International Journal of Pharmaceutics 515 (2016) 721–728

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Where does hydrolysis of nandrolone decanoate occur in the human body after release from an oil depot?

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ARTICLE INFO

Article history:
Received 4 October 2016
Received in revised form 27 October 2016
Accepted 28 October 2016
Available online 29 October 2016

Chemical compounds studied in this article:
Benzyl alcohol (PubChem CID: 244)
Nandrolone decanoate (PubChem CID: 9677)
Nandrolone (PubChem CID: 9904)

Keywords:
Oil depots
Hydrolysis
Human blood
Carboxylesterases
Muscle
Subcutaneous
Nandrolone decanoate

ABSTRACT

Long-term therapy of nandrolone (N) is recommended to increase mineral density and muscle strength. Using a parenteral sustained release drug formulation with nandrolone decanoate (ND), therapeutic N levels can be achieved and maintained. Until now, it is unknown if hydrolysis of ND into N occurs in tissue at the injection site or after systemic absorption. Therefore, hydrolysis studies were conducted to investigate the location and rate of ND hydrolysis after its release from the oil depot.

ND hydrolysis was studied in porcine tissues, to mimic the human muscular and subcutaneous tissues. Additionally, the ND hydrolysis was studied in human whole blood, plasma and serum at a concentration range of 23.3–233.3 μM.

ND hydrolysis only occurred in human whole blood. The hydrolysis did not start immediately, but after a lag time. The mean lag time for all studied concentrations was 34.9 ± 2.5 min. Because of a slow penetration into tissue, hydrolysis of ND is found to be very low in surrounding tissue. Therefore the local generation of the active compound is clinically irrelevant.

It is argued that after injection of the oil depot, ND molecules will be transported via the lymphatic system towards lymph nodes. From here, it will enter the central circulation and within half an hour it will hydrolyse to the active N compound.

1. Introduction

Androgens can be used to increase bone mineral density and muscle strength (Crawford et al., 2003; Erdtsieck et al., 1994; Frisoli et al., 2005; Notelovitz, 2002). For this purpose, long-term therapy of nandrolone is recommended. Therapeutic nandrolone levels in blood can be maintained using a parenteral sustained release drug formulation (Bagchus et al., 2005; Kalicharan et al., 2016c; Minto et al., 1997; Wijnand et al., 1985). An example of such a parenteral drug formulation is an oil depot. In general, slow release from oil depots is a result of the high partition coefficient of lipophilic compounds; The release rate decreases when the compound is more lipophilic. Increased lipophilicity can be accomplished through esterification with a fatty acid. For nandrolone, the decanoate has been selected as the appropriate moiety. In contrast to nandrolone (the active parent compound), nandrolone decanoate (ND) is an inactive prodrug. Oil depots with ND have been applied in several clinical studies, in which they were administrated by intramuscular (i.m.) (Bagchus et al., 2005; Minto et al., 1997; Wijnand et al., 1985) or subcutaneous (s.c.) routes (Kalicharan et al., 2016c).

Although pharmacokinetic profiles of nandrolone depots have been published, the fundamental mechanisms of drug release and absorption into the central circulation have hardly been studied. In theory, ND is released from the oil depot into the interstitial (tissue) fluid. The rate at which this occurs is largely determined by the compound concentration in the oil formulation and its partition coefficient. Subsequently, ND is hydrolysed into nandrolone. Until now, it is generally assumed that ND is hydrolysed in serum and not in the tissue (fluid) at the site of injection (Wijnand et al., 1985).

Recently, we have demonstrated that there exists a delay (*lag time*) in the appearance of nandrolone in the central circulation

Abbreviations: BOH, benzyl alcohol; HB, human whole blood; HP, human plasma; HS, human serum; N, nandrolone; ND, nandrolone decanoate; NBCS, new born calf serum.

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http://dx.doi.org/10.1016/j.ijpharm.2016.10.068
0378-5173/

(Kalicharan et al., 2016c). Since nandrolone has a *log P* of 3.0 ("ChemSpider," 2015) and therefore will be absorbed relatively rapidly, this observed lag time indicates that immediate hydrolyses of ND does not occur. The lag time can be affected by several factors, such as diffusion in tissue fluid, cell membrane adsorption and cell absorption (Kalicharan et al., 2016c). On the one hand, at the site of injection, interstitial fluid transport is slower than blood flow. On the other hand, this transport is faster than diffusion. Another factor, cell membrane adsorption is relevant, because lipophilic prodrugs have high affinity with lipophilic cell structures such as cell membranes and membrane proteins. Due to the adherence to the cell membrane, cell absorption seems a logical consequence. Once absorbed, the lipophilic prodrug can be hydrolysed by esterases (if present) localized in cytosol and microsomes (Jewell et al., 2007; Li et al., 2005; Prusakiewicz et al., 2006). After hydrolysis, efflux of the active parent compound out of the cell must occur in order to reach the central circulation. All these factors may contribute to a prolonged residence time of the lipophilic prodrug in tissues and fluids around the injection site.

Hydrolysis can occur via chemical processes or by carboxylesterases (Imai and Ohura, 2010; Jewell et al., 2007; Prusakiewicz et al., 2006). These enzymes hydrolyse a different ester prodrug, haloperidol decanoate (Nambu et al., 1987; Oh-E et al., 1987). Because the ester bond in this prodrug is similar to the ester bond in ND, it is likely that ND hydrolysis also occurs by carboxylesterases. To our knowledge, this has however never been published yet and will be studied in this paper.

Interestingly, carboxylesterases are inhibited by benzil (Hatfield and Potter, 2011). This compound shows great similarity on molecular structure with a commonly added oil depot additive: benzyl alcohol (BOH). Although BOH is processed in a significant quantity in oil depots of 1–10% (m/v) (Bagchus et al., 2005; Kalicharan et al., 2016c; Minto et al., 1997; Van Weringh et al., 1994; Wijnand et al., 1985), any inhibitory effect of BOH on carboxylesterases is yet unknown but can be clinically relevant if it inhibits carboxylesterases.

The time period between ND release from the oil depot and metabolism in the liver may account for the complete lag time, but it is also well possible that hydrolysis occurs earlier in the absorption phase. Until now, this has never been unambiguously demonstrated.

The aim of this research was to determine whether ND hydrolysis occurs at the injection site after its release from an oil depot. This paper covers the ND hydrolysis in human blood, plasma, and serum. Hydrolysis in interstitial fluid was also indirectly studied, as interstitial fluid originates from blood plasma (Charman and Stella, 1992; Wiig et al., 2012). Furthermore, the rate of ND hydrolysis is studied in muscle and subcutaneous tissue from pigs to mimic the injection site in respectively human muscle and subcutaneous tissue. Usability of carboxylesterases from porcine liver was evaluated in an experimental setup using new born calf serum (NBCS). This was also used to check whether carboxylesterases from porcine could induce ND hydrolysis in human intravascular fluids, as a positive control in the experimental setup. The evaluation took into account the effects of BOH on carboxylesterases.

2. Method

2.1. Chemicals and reagents

Nandrolone decanoate (ND) (Ph. Eur. quality) was purchased from MSD (Oss, the Netherlands). Nandrolone (N) (analytical standard), spironolactone (analytical standard), benzyl alcohol (BOH) (analytical standard), zinc sulphate (analytical standard), RIPA-buffer and carboxylesterases from porcine liver (lyophilized powder, ≥15 units/mg solid) were purchased from Sigma Aldrich, USA. HPLC-grade methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). Phosphate-buffered saline (PBS) was manufactured under current Good Manufacturing Practice conditions in the hospital pharmacy at the University Medical Center Utrecht, the Netherlands.

2.2. Biological materials

New born calf serum (NBCS) was purchased from Life Technologies (Carlsbad, CA).

Human blood (HB) was obtained by drawing blood into a BD Vacutainer® with anticoagulant (18.0 mg K_2EDTA). Human plasma (HP) was obtained by centrifuging HB for 10 min at 4000 rpm. Human serum (HS) was obtained by drawing blood into a BD Vacutainer® (REF 367896) with clot activator (micronized silica particles).

Muscle (gluteus maximus) and subcutaneous tissues were obtained from porcine (hind left leg) at the Central Laboratory Animal research facility (Utrecht University, the Netherlands). Before euthanizing with pentobarbital (barbiturate), esketamine (anaesthetic) and midazolam (benzodiazepine) were administered to the animal. Directly after termination, the tissues were cryopreserved with liquid nitrogen until usage.

2.3. HPLC system and conditions

The reversed phase-HPLC system was from Agilent Technologies 1100 series with a UV–vis detector (G1314A VWD). A Phenomenex guard column (C18, 4 × 2 mm ID, 5.0 μm particle size) was used to filter out contaminants from each injection. Compound separation was carried out on a LiChrospher 100 RP-C18 column (125 × 4 mm ID, 5.0 μm particle size). The column temperature was kept at 30 °C during analysis. The autosampler kept the samples at 4 °C. A volume of 15 μL for samples originating from serum/plasma or 30 μL for samples originating from blood were injected per run of 10 min. The flow rate was set at 1.0 mL/min. The eluted peaks were subsequently detected at a wavelength of 240 nm. The mobile phase was set as a gradient with a mixture of methanol absolute/distilled water, as listed in Table 1. Software used for equipment control and data acquisition was Chromeleon, version 7.1.3.2425 from ThermoFisher Scientific (Waltham, MA).

2.4. Standards

A calibration curve of the mixed stock solution was made of 2, 25, 100, 250, 500 and 1000 μg/mL for both ND and N in ethanol absolute. Internal standard (IS) spironolactone was prepared as a 25 μg/mL acetonitril solution. A stock concentration of 300 units/mL carboxylesterases (originating from porcine liver) was prepared in 10 mM borate buffer (pH 6.0) conform protocol Sigma

Table 1
Schematic representation of the used gradient. The gradient allowed elution within 10 min of the three substances: spironolactone, nandrolone and nandrolone decanoate.

Time (minutes)	Distilled water (%)	Methanol (%)
0.00	34.0	66.0
3.30	34.0	66.0
3.40	2.0	98.0
7.00	2.0	98.0
7.10	34.0	66.0
10.00	34.0	66.0