and the lung were removed and immediately frozen. Samples for HPLC analysis were prepared by homogenizing the organs with isotonic phosphate buffer (pH 7.4). The final concentration of the suspension was 20% (w/w). 400 µl of each of these suspensions were prepared as described previously for HPLC determination.

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