



Fundamental understanding of drug absorption from a parenteral oil depot



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ABSTRACT

Oil depots are parenteral drug formulations meant for sustained release of lipophilic compounds. Until now, a comprehensive understanding of the mechanism of drug absorption from oil depots is lacking. The aim of this paper was to fill this gap. A clinical study with healthy volunteers was conducted. An oil depot with nandrolone decanoate and benzyl alcohol was subcutaneously administered in the upper arm of female volunteers. Pharmacokinetic profiles of both substances were related to each other and to literature data. Benzyl alcohol absorbs much more rapidly than nandrolone. In detail, it appears that benzyl alcohol enters the central compartment directly, while nandrolone decanoate is recovered in serum after a lag time. This lag time is also seen in literature data, although not reported explicitly. The absorption of nandrolone is enhanced by the presence of benzyl alcohol. This is most likely an effect of altered oil viscosity and partition coefficient between the oil and aqueous phase. The absorption rate constant of compounds is found to be related to the logP of the solubilized prodrug. The absorption rate is however not only determined by the physico-chemical properties of the formulation but also by the tissue properties. Here, it is argued that lymphatic flow must be considered as a relevant parameter.

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1. Introduction

Oily solutions of lipophilic compounds are widely used as a sustained release formulation. Although this pharmaceutical approach has been applied for several decades already, relatively little research has been published on the fundamental parameters that determine the absorption characteristics.

Generally, the formulation of an oil depot contains arachis or sesame oil as well as an amount of benzyl alcohol (BOH) which increases the solubility of the (pro)drug in the oil. In addition to these excipients, the formulation contains the active compound, most often as the esterified substance. Theoretically, there are a number of factors that determine drug absorption from a parenteral oil depot:

- 1) The drug dissolved in the oil is released as a result of the concentration gradient. Relevant parameters are a) the concentration in the oil, b) the thickness of the diffusion layer as well as the diffusion coefficient in the oil, c) the surface area of the depot, d) the partition coefficient (P) between oil and tissue fluid and finally e) the thickness of the diffusion layer in the aqueous phase as well as the diffusivity in this compartment. Basically, this represents the rate at which the

drug is transported through the tissue (Kadir et al., 1990, 1992; Minto et al., 1997; Tanaka et al., 1974; Zuidema et al., 1994, 1988). A simplification of the real vivo situation is depicted in Fig. 1. The oil liquid is not injected directly in the blood stream, while yet the absorption is normally measured in this central compartment (C_{serum}). Therefore, a membrane should be included in this model representing the tissue which is situated in between the oil and the central circulation.

- 2) BOH exhibits not only a significant solubility in the oil phase, but does also dissolve in the aqueous phase. Consequently, it will also be released out of the depot. Because of the different physico-chemical properties, it can be expected that BOH shows a completely different release profile than the prodrug. BOH in turn has a significant influence on the solubility of the active compound in both the oil and the aqueous phase (Rowe et al., 2009). Therefore, it is obvious that the partition coefficient is not a constant value during the release from the depot. This is presented as the partition coefficient between the concentrations at the interface in Fig. 1.
- 3) In most cases a lipophilic ester is used as a prodrug. After release out of the oil, this ester has to be hydrolysed to the parent drug. The prodrug exhibits a significantly higher logP than the parent compound. As a consequence, the transport through tissue can be considerably different; highly lipophilic drugs show both retardation by tissue absorption effects and lymphatic transport whereas less lipophilic compounds diffuse directly to the central circulation. Hence, the speed and the place at which (enzymatic) hydrolysis occurs may have impact on the rate of absorption.

Abbreviations: BOH, benzyl alcohol; ND, nandrolone decanoate; P, partition coefficient.

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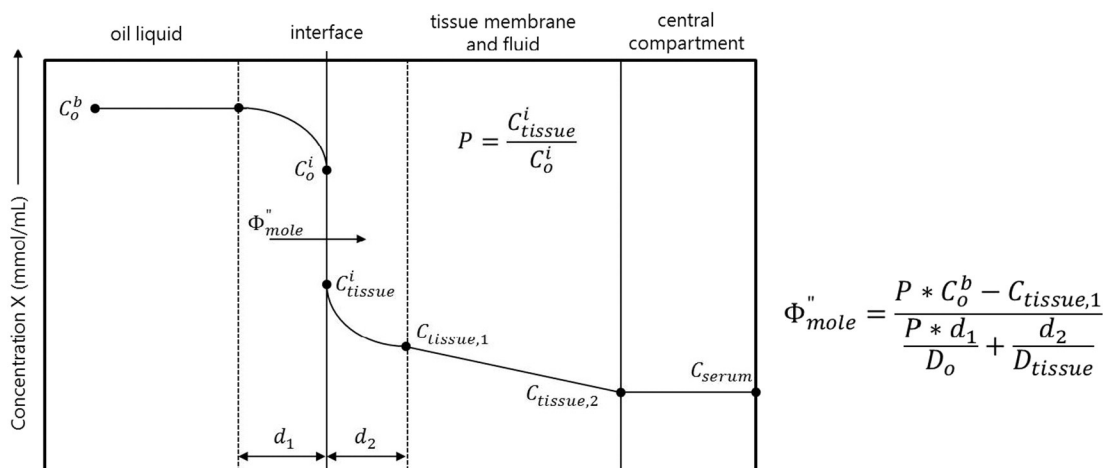


Fig. 1. Schematic overview of the vivo situation (left). Equation on the right presents the parameters which contribute to the mole flux (Φ_{mole}^*). Abbreviations: bulk concentration (pro)drug in oil (C_o^b), at oil interface (C_o^i), at tissue interface (C_{tissue}^i), in tissue beginning ($C_{tissue,1}$), before entering central compartment ($C_{tissue,2}$) and in serum (C_{serum}); d = diffusion layer in oil (d_1) and tissue fluid (d_2); P = partition coefficient; D = diffusion coefficient in oil (D_o) and in tissue fluid (D_{tissue}).

Other factors that may also contribute to the absorption rate are: injection depth (Ronald et al., 1993), site of injection (Soni et al., 1988; Vukovich et al., 1975), lymphatic absorption (Zuidema et al., 1994), massage before injection (Soni et al., 1988) and muscle activity (Soni et al., 1988). Although these suggested and obvious factors could lead to a complete understanding of drug absorption from oil depots, no studies have been published on this topic so far. This article makes a distinction between *release* and *absorption* kinetics: substance *release* from the depot can be translated from the mass flux from oil towards the aqueous phase, while *absorption* represents the entire process in which the substance enters in the central compartment. Hence, absorption includes the release out of the oil and the subsequent transfer through the tissue to the blood stream.

The current study started with a clinical trial in which an oil depot containing nandrolone decanoate was used. Frequent sampling of volunteers enabled us to monitor the absorption phase in detail. The aim of this paper is to create further understanding of the fundamental mechanisms that determine the drug absorption from a parenteral oil depot. Second, this paper elucidates the effect of BOH on nandrolone absorption. The observations are compared with results reported in literature and put into perspective with the pharmacokinetic profile of BOH that has been published separately [Kalicharan, BOH article].

2. Materials and methods

2.1. Experimental design

Drug product was manufactured under current Good Manufacturing Practice conditions in the hospital pharmacy at the University Medical Center Utrecht, The Netherlands. Each 1.0 mL of the solution contained 117 μmol nandrolone decanoate (ND), 28,000 IU cholecalciferol, 926 μmol (10% (m/v)) BOH and ad 1.0 mL sesame oil. In this study, 0.5 mL of the solution was subcutaneously (s.c.) injected in the upper arm. Fourteen female volunteers participated in this study. Full informed written consent was obtained from the volunteers, conforming to the Declaration of Helsinki. Inclusion criteria were: good physically and mentally healthy Caucasian females with an age between 65–80 years old and a body mass index (BMI) between 20–30 kg/m^2 . Volunteers were excluded when using any drug, food or beverages that influence the metabolism of ND from 2 weeks or 5 half-lives of the medication (whichever is longer) prior to drug administration. Smoking was allowed, provided no more than 4 cigarettes or equivalents were used per day.

A validated LC–MS/MS bioassay was used to determine serum nandrolone concentrations (LoQ = 0.12 pmol/mL). Blood samples were taken directly after injection (0 h) and 2, 4, 8, 12, 15, 22, 24, 36, 48, 72, 96, 168, 216, 264, 360, 456, 552, 648 and 840 h after injection.

2.2. Pharmacokinetic analysis

Pharmacokinetic parameters were examined using Microsoft Excel 2010. The following parameters were determined: maximum serum concentration (C_{max}) and time to reach this concentration (T_{max}); the area under the serum concentration–time curve (AUC) was calculated using the linear trapezoidal rule. All data are expressed as mean \pm standard error of the mean (SEM).

2.2.1. Absorption analysis

Drug absorption from the depot was estimated by converting serum levels to total amount of absorbed drug. All serum levels were converted to molar concentrations. The amount of absorption was calculated using the Wagner–Nelson method as described previously (Wagner, 1974). Analysis was performed by the following equation:

$$\frac{A}{V_d} = C_{serum} + k_e * \int_0^t C_{serum} * dt \quad (1)$$

wherein the maximum (cumulative) amount absorbed into the central compartment equals cumulative amount released by the depot, which was calculated according to

$$\frac{A_{max}}{V_d} = k_e * AUC_{0-\infty} \quad (2)$$

$$AUC_{0-\infty} = \left[\int_0^t C_{serum} * dt \right] + \frac{C_{serum}^{final}}{\lambda_z} \quad (3)$$

where A and A_{max} are the amount absorbed at moment t and maximal amount absorbed, respectively; C_{serum} and C_{serum}^{final} are the serum concentration at time t and the last measured plasma concentration, respectively; λ_z is the apparent elimination rate constant due to flip-flop pharmacokinetics (see below); V_d is the distribution volume and k_e is the elimination rate constant after intravenous injection. Values for k_e in this study were for nandrolone 2.75 h^{-1} (Minto et al., 1997), haloperidol 0.03 h^{-1} (Kudo and Ishizaki, 1999) and testosterone 0.80 h^{-1} (White et al., 1999).

Absorption rate constant (k_a) was determined by calculating the elimination rate constant (λ_z) using the least square method. Wijnand et al. (1985) pointed out that drug release from sustained oil depots shows flip-flop pharmacokinetics. As a result, during flip-flop pharmacokinetics the rate of absorption equals the decline of the terminal slope in the serum concentrations plot (Yáñez et al., 2011).

Recovery was calculated by dividing A_{max} (μmol) by total injected dose (μmol). It should be noted that all determined concentrations represent the levels of the parent compound. The depot contained a prodrug, i.e. the decanoate ester of the parent compound nandrolone.

2.3. Viscosity measurement

The viscosities of the oil solutions were determined in an AR-G2 rheometer (TA Instruments, New Castle, USA) at 23 °C. The shear rate was 100 s^{-1} and rotating frequency of 2.0 rad/s. Sesame oil mixed with different concentrations of BOH was measured in three fold.

2.4. Inclusion criteria of literature

A literature search was conducted within the PubMed/Medline database from January 1965 to May 2015. Mesh search query: (“Injections, Intramuscular”[Mesh]) OR “Injections, Subcutaneous”[Mesh]) AND

“Delayed-Action Preparations”[Mesh] AND “Pharmacokinetics”[Mesh]). The following filters were applied: “Clinical trial”, “Review”, and “Humans”. Articles were eligible for inclusion if they met: 1) The used injections had to be oil depots; and 2) pharmacokinetic intravenous data of the APIs were known, otherwise data analysis could not be conducted.

3. Results and discussion

The baseline characteristics of the fourteen females were (mean \pm standard error): Age = 70.7 ± 4.3 year, length = 1.65 ± 0.06 m, weight = 68.0 ± 9.0 kg and BMI = 24.8 ± 2.3 kg/m². All subjects were included in the following analysis. No adverse reactions were reported during or after the study.

3.1. Pharmacokinetic profile of nandrolone

The serum profile of nandrolone is presented in Fig. 2. The pharmacokinetic parameters are summarized in Table 1. Remarkably, Fig. 2 shows two distinct C_{max} values; there is a peak at 22 h while a second maximum is reached between 9–15 days post-injection. Surprisingly, literature data (Table 2) show these double peaks in all nandrolone oil depot studies, although it is not mentioned explicitly. In clinical papers

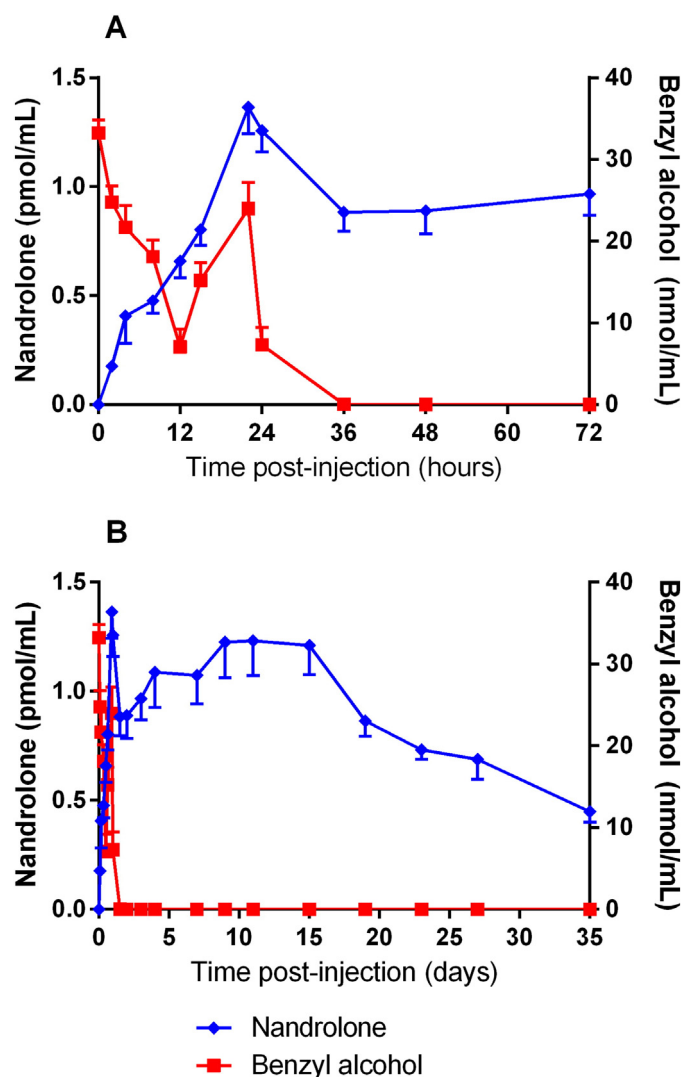


Fig. 2. Nandrolone (♦) serum levels after subcutaneous injection of 58.3 μmol of nandrolone decanoate ($n = 14$). Benzyl alcohol (■) serum levels were determined in another study [Kalicharan et al., BOH article]. Results expressed as mean and standard error of the mean, unless standard error is smaller than the symbol.

Table 1

Summary of pharmacokinetic parameters for nandrolone in serum (n = 14).

Variable	(Mean ± SEM)
C_{\max} (pmol/mL)	
First peak	1.42 ± 0.11
Second peak	1.50 ± 0.16
T_{\max} (hours)	
First peak	24.4 ± 1.8
Second peak	274.3 ± 38.0
AUC (pmol/mL × h)	
0–840 h	726.5 ± 52.4
0–∞	1035.2 ± 74.7
Amount absorbed (μmol)	
0–840 h	22.9 ± 1.7
0–∞	32.6 ± 2.4
k_a (h ⁻¹)	0.0021 ± 0.0003
Recovery (%)	
0–840 h	39.3 ± 2.8
0–∞	56.0 ± 4.0

Abbreviations: C_{\max} = maximum serum concentration; T_{\max} = time post-injection to reach C_{\max} ; AUC = area under the serum concentration–time curve; and k_a = absorption rate constant.

about oil depots containing antipsychotics, the occurrence of two peaks has been noted however (Jann et al., 1985). Here, the first peak was thought to be associated to the presence of a certain quantity of parent compound, i.e. the substance without the ester moiety. In our clinical study, no additional peaks were obtained during quality control analysis of the investigational medicinal product (IMP). This indicates no significant amount of parent compound in the IMP, which may cause the first nandrolone peak. This phenomenon will be discussed further below.

Fig. 2 also shows the serum profile of benzyl alcohol. This has been reported earlier [Kalicharan et al., BOH article], in a paper which also reports the development of a bioassay for BOH. As can be seen, BOH shows a completely different pharmacokinetic profile compared to the nandrolone profile; BOH is present in the central circulation at the earliest moment samples were taken, i.e. only a few minute after the moment of injection. This suggests principally different mechanisms of absorption compared to nandrolone; BOH appears to absorb fairly rapidly, whereas nandrolone exhibits a slow absorption. Another observation is that the first nandrolone peak at 22 h post-injection coincides with a peak in BOH level, after which both substances show a decline

in serum concentration (Fig. 2A). Furthermore, the absorption of nandrolone is slower after 36 h post-injection compared to the period directly after injection. BOH was only recovered during the first 36 h post-injection, which may suggest that the initially increased absorption of nandrolone is related to the quick release of BOH; the obvious explanation can be that this will lead to a decrease in concentration of the solubilizing compounds in the oil phase while it will have the reverse effect in the aqueous phase at the same time. Basically, this means that the partition coefficient between oily and aqueous phase changes during the release of BOH until there is a full depletion. In addition, also the viscosity of the oil depot changes during BOH release (Fig. 3). As expressed by the Stokes–Einstein law, the diffusion coefficient decreases upon increasing viscosity.

After the depletion of BOH, nandrolone clearly absorbs more slowly, resulting in a second prolonged serum peak, representing a steady state situation. Fig. 4A depicts the cumulative absorption profile of nandrolone. These results were obtained by converting the serum level (Fig. 2) to the amount of absorbed nandrolone (Fig. 4) using the Wagner–Nelson method. Also the cumulative release of BOH is given. This is in this case expressed as area under the curve instead of the percentage absorbed, because the Wagner–Nelson method could not be applied; the kinetic parameters *elimination constant* and the *distribution volume* are unknown for BOH. After 52 h post-injection, no BOH was detected in serum anymore, which implies that the depot at this point is depleted of BOH. Therefore, we assume a nearly 100% recovery of BOH from the depot.

3.2. Lag time

As can be seen in Fig. 4B, the absorption phase for BOH is quick and complete. In contrast, the absorption of nandrolone is principally different; the exposure is not instantaneous. There appears to be a lag time before nandrolone enters the central circulation. From a physical perspective, it is obvious that the compound release starts immediately after injection. This has been confirmed in *in vitro* studies (Larsen et al., 2006; Thing et al., 2012). It must be noted that these *in vitro* models did not include a membrane. BOH seems to behave according to these *in vitro* models, but knowing that there is no direct contact between blood and oil, it should be concluded that the lag time for BOH is extremely short. Hence, the transport through the tissue layer occurs very rapidly. The transport of ND from the oil to the central circulation

Table 2

Injections were administered in several muscles. Different vehicles (oil types and percentage benzyl alcohol) and chemical parameters (partition coefficient and molar weight) are shown of prodrug and corresponding parent compound. Abbreviations: API = active pharmaceutical ingredient; ND = nandrolone decanoate; NPP = nandrolone phenyl propionate; HD = haloperidol decanoate; TD = testosterone decanoate; k_a = absorption rate constant and BOH = benzyl alcohol.

Reference	Depot			Injection site	Type of oil	BOH (%)	logP ^{a,b}		Molecular weight ^c (g/mol)		Lag time (h)	k_a (h ⁻¹)	Recovery (%)
	API	Dose (mmol)	Injection volume (mL)				Prodrug	Parent	Prodrug	Parent			
Wijnand et al. (1985)	ND	0.23	1	Vastus lateralis	Arachis	10	7.9	2.6	428.6	274.4	2.7	0.0082	34
	ND	0.47	2	Vastus lateralis	Arachis	10	7.9	2.6	428.6	274.4	2.6	0.0055	22
Minto et al. (1997)	NPP	0.25	4	Gluteal	Arachis	10	6.0	2.6	406.6	274.4	4.9	0.0112	45
	ND	0.23	4	Gluteal	Arachis	10	7.9	2.6	428.6	274.4	14.6	0.0056	45
	ND	0.23	1	Gluteal	Arachis	10	7.9	2.6	428.6	274.4	11.4	0.0051	61
	ND	0.23	1	Deltoid	Arachis	10	7.9	2.6	428.6	274.4	26.4	0.0036	49
Bagchus et al. (2005)	ND	0.12	1	Gluteal	Arachis	10	7.9	2.6	428.6	274.4	10.8	0.0053	42
	ND	0.23	1	Gluteal	Arachis	10	7.9	2.6	428.6	274.4	12.0	0.0058	43
	ND	0.35	1	Gluteal	Arachis	10	7.9	2.6	428.6	274.4	15.9	0.0051	41
van Weringh et al. (1994)	HD	0.38	2	Gluteal	Sesame	1.5	6.9	3.2	530.1	375.9	29.8	0.0107	25
	HD	0.57	3	Gluteal	Sesame	1.5	6.9	3.2	530.1	375.9	27.3	0.0122	29
Morgentaler et al. (2008)	TD	1.69	1	Gluteal	Castor	0	8.6	3.3	442.6	288.4	24.2	0.0022	42

^a Data obtained from Clarke's Analysis of Drugs and Poisons (Moffat et al., 2003).

^b Data obtained from "ChemSpider" (2015).

^c Data obtained from The Merck Index, 14th ed (O'Neil et al., 2006).



Fig. 3. Viscosity of different concentrations benzyl alcohol mixed with sesame oil. No other substances were added. Results expressed as mean and standard error of the mean ($n = 3$), unless standard error is smaller than the symbol.

appears to be slower however. Obviously, there is a retarding factor in the tissue and therefore it is clear that this lag time period reflects the mechanism of transport in the tissue. There were no relationships found between the volunteers' baseline characteristics (e.g. BMI, weight, length or age) and the lag time. In this study, nandrolone injected subcutaneously as decanoate, exhibits a lag time of about eight hours (Fig. 4B).

Literature demonstrates that this phenomenon is not unique to a specific oil depot. A lag time is seen in all parenteral oil depot formulations (Table 2). This is not a constant interval however.

Several endogenous factors may contribute to this delayed absorption: (temporary) cell membrane adsorption, cell absorption, esterase activity, interstitial and/or lymph flow and/or alternative pathways. After injection of the depot into the tissue the prodrug starts to release and enters the interstitial space, in which the depot is injected. These prodrugs, being ester compounds, can spontaneously be hydrolysed with water or actively be hydrolysed by esterases. Van der Vies (1970) published a half-life hydrolysis of 4 min for 2.5×10^{-3} $\mu\text{mol/mL}$ (1 $\mu\text{g/mL}$) nandrolone phenyl propionate in rat plasma. Although no hydrolysis data on nandrolone decanoate in human fluids have been

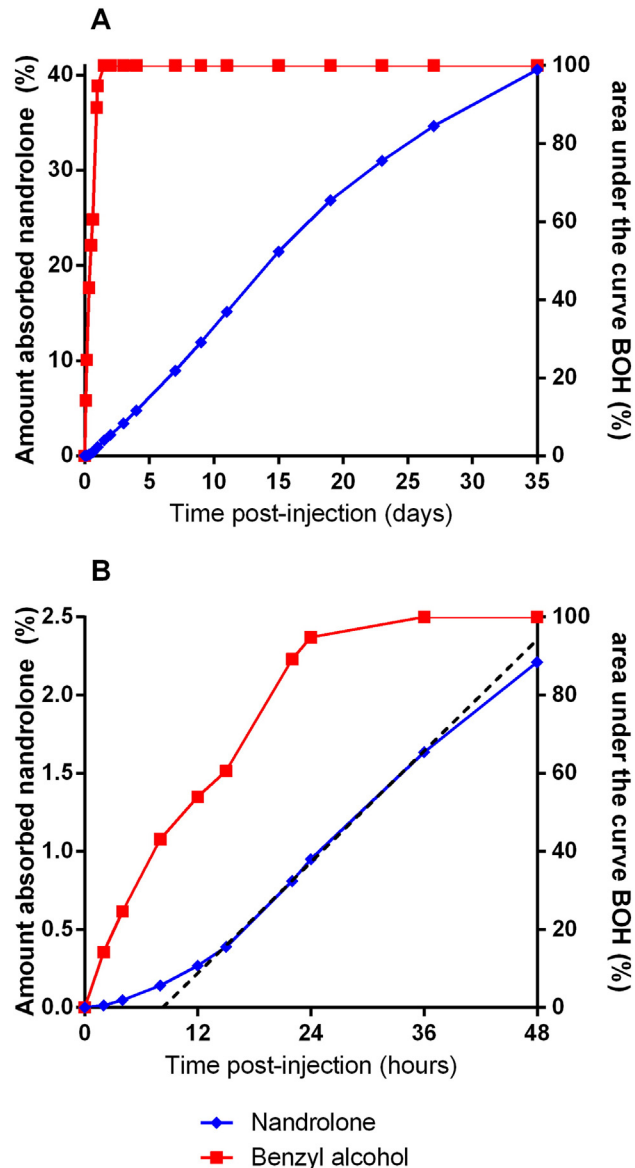


Fig. 4. Cumulative amount of absorbed nandrolone (◆) is represented as percentage of the recovery in serum at the left y-axis. The right y-axis shows the cumulative AUC of BOH (■) as percentage of total AUC. (B) The intercept of the dashed line with the x-axis shows the lag time of nandrolone.

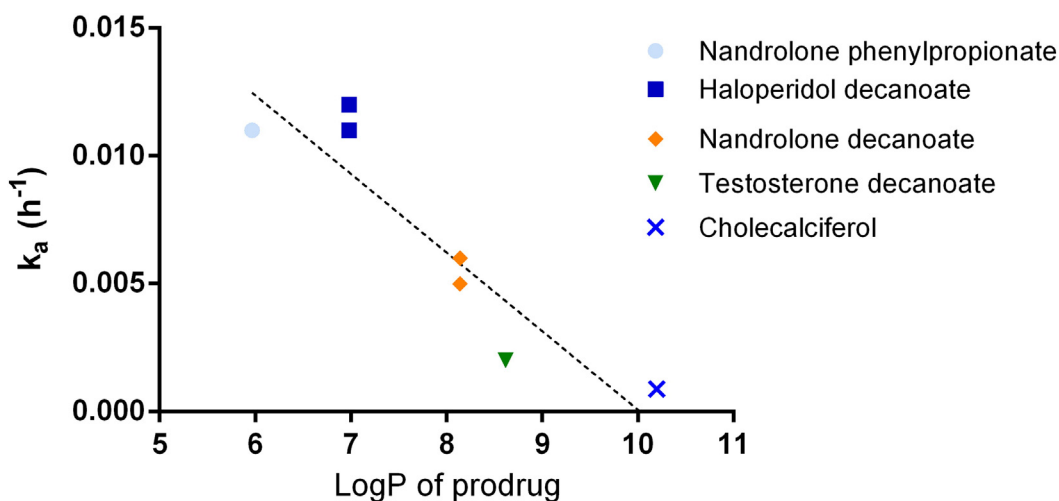


Fig. 5. Absorption rate constant (k_a) of the parent compound as a function of the prodrug partition coefficient. Figure includes only depots administered in the gluteal muscle. The dashed line represents the 'best-fitted' relation of prodrug logP with k_a . Here, logP is the theoretical distribution between octanol and water. See Table 2 for raw data and references (cholecalciferol (32)).

published yet, Wijnand et al. (1985) assumed that the time needed for hydrolysis of 2.3×10^{-5} $\mu\text{mol/mL}$ ($=0.01$ $\mu\text{g/mL}$) nandrolone decanoate in serum is presumably below one hour. They based their assumptions on data published by Van Der Vies 1970. In the present study, released nandrolone decanoate will then be hydrolysed within one hour in plasma, because all nandrolone serum concentrations were below 1.0×10^{-5} $\mu\text{mol/mL}$ (Fig. 2B). Yet a delayed absorption is seen, indicating that ester hydrolysis is not occurring in the interstitial fluid, but probably only in the central circulation. When hydrolysis is not immediate, the prodrug may flow towards lymph vessels, whether or not linked to proteins such as globulins. Interstitial space consists of reticular and collagenous fibers (Olszewski, 1985), to which molecules could be adsorbed. The absorption rate into the lymph depends on the rate of diffusion through the interstitium (O'Hagan et al., 1992) and pressure gradients (Swartz and Fleury, 2007). It has already been suggested by Zuidema and colleagues that the interstitial space can be compared to reversed-phase chromatography (Zuidema et al., 1988): the interstitial fluid acts as the mobile phase, whereas the cells and fibers represent the stationary phase. The lag time is probably a combination of mentioned factors above.

The nearly immediate absorption of BOH suggests a fast pathway to the central compartment (Fig. 1). Oil depots injected into muscle or subcutaneous tissues are surrounded by (micro) blood vessels. It is well known that blood vessels are natural barriers, because they are constructed out of a concatenation of endothelial cells. Porter and Charman showed that small molecules (<2000 molecular weight) with a logP (octanol/water) under five enter the systemic circulation directly after oral ingestion (Porter et al., 2007). These substances can pass capillary walls which result in instantaneous absorption into the systemic circulation (Porter and Charman, 2000). Analogous to oral absorption, parent compounds can immediately enter the bloodstream when they meet the two chemical properties molecular weight and logP.

It is interesting to realize that drug absorption from an oil depot is not well described by a simple two phase mass transfer model. There is obviously a considerable resistance towards mass transfer in the tissue surrounding the depot, which is given in Fig. 1 as a tissue membrane. The presence of this membrane fits with the finding of a substantial lag time for nandrolone. The difficulty with this theoretical presentation is that this membrane represents body tissue where not

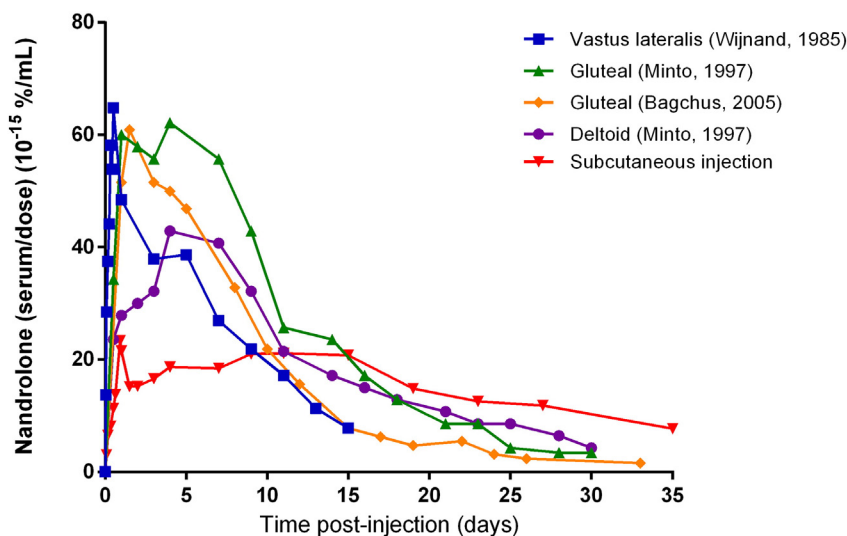


Fig. 6. All i.m. administered injections (deltoid (●), gluteal (◆ and ▲), vastus lateralis (■)) were 1 mL at a concentration of 233 $\mu\text{mol/mL}$ nandrolone decanoate. The 0.5 mL s.c. injection (x) had a concentration of 117 $\mu\text{mol/mL}$ nandrolone decanoate. Y-axis shows the concentration in percentage of serum level divided by amount administrated. See Table 2 for raw data and references.

only diffusion takes place, but where also lymphatic transport and hydrolysis may play a role.

3.3. Absorption

After the lag time period, a steady absorption is established (Fig. 4), resulting in a plateau as seen in Fig. 2B. In general, the obvious parameters that have influence on drug release can be understood from Fig. 1; the higher the concentration in the depot, the higher the driving force for release (Bagchus et al., 2005). The same holds for the surface area. Minto et al. (1997) have shown that a simultaneous increase in volume and proportional decrease in concentration does not change the absorption significantly (Table 2). It has been postulated (Tanaka et al., 1974; Weng Larsen and Larsen, 2009) that the digestion of oil would play a role in the rate at which compounds are absorbed, but that is not confirmed by our data.

After approximately two weeks, the plasma profile declines, which in fact reflects the depletion of the depot; the concentration gradient gradually decreases because of the amount of drug that already has been released. This is generally referred to as ‘flip-flop’ pharmacokinetics; what might be perceived as the elimination rate constant basically equals the absorption rate constant (k_a) from the formulation (Wijnand et al., 1985; Yáñez et al., 2011). In the case of the oil depots studied, a higher k_a reflects a faster depletion of the depot, which actually means that the resistance towards mass transfer in the surrounding tissue is relatively low. As discussed above, this mass transfer is determined by e.g. the diffusion through the interstitial fluid and the flow of the lymphatic system into the circulation.

Fig. 5 shows that the absorption of ingredients from depots injected in the gluteal muscle is determined by the partition coefficient of the prodrugs. The role of logP is twofold; it determines the concentration C_{tissue} , which means that a high logP will yield a low C_{tissue} and subsequently a low driving force for mass transfer in the aqueous phase (Fig. 1). At the same time, a high logP results in increased absorptive and adsorptive interactions with tissue components by which the permeability decreases. Similar relationships have been published for intramuscularly injected beta blockers (aqueous solutions) in pigs (Kadir et al., 1990) where the absorption rate appeared also to be affected by the lipophilicity. Remarkably, there is no such relationship as depicted in Fig. 5 for the parent compound, which seems to confirm our conclusion that it is the prodrug that is transported through the tissue and that conversion to the parent compound takes place in the central compartment.

Absorption rate constants of different compounds were compared after administration in the gluteal muscle (Fig. 5). In contrast, Fig. 6 shows the normalized profiles of equal formulations injected in different muscles: As can be seen, the site of injection appears to have a considerable influence on the absorption. For comparison, also the subcutaneous injection of the present study is given. It must be noted however that used oil as well as volume of injection were different. Our own experiments (based on Andrés et al., 2015, procedure 2) showed that the type of oil does not affect the partition coefficient of nandrolone decanoate: $\log P(\text{sesame oil:serum}) = 2.36$ and $\log P(\text{arachis oil:serum}) = 2.44$ (both mixed with 10% BOH), which emphasizes the low absorption from the subcutaneous injection.

The i.m. depot formulations were equal, i.e. they contained the same compound at the same concentration in the same volume. This limits the amount of explanations for these differences. First, it can be speculated that the surface areas of the depots were different in the three muscles because of differences in spreading. The larger the surface, the larger the total mass flux (Φ''). Unfortunately, the surface areas of injected oil depots are unknown. This has been a reason for us to start a study to visualize the fate of oil when injected in muscles, results of which will be reported later. The role of surface area may also be relevant for the s.c. injection; the absorption was significantly lower, which can be attributed to the smaller surface area of the depot as it is likely that the shear forces in subcutaneous tissue are substantially lower than those in muscles. In addition, the way the compound is being transported through the different tissues may be different. In this respect, the lymphatic flow rate may therefore be interesting to study as well.

Fig. 6 shows that absorption from the deltoid muscle is much lower compared to that from gluteal and vastus lateralis muscles. This is counterintuitive, since blood flow in deltoid muscle is higher than the flow in gluteal and vastus lateralis (Evans et al., 1975). However, as has been argued, it is not the blood perfusion but the lymphatic drainage that determines drug absorption in this case. Table 2 shows that the ND absorption rate constant from the deltoid muscle is lower compared to the gluteal and vastus lateris muscle. There appears to be a relationship between the absorption rate constant and the lag time within one muscle group (Fig. 7). The reasonable explanation for this must be that both parameters may be a result of the same variable. For example, when the differences in release are due to different surface areas, the lag time would be the same, since the way the compound is transferred to the blood stream would not be changed. Therefore, it is more likely that the differences in transport through tissue, such as lymphatic transport

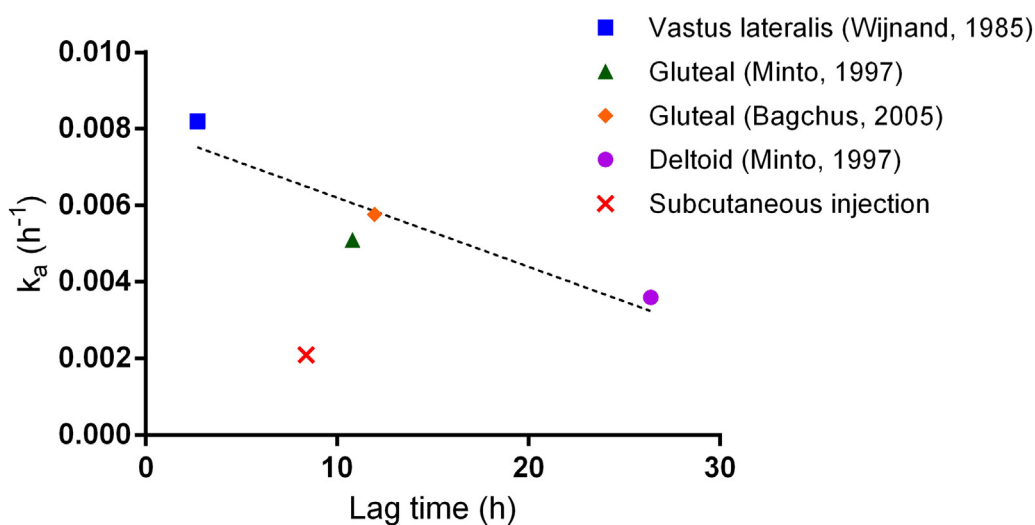


Fig. 7. Plot of absorption rate constant (k_a) and lag time of nandrolone administered at different injection sites: deltoid (●), gluteal (◆ and ▲), vastus lateralis (■) muscle and subcutaneous tissue (x). All i.m. injections were 1 mL at a concentration of 233 $\mu\text{mol/mL}$ nandrolone decanoate. The s.c. injection (0.5 mL) had a concentration of 117 $\mu\text{mol/mL}$ nandrolone decanoate. See Table 2 for raw data and references.

may determine the absorption phase. This would also explain the considerably deviating results of the s.c. injection; the low release rate may be attributed to a low surface area, whereas the relatively short lag time may be a result of relatively high lymphatic flow or shorter lymph vessel. Unfortunately, little is known about the human lymphatic flow rate in the studied muscles, although one article reported a lymph flow rate of 0.25–0.41 mL/h in the superficial lymph vessel of the leg (Olszewski and Engeset, 1980). In rats and rabbits however, skin lymph flow (measured as albumin clearance rate) is shown to be higher compared to the muscle lymph flow (Bach and Lewis, 1973; Renkin and Wiig, 1994). Of course, this does not necessarily mean that this also applies to the human situation.

Another approach to verify the suggested mechanism would be to change the lymphatic flow. Two studies report a raised lymph flow (measured as albumin clearance rate) in the lymphatic vessels of the vastus lateralis muscle during exercise (Havas et al., 1997) or during massage (rabbits) (Ikomi et al., 2014). However, Soni et al. (1988) described no increased absorption of fluphenazine after exercise or massage from a fluphenazine decanoate sesame oil depot, injected in the tight and buttock skeletal muscle. Clearly, there is no unambiguous explanation for the observed differences at this moment. Future studies on this subject should provide clarity.

4. Conclusions

This study discusses critical parameters that determine the release and absorption mechanisms of (active) substances from oil depots. It is shown that small molecules (e.g. BOH) are directly and fully absorbed, while larger, more lipophilic substances (e.g. prodrugs) exhibit an incomplete and slow absorption pattern. A lag time is seen, which is a critical parameter for absorption into the systemic circulation. This means that the absorption of compounds from a depot is significantly affected by the mass transfer in the tissue. The more lipophilic the compound, the more this plays a role. It is suggested that concentration of the compound in the oil, the in situ surface area of the depot as well as the partition coefficient of the compound are the most important formulation parameters. The mass transfer is mainly determined by the lipophilicity of the compound, while also the lymphatic flow is suggested to be relevant for drug absorption. In oil depots, BOH is often used as an excipient. It appeared that the absorption of nandrolone is enhanced by the presence of benzyl alcohol in the first few days. Subsequently, upon BOH depletion, a change in absorption of nandrolone is seen. Injections of equivalent formulation in different muscles demonstrate that the mass transfer through these tissues is not the same. It is argued that this may be due to differences in lymphatic transport.

Conflicts of interest

The authors declare no conflict of interest.

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