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Invited Review

Release and absorption rates of intramuscularly and subcutaneously injected pharmaceuticals (II)

J. Zuidema *, F. Kadir, H.A.C. Titulaer, C. Oussoren

Department of Biopharmaceutics, Faculty of Pharmacy, Department of Biopharmaceutics PO Box 80082, Utrecht University, 3508 TB Utrecht, The Netherlands

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Abstract

The rate and extent of intramuscular (i.m.) and subcutaneous (s.c.) drug absorption are very erratic and variable. The lipophilicity of the compound plays an important role. Aqueous drug solutions and suspensions of the more lipophilic compounds are often absorbed incompletely within the therapeutically relevant time. More hydrophilic compounds are absorbed completely. Injection depth, drug concentration and vehicle volume, pH-pK_a relation, vehicle, cosolvents and surfactants have strong influences on the absorption profile of lipophilic drugs. Aqueous solutions of hydrophilic drugs are less sensitive to these factors. Drug solutions in oil and even suspensions in oil are often thought to be sustained release preparations. In fact, rapid absorption has often been observed. Slow release is not a property of the oily vehicle but is achieved by a high lipophilicity of the dissolved or suspended compound. Liposomal preparations are currently under investigation as i.m. and s.c. injectable sustained release preparations. Factors that induce drug release at the injection sites are the proteins and especially lipoproteins in the interstitial fluids, originating from serum filtrate and from turnover of inflammatory cells. Phagocytosis by macrophages and fat cells may play an important role in the local clearance of liposomal material from the injection site. Sustained release of some pharmaceuticals with normal or long half-lives appeared in specific cases preferable to rapid release. In addition, high arterial drug concentrations during the absorption phase may result in undesired effects even when venous drug concentrations are within the safe range.

Key words: Drug absorption; Intramuscular administration; Subcutaneous administration; Absorption rate

1. Introduction

The intramuscular and subcutaneous routes of drug injection are often used when drugs cannot be injected intravenously because of their low aqueous solubility and/or when high peak concentrations, resulting in local or systemic side effects, occur with intravenous injection. Moreover, additional advantages of these routes include greater convenience, less problems with compatibility of the injection components with full blood in the ciculation and often less frequent administration when compared to intravenous administration.

Many variables are known to affect drug release after intramuscular or subcutaneous injection. Factors such as molecular size, pK_a, drug

* Corresponding author.

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solubility, initial drug concentration, injection depth, body movement, blood supply at the injection site, injection technique and properties of the vehicle in which the drug is formulated have been discussed extensively in a previous review (Zuidema et al., 1988). This article is an update with emphasis on factors related to drug transport through the tissue, the role of drug lipophilicity, recent technology to modulate drug absorption from intramuscular and subcutaneous injection sites by carrier systems such as liposomes, absorption by the lymphatic system and clinical implications.

Drugs such as antibiotics, anti-asthmatics, anticonvulsics, anxiolytics and analgesics are often administered intramuscularly in severe disease states. A generally held viewpoint is that the drug is rapidly and completely absorbed from the injection site. Previously published data have already demonstrated that complete absorption during a time relevant for therapy is not true in every case (Ballard, 1968; Dundee et al., 1974; Kostenbauder et al., 1975; Tse and Welling, 1980), however, recognition of their significance is lacking. Such findings may have important clinical implications.

Consequently, this article is aimed at reviewing the relevant literature, in order to provide and to discuss material for the rational design of intramuscular and subcutaneous drug formulations and to examine the clinical aspects of these types of injections. In contrast to the former review which was organised in order of the types of injection, this article is mainly ordered with respect to elements of the mechanism and further subdivided in types of injection.

2. Drugs in conventional systems

Conventional systems are solutions, emulsions and suspensions in aqueous or in oily vehicles.

2.1. Drugs in rapidly releasing systems

2.1.1. Aqueous injections; variability in absorption rate

It has frequently been reported that absorption after intramuscular and subcutaneous injection is very variable (Gibaldi, 1977). This is first illustrated with some aqueous solutions.

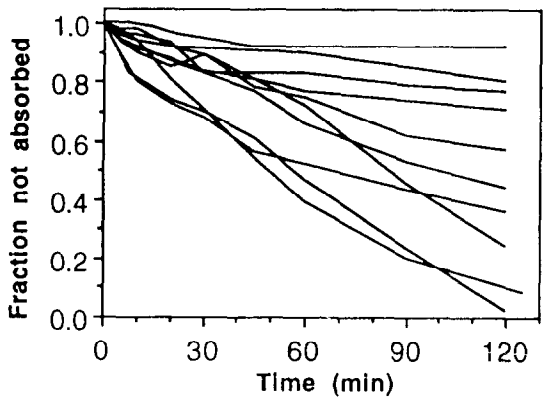

Fig. 1. Fraction remaining to be absorbed after intramuscular injection of 20 mg/kg sodium artelinate aqueous solution in rabbits ($n = 10$).

Artelinic acid is a water-soluble derivative of artemisinin, an antimalarial drug, of the fast-acting schizontocidal type. Artemisinin and its derivatives are important new drugs, especially for the treatment of life-threatening states of the disease. Artelinic acid is even more active than artemisinin itself, but is very rapidly eliminated after intravenous injection. Rapid and complete absorption is therefore essential. After intramuscular injection in rabbits the absorption rate appears to be very variable (Titulaer et al., 1993). This is depicted in Fig. 1 where the fractions not absorbed are plotted vs time.

The curves representing the fraction remaining to be absorbed suggest an apparent zero-order absorption. This is a rather unexpected phenomenon, since diffusion is characterised by a first-order mechanism. A possible explanation is a solvent flow dependent paracellular transport of this highly hydrophilic solute. This transport capacity is very variable, at least between subjects, and it appears that it is influenced by several factors including muscle activity, inflammation and flow of the tissue fluid (Zuidema et al., 1988a). This explanation is supported by the next example.

Relevant information on kinetic behaviour of i.m. and s.c. injections has often originated from

veterinary studies. The risk of residual drug at injection sites is a considerable problem in meat consumption. Fig. 2 shows as a second example the large variation in absorption parameters after intramuscular and subcutaneous injection, in a fat-rich region, also referred to as intra-adipose injection (Kadir et al., 1990a). Carazolol is a β-blocking agent which is used in veterinary practice as a tranquillising agent in cattle and pigs. The fraction of carazolol absorbed during the first 24 h from an aqueous solution varied from 24 to 59% after intramuscular and from 25 to 66% after intra-adipose injection.

Many factors which influence the variability in the rate and extent of absorption can be postulated. Firstly, a difference between intra- and intermuscular injection is postulated and defined as injections within and between the muscle fibrils, respectively (Groothuis et al., 1980). Such a supposition must be supported by a bimodal statistical distribution in absorption rate. In the literature, however, experimental evidence for this contention is lacking. Secondly, differences in drainage and blood flow are possible explanations. The cause of these differences, however, remains unclear. Thirdly, differences in absorption rate might also be result from differences in osmolality and other formulation factors, however, such factors cannot explain variability with the same preparation and batch. Physiological circumstances that vary randomly and physiological reactions to the injection trauma might influence absorption.

A more likely explanation than those mentioned above is a variation in the shape of the depot. The shape may vary from merely spherical to almost needle-shaped in different subjects. These differences depend on the local cohesion between the muscle components and the tendency to be torn open by the injection procedure. Differences in shape are accompanied by differences in the depot surface (and therefore in the effective permeation area), the interface between depot and tissue and the absorption rate.

2.1.2. Drug lipophilicity in aqueous systems; extent of absorption and absorption rate

Lipophilic compounds are slowly absorbed from intramuscular and subcutaneous injection sites (Zuidema et al., 1988). Recent findings show that absorption under such conditions often seems to be incomplete as well. It appeared that the apparent half-lives of midazolam in patients after intramuscular injection are much longer than after intravenous injection, due to rate-limiting sustained release from the intramuscular injection site (Raeder and Nilsen, 1988). In a former study by the same group, a reduced apparent bioavail-

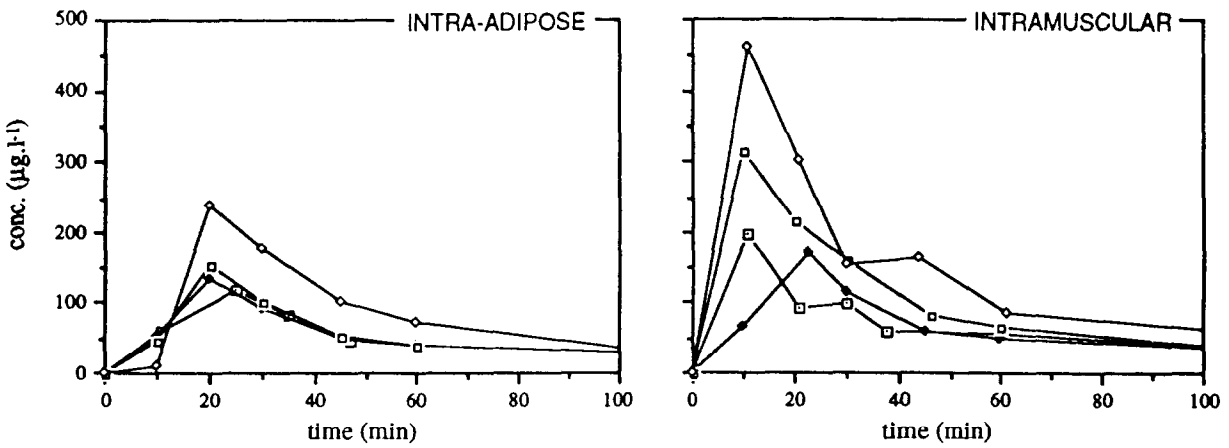

Fig. 2. Individual concentration-time curves of carazolol in the serum of four pigs following intramuscular and subcutaneous administration of 0.025 mg/kg in a fat layer (intra-adipose injection).

ability of midazolam under these conditions was also observed (Raeder and Breivik, 1987).

Phenobarbital appeared to be completely absorbed after intramuscular injection in deltoid muscle in children, however, the bioavailability was only 80% relative to oral administration in adults (Viswanathan et al., 1978). Nevertheless, the number of investigated subjects was too small to permit definite and statistically warranted conclusions. A lack of stability of phenobarbital (the amide bonds might be hydrolysed at the injection site) might be proposed as an explanation for the incomplete bioavailability. This possibility cannot excluded, however, it does not explain the difference between children and adults nor the findings in the midazolam study. Further information is needed for a better understanding of the factors which determine bioavailability in these specific cases.

The β-blocking agents are an ideal group for studying drug lipophilicity and release from intramuscular and subcutaneous injection sites, since they have similar molecular weights and pK_a values but differ markedly in lipophilicity. Studies in pigs using crossover experiments with propranolol, atenolol, carazolol, metoprolol and alprenolol have recently been published (Kadir et al., 1990a,b).

The curves representing the fraction remaining to be absorbed of the β-blocking agents, constructed from intramuscular and subcutaneous (intra-adipose) plots and using intravenous data as references, demonstrate biphasic declines; a rapid first phase followed by a very slow second phase (Fig. 3). Initial release rates appeared to be negatively correlated with drug lipophilicity expressed as fat-buffer partition coefficients, especially after injection in the subcutaneous fat layers, also called intra-adipose layers. Propranolol showed greater and faster absorption than expected from its lipophilicity only after intramuscular, but not after intra-adipose, injection. Propranolol is known to possess irritating properties which may improve blood perfusion in the muscles and account for the deviation in behaviour after intramuscular injection. The subcutaneous fat layer or adipose layer is less sensitive to such irritating properties and is less perfused.

The extent of drug release within the mentioned 24 h also transpired to be dependent on the lipophilicity of the compound: the more lipophilic the compound, the lower the bioavailability at 24 h after injection (the observation period) (Fig. 4). The most hydrophilic compound, atenolol, was the only one which was completely absorbed or bioavailable within 8 h after intramuscular injection and within 24 h after subcutaneous injection (Fig. 3).

Injected drugs are probably rapidly absorbed, provided sufficient vehicle is present to maintain the drug in solution or to drive the absorption process. After the vehicle has been absorbed the

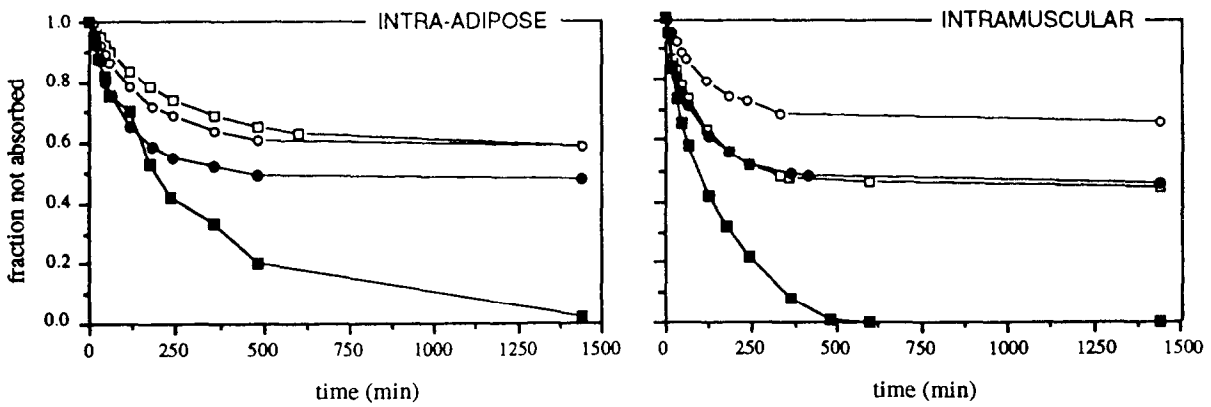

Fig. 3. Fraction remaining to be absorbed curves (drug vs time) after intramuscular and intra-adipose administration of a series of β-blocking agents. (□) Propranolol, (○) alprenolol, (●) metoprolol, (■) atenolol.

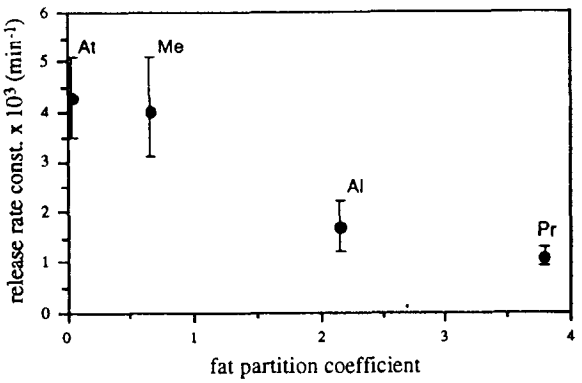

Fig. 4. Correlation between the fat-buffer distribution constants and release rates on intra-adipose administration of atenolol (At), metopropol (Me), alprenolol (Al) and propranolol (Pr).

absorption rate of the drug decreases rapidly. This theory explains the midazolam studies but is also relevant in the case of the rapid absorption of artemisinin from an intramuscularly injected suspension in oil and the low and erratic absorption of artemisinin from a suspension in water as shown in the following (Fig. 5).

2.1.3. Oily injections; influence of the lipophilicity of the vehicle on the absorption rate

The next example is an oily injection. Artemisinin is rather lipophilic and not soluble in water, however, it is also sufficiently insoluble in oil to allow its preparation as a dissolved injection as of a conventional oil system with a sufficiently high dose.

Oil systems and suspensions for injection are generally considered to be sustained release formulations. Therefore, the rapid onset of absorption shown in Fig. 5a with the artemisinin suspension in oil is striking (Titulaer et al., 1990b). The oily vehicle is absorbed only very slowly and remains present at the injection site for several months. Apparently, artemisinin dissolves rather rapidly in the oil phase and the dissolved fraction is then depleted by further absorption. In the case of artemisinin, this depletion is apparently a rapid transit process over the oil to the water interface to the tissue fluids. This is less favoured in the case of highly lipophilic substances.

In contrast to the oily system, the rate of dissolution of artemisinin in the aqueous injection is slow and the process appears to cease almost completely within the first few hours, the time during which the aqueous vehicle has been absorbed.

In the preceding section, studies have been discussed in which the lipophilicity of a drug or model compound was the variable in a given aqueous medium. Interestingly, a study has appeared in which the drug was chosen as the constant and the lipophilicity of the oily vehicle was the variable (Table 1) (Al-Hindawi et al., 1986). The in vivo release of testosterone propionate in a number of oily vehicles was investi-

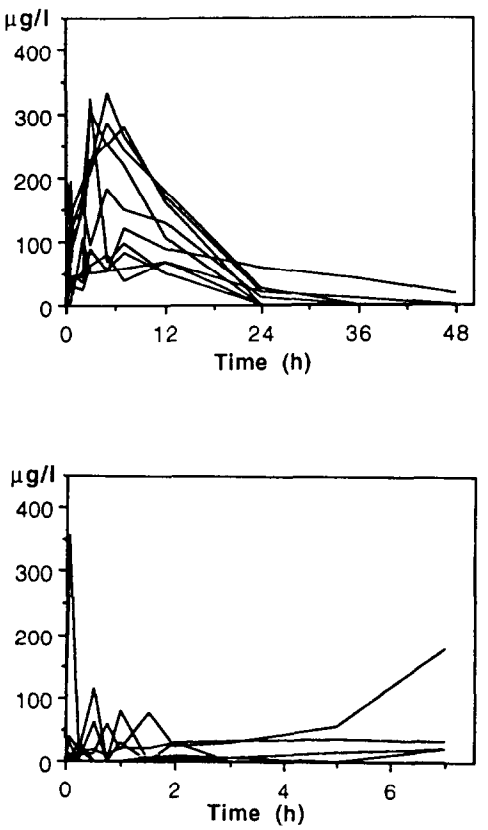

Fig. 5. Plots of artemisinin concentrations in serum vs time ($n = 10$) after a dose of 400 mg artemisinin to human volunteers: (a) suspension in oil intramuscularly; (b) suspension in an aqueous vehicle intramuscularly.

Table 1
Testosterondecanoate fat/phosphatebuffer partition and absorption rate expressed as half-life in the muscle

Solvent	Partition coefficient ($\times 10^{-3}$)	$t_{1/2}$ in muscle (h)
Ethyl oleate	6.3	10.3
Octanol	5.3	9.7
Isopropyl myristate	4.3	7.8
Light liquid paraffin	1.3	3.2

gated. Disappearance from the injection site appeared to be proportionally related to the in vitro partition coefficients. This study illustrates and emphasises the importance of the vehicle and the affinity of the drug to the vehicle for the disappearance process in the muscles after injection.

2.1.4. Influence of injection volume and drug concentration

Data on the influence of injection volume and drug concentration seem to be contradictory. They can be classified into the following four groups of results on: (i) hydrophilic drugs in aqueous systems (concentrations are much lower than drug saturation in the vehicle); (ii) lipophilic drugs solved in oily systems; (iii) lipophilic drugs in aqueous systems (concentrations are close to drug saturation in the vehicle); and (iv) drugs in suspension. The latter is discussed in section 2.2, since most of the suspension formulations are intended to be sustained release formulations. The available information on the first three groups is summarised below.

Atropine, sodium chloride, sugars and polyols such as mannitol and sorbitol, all *hydrophilic drug solutions in aqueous systems*, were reported to be absorbed more rapidly when the compounds were administered in smaller injection volumes (Warner et al., 1953; Schriftman and Kondritzer, 1957; Sund and Schou, 1964). The common properties of these compounds are that they are very hydrophilic, readily soluble in water and have low molecular weights. Examples of compounds with higher molecular weights are the dextrans, which appear to behave similarly but have a slower rate of absorption. The molecular weight appears to be inversely related to the absorption rate. The higher absorption rate in smaller volumes can be explained by the greater diffusion potential. Obviously, the absorption of this type of compound is controlled by passive diffusion or by paracellular transport.

Results with amikacin, an aminoglycoside, are often misinterpreted (Pfeffer and Harken, 1981). In the available literature, these results are often wrongly discussed together with the first group. Amikacin is a hydrophilic compound but with a high molecular weight. In aqueous systems it is a suspension, which is slowly absorbed from a single large depot. It is preferably given in multiple, simultaneously administered separate injections, in order to provide sufficiently high concentrations in serum. The solved amikacin fraction is constant and concentration is therefore not a useful absorption rate-determining variable variable. This in contrast to the situation with atropine, sugars and polyols as discussed above.

The effects of drugs which depress absorption rate are illustrated with atropine. At higher doses it exerts a self-depressive effect on the absorption rate by its parasympathicolytic activity. Effects of this type have scarcely been investigated and literature data are sparse.

Testosterone and some other model compounds represent examples of the second group, *lipophilic substances in oil*. They have been studied in several oily vehicles (Tanaka et al., 1974). This type of compound is absorbed more rapidly when it is dosed in smaller volumes of an oily vehicle. Again the diffusion potential is obviously the dominating absorption rate-determining factor.

The influence of injection volume on the bioavailability of *lipophilic drugs in aqueous solvents*, the third group, has been studied in rats (Kadir et al., 1992c). The aforementioned study was performed in order to find an explanation for the incomplete absorption of the more lipophilic β-blocking agents as described in section 2.1. Propranolol was used as the model compound.

Both the rate and the extent of absorption at 8 h appeared to increase with increasing injection volume. When the vehicle volume is increased the residence time of the vehicle at the injection site increases, maintaining the drug in solution

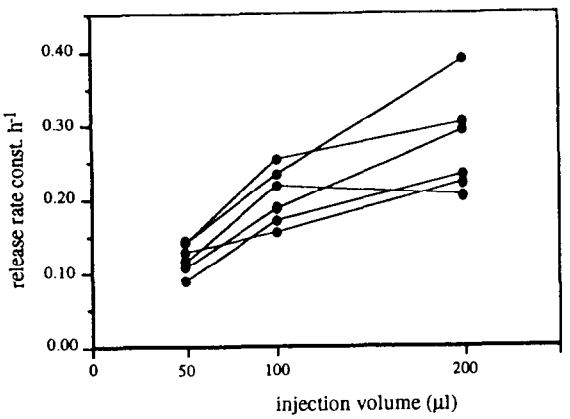

Fig. 6. Individual release rate constants after intramuscular injection of 3 mg propranolol HCl in 50, 100 and 200 µl aqueous solution in rats. Lines connect the individual values for the same rat ($n = 6$).

for a greater length of time; in other words, it remains dissolved over a longer time and absorption is faster from the dissolved state (Fig. 6). Moreover, the increasing volume may increase the vehicle flow away from the depot This influence is in a certain sense comparable with the action of the mobile phase in a chromatographic system. In HPLC terms: increasing solvent flow diminishes the retention time. This effect may be a possible explanation for the greater absorption rate during the initial phase.

2.1.5. *Influence of pH and / or cosolvents*

Cosolvents such as ethanol, glycerol, propylene glycol and also polyethylene glycol 400 are used as solvents together with water, in order to enhance the solubility of certain drugs for injection (Chen-Der and Kent, 1982). They have varying influences on the absorption of drugs. Ethanol is better discussed separately in the next survey, since the mechanism of its influence deviates from those of the other cosolvents.

Ethanol is a liquid of very low viscosity and exerts only a minor influence on the viscosity of the vehicle in mixtures with water. When used at high concentrations it has a denaturing effect on proteins at the injection site. It appears to have an inhibitory effect on the absorption rate of water-soluble ionic as well as non-ionic model drugs such as isonicotinamide, methylisonicotinate, isonicotinic acid and procaine hydrochloride (Kobayashi et al., 1977). This influence could be attributed to alterations in the permeability of the connective tissue which was significantly decreased. In chromatographic terms: ethanol seems to change the properties of the 'stationary phase', probably by protein denaturation.

Other cosolvents such as propylene glycol, glycerol and polyethylene glycol 400 have been reported contradictorily to diminish and to enhance the absorption rate of model compounds (Kakemi et al., 1972; Cheng-Der and Kent, 1982). The diminishing effect is ascribed to the viscosity-enhancing influence of these compounds on the vehicle.

Effects of cosolvents may partly be explained by a change of the properties of the 'mobile phase'. The dielectric constant and consequently the hydro/lipophilicity of the solvent is changed to that of water. The enhancing effect of cosolvents and the properties of the systems where enhancement occurs are discussed below, together with the effects of pH-pK_a relationships.

Additives with high molecular weights, known as viscosity enhancers, such as cellulose derivatives, dextrans or long-chain polyethylene glycols resulted in a degree of inhibition that was less than expected. This suggests that macromolecules apparently do not interfere with the absorption mechanisms.

The absorption rate of cefonicid has been described as being pH-dependent (Brumfitt et al., 1988). However, the authors failed to give an explanation nor is it possible to analyse their results, since the experimental data were lacking in detail. Other studies shed more light on the pH dependency in i.m. and s.c. absorption processes.

Salts with an alkaline or acidic reaction which can be neutralised by the tissue fluids have the potential to precipitate after injection due to the neutralising or buffer capacity of the tissue fluids. This has been briefly mentioned in the literature for quinidine hydrochloride as well (Tse and

Welling, 1980). The effect, however, is clearly illustrated in the study using human volunteers by Kostenbauder et al. (1975) with intramuscular phenytoin (see section 1). Phenytoin is absorbed over a period of approx. 5 days. Even after 40 h 20% of the drug remained unabsorbed. Phenytoin is a drug which is dissolved for injection in relatively high concentrations at pH 11 or higher, and also using cosolvents and/or complexing agents.

The authors reported that precipitation and slow redissolution of the drug by tissue fluids at the injection site could explain their results and developed a mathematical model based on this concept. The observed drug concentration curves in plasma fitted well with this model. Precipitation probably has two causes, i.e., the pH neutralising effect of the tissue components and the rapid absorption of some of the solvents.

The influence of cosolvents on the absorption of salts is illustrated by a study of the effect of propylene glycol on the absorption of benzimidazole hydrochloride (Cheng-Der and Kent, 1982). It appeared that propylene glycol, which apparently is absorbed more slowly than water, may prevent in certain circumstances, at least partly, the precipitation of the free base (or free acid). In this manner, it might *enhance* the absorption of the drugs in question.

2.1.6. Influence of surfactants

The influence of surfactants and injection depth on the kinetics of absorption has been discussed previously (Zuidema et al., 1988). Non-ionic surfactants at low concentrations probably exert a retaining effect on well-absorbed water-soluble drugs, however, the promotion of absorption has been reported for a poorly absorbed high molecular weight polypeptide. The mechanisms underlying this phenomenon are still not well understood. Again the model of reversed-phase chromatography may be helpful.

Surfactants in the mobile phase might have a coating effect on the lipophilic stationary phase, making the stationary phase more hydrophilic. This might explain the retaining effects on hydrophilic drugs. Such a model predicts the reverse effect on lipophilic drugs.

2.1.7. Influence of injection depth

In the articles discussed above, the difference in absorption rate between deep intramuscular injections and shallow subcutaneous or intra-adipose injections is shown in Fig. 3 (Kadir et al., 1990b). Even the hydrophilic atenolol is already completely absorbed 8 h after intramuscular injection whereas this lasts about 24 h after subcutaneous or intra-adipose injection. The fatty subcutaneous connective tissues and adipose layers are more lipophilic and perfused less than muscular tissues. These phenomena have been discussed extensively previously (Zuidema et al., 1988).

2.2. Long-acting systems

Long-acting systems consist either of lipophilic drugs in aqueous solvents as suspensions or of highly lipophilic drugs dissolved or suspended in oil. In the first case, the release or absorption is dissolution rate controlled, while in the latter, the compounds with lower molecular weights, which are soluble in oil, are 'phase transfer controlled' released from the system (Zuidema et al., 1988); the compounds with high molecular weights, which are not soluble in the oil, are released by dissolution and/or phase transfer control, these factors alone or in combination.

Another possible absorption mechanism is phagocytosis. The participation of direct absorption of fine particles by phagocytosis appears to be possible in certain cases but the contribution to the overall absorption process can mostly be neglected. Absorption via the mechanisms of lymphatic transport and inflammation-mediated appearance of phagocytosing macrophages (24–48 h after injection) have been demonstrated for iron complexes (Beresford et al., 1957).

Long-lasting residues after intramuscular injection or subcutaneous injection, of water-insoluble penicillin derivatives and dihydrostreptomycin in aqueous vehicle in cattle have been detected 30–45 days after administration (Mercer et al., 1971). Examples of long-acting systems in oil are the depot neuroleptics as discussed previously (Zuidema et al., 1988).

Ober et al. (1958) stated that aggregation in

concentrated systems often gives rise to increased viscosities, specific rheological features and a diminished rate of dissolution after injection. It is known that viscosities and specific rheological features such as (pseudo)plastic behaviour in suspensions and emulsions increase with increasing concentration and with decreasing particle size.

In fact, this process is associated with the so-called flocculation phenomenon. This is because gel formation consists of aggregation to a large viscous aggregate in which vehicle might be included (structured water or, in oil, structured oil), which is a special case of flocculation of lyophilic dispersions. These systems display mostly (pseudo)plastic properties. This theory is largely expounded in the context of colloidal systems but is it also valid for systems of small non-colloidal particles.

When either lyophilic or lyophobic systems are concentrated by precipitation under gravitational forces or by other compression forces, they might develop a more compact form of aggregation, comparable with the phenomenon referred to as caking.

This theory, applied to suspension preparations for injection, was provided with a solid and mathematical foundation by the work of Hirano et al. (1981) and Hirano and Yamada (1982, 1983a,b). It is relevant to review this work in a little more detail, since it is also pertinent to the behaviour of liposomes, as discussed in section 3.

The authors studied the local clearance of suspensions of practically water-insoluble drugs injected intramuscularly or subcutaneously in the rat. They used these results to develop or to check their mathematical model. This work is of great importance for understanding the influence of the phenomenon of aggregation on the release kinetics. The theory is mainly applicable to those drugs for which the absorption into blood or lymph is controlled by dissolution. Phase transition of oily systems and blood supply are not thought to be relevant. As is clear from the former study, this is a simplification and not true for every system (Zuidema et al., 1988).

Hirano and co-workers expressed the influence of aggregation as a factor ϵ with two limits: no aggregation and complete aggregation. In the first case, the suspension particles behave independently, while in the latter, the aggregate behaves as a single clot with the clot surface acting as the effective area for release or in vivo dissolution. The parameter ϵ is governed by particle size, concentration, volume, hydrodynamic factors such as injection speed and pressure and histological and physiological states at the injection site. This means that particle size has two opposite influences on the dissolution rate: on the one hand, smaller sizes without aggregation lead to a greater effective dissolution surface and therefore to higher dissolution rates; on the other, smaller particle sizes may lead to stronger aggregation effects with more compact aggregates and subsequently to a smaller effective dissolution surface.

A deviation of this pattern occurs for particles smaller than about 2–3 μm, especially after subcutaneous injection. These particles appear to pass more easily through the fibrous networks accompanying the spreading of the dispersion medium during injection and seem to form consequently looser agglomerate. Differences between muscle or subcutaneous networks, however, were not investigated and therefore not discussed.

Dose adjustment can be effected as a change in drug concentration for a constant volume or as a change in vehicle volume at a constant drug concentration. In both cases, the absorption rate appears to decrease with increasing dose as a result of the aggregation phenomenon. The relation with volume is less pronounced than with concentration, however, rapid in vivo absorption of aqueous vehicle will lead to an increase in concentration. Hence, the absorption rate depends mainly on three factors: solubility, rate of diffusion through the surrounding medium and particle density.

This all has important consequences: firstly, increasing dosages will lead to a higher aggregation state and therefore a smaller effective dissolution area, consequently giving rise to increased sustained release characteristics of a suspension injection. On the other hand, reversibility of aggregation during an absorption process of this kind of injection may lead to deviations in the Noyes-Whitney dissolution behaviour of the suspension. Moreover, it is not the in vitro dissolu-

tion rate but rather the in vitro solubility which appears to have a positive relation to the in vivo absorption rate.

In addition, the kind of excipients used for stabilising the suspension is important. On the one hand, a better dispersed system will promote the individual behaviour of the particles, i.e., less aggregation and a greater absorption rate will occur. On the other, some excipients are known to promote the aggregation of particles, giving rise to lower absorption rates.

It can be concluded that the absorption rate of this kind of practically water-insoluble drug is obviously predominantly determined by the physical behaviour of the injection depot. This signifies that interspecies differences should not be expected, other than those caused by an increase in dose for larger animals (or humans). Studies in a range of rodents of different sizes are in line with this hypothesis.

3. Drugs encapsulated in liposomes

Liposomes are thermodynamically stable spherical phospholipid systems which can be used as drug carriers. Liposomal properties are dependent on the manner of production, size and number of concentric membranes and their composition. Liposomal membranes are built up as bilayers consisting of phospholipids and often stabilising additives such as cholesterol; they include an aqueous interior. Gel-state liposomes are liposomes with a phase-transition temperature above 37°C, mostly consisting of saturated phospholipids and some additives; fluid-state liposomes are liposomes with a phase-transition temperature below 37°C, mostly consisting of unsaturated phospholipids. Hydrophilic drugs, diagnostics or experimental compounds can be included in the aqueous interior, while lipophilic types can be incorporated into the membrane or be adsorbed to the membrane. Fluid-state liposomes are more leaky than gel-state liposomes.

The different types of liposomes can be used for a large range of applications, mostly experimental. Interesting experiments with drug- or biotherapeutic-loaded liposomes have been described and reviewed in the literature (Crommelin et al., 1991; Storm et al., 1991).

Liposomal systems are biodegradable and can be used as systems for parenteral sustained release or drug targeting. After intravenous or intraperitoneal application the period of release of the drug from the liposomes is short, being determined by the elimination and termination of the liposomes by the reticulo-endothelial system (RES). The mean absorption times (MAT) are mostly in the order of magnitude of 1 day (Titulaer, 1990b). Recently, it has been recognised that the use of specialised phospholipids, esterified with hydrophilic groups such as polyethylene derivatives, enables RES elimination to be avoided and prolongs the in vivo circulation time.

The application of liposomes as drug delivery systems for intramuscular injection has been investigated, for example, by Arrowsmith et al. (1984), using large multilamellar vesicular (MLV) liposomes. The subcutaneous injection of liposomes has been reported by Kim and Howell (1987). The therapeutic results of these types of applications have been reviewed recently (Kadir et al., 1993). The release times of the drug from the liposomal formulation (mean absorption times) are several-fold greater than after intravenous administration of free drugs. Recent experimental data have been employed in the attempt to elucidate the release mechanisms. This is reviewed and discussed below.

3.1. Formulation factors

The more basic release phenomena of drugs from liposomal preparations are illustrated by the following study. Free chloroquine was compared with (gel-state and rather large-sized) chloroquine liposomes which were injected intraperitoneally, intramuscularly and intramuscularly in mice. The study belongs to a series on the relationship between the kinetics and minimal inhibitory concentrations of chloroquine against *Plasmodium berghei* in rodents (Titulaer et al., 1990a). As assessed from the curves of drug concentration in the blood, distribution data in the heart, liver and spleen and efficacy against the parasite, it appeared that after intramuscular or

subcutaneous administration, in contrast to intraperitoneal administration, liposomes were not cleared by the liver and spleen and therefore not absorbed intact from the injection site and that the liposomes released chloroquine over prolonged periods. In this study, the maximal release time was not determined, however, the protective effects of chloroquine against challenging with parasites lasted a few weeks.

Interestingly, the concentrations after higher liposomal dosages appeared to be lower than after the lower dosages, suggesting more sustained release characteristics. During the first few hours after administration, a peak was observed in the drug concentration-time curves in plasma. Without knowing the exact cause, this is nevertheless often called a 'burst effect'. In fact, the inverse relation between dose and release rate is explained on the basis of a higher aggregation state. This is in accordance with the findings of Ober et al. (1958) and Hirano et al. (1981) and Hirano and Yamada (1982, 1983a,b) with suspensions, as discussed above. This observation stimulated more focused research on this subject and is discussed in the ensuing section.

3.1.1. Influence of injection volume

If the theories of Ober et al. (1958) and Hirano et al. (1981) and Hirano and Yamada (1982, 1983a,b), developed for solid drug particles in suspensions, essentially hold true for the more flexible particles of liposomes, then an increased volume at a fixed concentration must result in an increased rate of absorption and a greater dose or liposome concentration must correspondingly lead to a decreased absorption rate. In fact, the results might be more pronounced because the flexible structure of liposomes will give rise to a smaller effective release surface at a certain degree of aggregation ϵ. Studies with intramuscular and subcutaneous injections of liposomal chloroquine in mice have confirmed this idea (Kadir et al., 1991). A liposomal dose, corresponding to 0.6 mg chloroquine, was administered in different volumes. Subcutaneous injection resulted in lower concentrations than intramuscular administration. The absorption rate constants showed a positive correlation with injection volume after

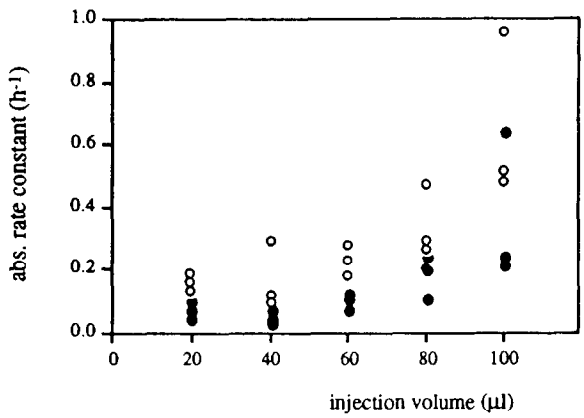

Fig. 7. Initial absorption rate constants after injection of liposome-encapsulated chloroquine, corresponding to 0.6 mg chloroquine, in different volumes after intramuscular (○) and subcutaneous injection (●).

both routes of administration (Fig. 7). The results can be explained by assuming that an aggregation process occurs under the influence of the muscle tonus and that release from the aggregate at its surface is the rate-limiting step in drug absorption.

3.1.2. Influence of liposome concentration

The aggregation theory also appears to apply in a study in mice with increasing dosages administered by intramuscular or subcutaneous injection as reported recently (Kadir et al., 1992b). Fig. 8 demonstrates that increasing dosages resulted in increasing mean residence times and decreasing initial absorption rate constants.

Within the range tested, injection of higher doses of liposomal chloroquine exhibited prolonged and comparatively slower release of chloroquine. In addition, it also appeared that the prophylactic efficacy, i.e., the time that an animal is protected against infection as measured by repeated inoculation with *P. berghei* strains, increased with the liposomal chloroquine dose.

3.2. Release mechanisms

The liposome studies, described above, were performed with gel-state liposomes containing high percentages of cholesterol. Such liposomes

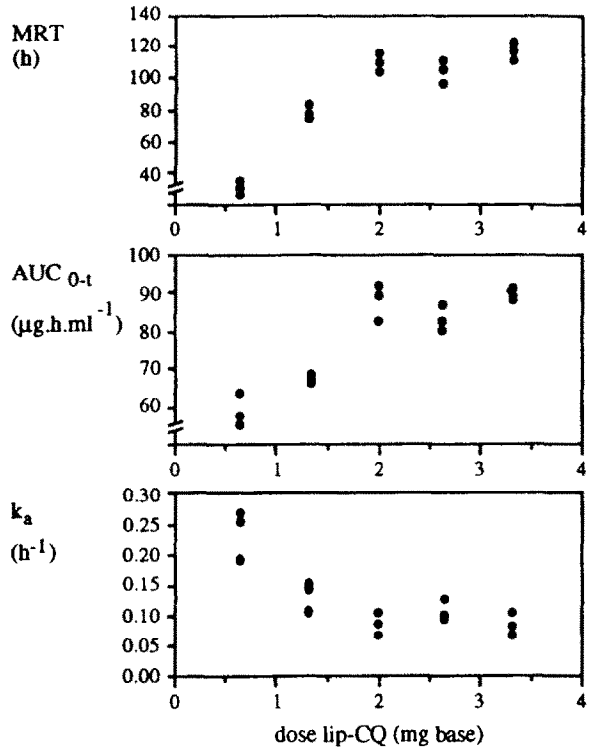

Fig. 8. Mean residence times (MRT), areas under the curve (AUC) and estimated absorption rate constants (k_a) from intramuscular injection of increasing amounts of liposome-encapsulated chloroquine, corresponding to 1.98, 2.63 and 3.30 mg chloroquine, respectively. Each dose was administered to three animals.

are very stable in vitro in buffer systems. After injection, however, liposomes lose their stability, depending on the lipid composition and additives, and release the drug more or less rapidly. This may occur rather rapidly after intravenous or intraperitoneal injection but more slowly after intramuscular or subcutaneous injection. The subsequent text provides a survey of current knowledge on the release-triggering or liposome-destabilising factor in vivo. The question is less relevant in the case of fluid-state liposomes which are much less stable in vitro and do not show a stability difference between the in vivo and in vitro situations to the same extent.

3.2.1. Enzymatic triggered release

An influence of the muscle components on the phospholipid membranes of liposomes is one of the possibilities that have been proposed to explain the loss of stability of liposomes after injection. In order to examine this idea, a study has been performed with in vitro incubation of chloroquine-containing liposomes in buffer solution, in diluted muscle homogenates and in cultured muscle cells, simulating various environments which the liposomes may encounter upon intramuscular injection (Kadir et al., 1992d). In all cases, a small amount of the encapsulated chloroquine was released during the first few minutes but thereafter the liposomes appeared very stable. Only in cultured muscle cells was a slight tendency toward greater release obserbed, however, the sample size of the experiment did not allow significant conclusions to be drawn. This study suggests that tissue components are not important release rate controlling factors. However, the experiments with the muscle homogenates were performed with diluted systems, thus hindering a definite interpretation of the results.

Experimental data with muscle interstitial fluids are not available; interstitial fluid is a filtrate of serum or plasma and contains all fractions of serum proteins, however, their concentrations are low and depend on the site. Serum or plasma appears to have liposome leakage-inducing properties. From experiments with a series of serum dilutions the leakage rate appeared dependent on the concentrations and showed saturation effects (Titulaer, personal communication). Thus, liposomes deplete plasma of its destabilising factors (Hunt, 1982).

Leakage is also dependent on the composition of the liposomes; it could be reduced by the incorporation into the liposome membranes of cholesterol or sphingomyelin and leakage is increased by lysophospholipids or an osmotic gradient across the membrane (Kitagawa et al., 1976; Finkelstein and Weismann, 1979; Allen and Cleland, 1980; Kirby et al., 1980). The leakage-inducing roles of free fatty acids, lysophospholipids, plasma proteins and (apo)lipoproteins have been investigated extensively (Black and Gregoriadis, 1976; Tyrell et al., 1977; Zborowsky et al., 1977; Kimelberg and Mayhew, 1978; Hoekstra and Scherphof, 1979; Jackson et al., 1979; Guo et al.,

1980). In particular, high density lipoproteins are potent liposome-destabilising factors (Scherphof et al., 1978; Damen et al., 1980; Tall and Green, 1981). Destabilising factors such as proteins and lipoproteins in the muscle may originate from tissue fluids but may also be the result of cell turnover. This subject is discussed further in section 3.2.2. However, when liposomes are degraded, secondary destabilising factors may arise from their own metabolites (e.g., lysophospholipids).

3.2.2. Metabolising cells and local clearance

Another point of interest is the possible role of metabolising cells in the induction of release from liposomes. Evidence of the migration of granulocytes and macrophages to liposomal depots was provided by another study (Kadir et al., 1992a).

In this work, primarily designed to investigate the protection of muscle tissues against strongly irritating drugs by liposomal encapsulation, the liposomal depots appeared to attract granulocytes and later macrophages, irrespective of the drug content of the liposomes. Rapid accumulation of granulocytes was observed after injection and, from day 7 on, lipid-containing macrophages and clusters of fat cells were seen in the histological preparations. Macrophages are possibly involved in the process of clearance of the liposomal material by phagocytosis. Whether or not the fat content of the macrophages and fat cells that was found is of liposomal origin was not definitely proven.

Phagocytosis by macrophages, however, cannot be the definitive explanation for drug release from liposomes. It is clear that the onset of this supposed mechanism is much slower than that of release. Release is already significant within the first hours and days after administration whereas the appearance of macrophages spans a matter of days. Moreover, it is open to doubt whether the phagocytosed material is again rapidly released from the macrophages. To summarise, cell-mediated drug release, should it exist, is a slow process which cannot explain the early drug release.

The possible role of granulocytes, which appear rapidly after injection in the tissues surrounding the depot, is difficult to interpret but very interesting. Granulocytes also have phagocytosing capacity. Granulocytes and other inflammation cells show a high turnover. (Lipo)proteins originating from turnover and other cell degradation products might be involved in the induction of release from the liposomes.

The burst effect is an indication that a certain amount of release-inducing material is already present in the interstitial fluid at the time of injection, originating from serum filtrate, cell turnover or injection trauma. This amount is probably rapidly depleted after which further release is dependent on turnover and supply of new systemic factors. The complete removal or clearance of lipid material is probably a much slower process, essentially processed by the macrophages and fat cells.

4. Absorption via the lymphatic system after intramuscular and subcutaneous injection

The venous system is not the only one which drains the tissue fluids and transports injected drugs to the circulation. Another is the lymphatic system, a low-pressure system with filter stations, the lymph nodes, in which macrophages are active. The lymphatic system converges into large vessels flowing into the vena cava. The flow in the lymphatic system is low, in fact much lower than the blood flow in the capillaries, and derives its energy mainly from muscle motility, however, the large vessels have their own contractile elements with valves. The flow is dependent on body activity and on humoral factors.

Intended uptake of drugs via the lymphatics is mostly accomplished by subcutaneous or intraperitoneal injection. It is used therapeutically to deliver antivirals or antibiotics in order to treat lymphatic infections or to deliver antineoplastics to lymphatic tumours or metastases of primary tumours in the lymphatics. Metastases migrate from the primary tumour via the interstitial fluid to the lymphatics; they are often filtered by the lymph nodes and therefore often become localised in the lymph nodes.

Lymphatic transport after parenteral drug administration has been reviewed very recently in

the literature and will be treated here only very briefly (Takakura et al., 1992). The absorption pathway of topically injected pharmaceuticals depends on injection depth, lipophilicity of the compound and size of the particles or carrier.

Absorption into the lymphatics takes place more readily from the subcutaneous layers than from the muscular injection sites, since the lymphatic system is better developed in the subcutaneous layers. Highly lipophilic compounds are preferentially absorbed into the lymphatics, whereas more hydrophilic compounds are better absorbed by the venous capillaries. Molecules smaller than 5 kDa favour absorption by the capillaries whereas species larger than 16 kDa are preferentially taken up by the lymphatics. Liposomes and other particles up to 200 nm in size are preferentially drained by the lymphatic system. Thus, liposomal preparations developed as sustained release systems for intramuscular or subcutaneous injection must be larger than 200 nm. This type of preparation is discussed in the preceding section. Small liposomes, macromolecules larger than 16 kDa and emulsions are suitable carriers for lymphatic targeting.

5. Discussion

5.1. Mechanistic considerations

Many factors may affect the release from an intramuscular or subcutaneous injection site. It was the aim of this article to review current opinions. Ionisation and binding to tissue components play a role (Sund and Schou, 1964), but in general a cohesive picture seems to have emerged with a central theme: for the rapid and complete absorption of a compound, it is important to keep a drug, which is intended to act rapidly, in a real solution at the injection site as long as is possible. In oily systems this is not a problem at all, since oil is cleared from the injection site at a rate almost always slower than that of clearance of the drug from the injection site. However, the problem is much greater for drugs in aqueous systems.

Absorption from aqueous systems is characterised by a biphasic absorption process: a relatively rapid initial phase during the period of absorption of water from the vehicle and a very slow second phase (Fig. 3). The latter can clinically be interpreted as corresponding to incomplete bioavailability for drugs which are administered for rapid action. The hang-over effect of the drug, however, during the second absorption phase might be a source of unwanted side effects.

The observed relation between drug lipophilicity and initial absorption rate could be explained by assuming that the fat-containing parts of the tissue have stronger retarding effects on more lipophilic than on hydrophilic compounds. This part of the process can be compared favourably with partition chromatography (of the reversed-phase type) with the paracellular route as the mobile phase and the cells as the more lipophilic stationary phase (Kadir et al., 1990b). The composition of the mobile phase (the injection vehicle) and possible alterations of the stationary phase (the cell material) by injection components such as surfactants determine the initial absorption rate.

Hydrophilic paracellular transport is probably the predominant process for hydrophilic compounds, whereas more lipophilic compounds are retarded by 'partitioning' over the transcellular route of transport. Variation in the length of the 'chromatographic column', the distance from the injection site to the circulation, is one of the likely factors determining the variation in absorption rate. In the case of hydrophilic compounds the drainage capacity may be rate limiting in certain cases, causing apparent zero-order absorption kinetics (Titulaer et al., 1993).

After the aqueous vehicle has been absorbed, two aspects are important. Firstly, its contribution to the dissolved state of the drug is less and secondly the vehicle flow to the circulation stops, further absorption from that time on being only dependent on the comparatively low flow of the tissue fluids and normal diffusion processes. Tissue binding probably plays only a minor role: tissue binding is saturated at relatively low concentrations compared with the total drug load at the injection site and is largely a dynamic equilibrium. These facts are in contradiction with the observed phenomena. Larger volumes lead to greater and longer duration of vehicle flows and

longer times to maintain the drug in the dissolved state. At a higher flow, the chromatographic analogy predicts the smaller retention times found. In addition, the increased solvent drag induced by injection of larger volumes may alter the relative contributions of the different transport routes, i.e., the transcellular and the paracellular pathways.

Another explanation for the increased absorption with increasing injection volume is the larger absorption surface when greater volumes are injected. This will be especially important when phase transit is the rate-determining step. This theory is in line with a third explanation, a variation in depot shape, for variation in absorption rate. The explanations might be relevant alone, or in combination, depending on the nature of the vehicle.

The precipitation theory of Kostenbauder et al. (1975) is consistent with this complex picture and very plausible for more highly concentrated drug systems such as phenytoin: phenytoin is a drug which is dissolved for injection at a higher concentration than the model drugs in the studies with the series of β-blocking agents (Kadir et al., 1990b). It is dissolved at a very high pH often using cosolvents and/or complexing agents. Both diffusion of the solvents and other excipients away from the injection site and rapid pH neutralisation by tissue fluids or components undoubtedly result in precipitation of phenytoin at the injection site. The above cannot be excluded and should be consistent with the theory that an oil-system with phenytoin-acid should result in more rapid release and absorption, or in other words better biopharmaceutical quality of this relatively important injection.

In vitro release studies have only limited value for the in vivo behaviour of suspended pharmaceuticals. In vitro solubility is in fact the parameter which has a positive correlation with the in vivo absorption rate of suspensions and not the dissolution rate (Ober et al., 1958; Hirano et al., 1981; Hirano and Yamada, 1982, 1983a,b). At present, in vitro-in vivo studies fail to show any correlation for liposomally encapsulated pharmaceuticals. The only fact that is known is that again the in vivo release rate has only limited value.

The conclusion that the release of active material is confined to the surface of the liposome aggregate is justifiable. The available data suggest that liposomes also behave more or less as suspensions, obeying the rules forthcoming from the theories of Ober et al. (1958) and Hirano et al. (1981) and Hirano and Yamada (1982, 1983a,b). Differences with the solid drug suspensions with respect to the quantitative relationships are likely because liposomes are more flexible structures, the flexibility being dependent on their composition. Differences between types of liposomes might also be likely. Since liposomes are flexible systems, aggregation phenomena will probably cause less porous caking structures, as aggregation forces are stronger and cause more intensive compaction.

In fact, the release from liposomes seems to be dependent on a release-triggering factor generated in vivo in the surroundings of the injection site. The nature of the interaction with its environment and the cause of the destabilisation have not yet been definitely clarified. The literature data obviously provide evidence for the theory that at least serum components and especially lipoproteins are able to destabilise liposomes. It is a likely supposition that the tissue fluids such as filtrates of serum have more or less retained this property. In addition, the turnover of cells surrounding the injection site, including inflammation cells, may provide release-inducing factors.

With respect to the possible mechanism of phagocytosis by macrophages two problems can be discussed. Firstly, macrophage-mediated release cannot explain the observed drug release from the liposomes during the first few hours after injection (Titulaer et al., 1990a), since macrophages were only seen 7 days after injection. Secondly, after phagocytosis the liposomes must undergo degradation in the macrophage by lysosomal enzymes and the drug must subsequently be released from the macrophage. The concept of macrophage-mediated sustained release has been discussed previously for intravenously injected doxorubicin liposomes (Storm et al., 1989). These processes are recognised as being generally slow. If macrophages contribute

to the release process this would appear to be possible only for the late release; other explanations are necessary for the burst effect and release over the first few days. Nevertheless, it cannot be excluded that other cells such as granulocytes could be involved in this process.

Release and termination of the liposomal material in the muscles are probably comparable with those in the general circulation. Here, termination of the liposomes is caused by the RES, liver and spleen after attack by serum opsonins and is dependent on charge, size and composition (Juliano and Stamp, 1975; Gregoriadis and Senior, 1980; Allen and Everest, 1983; Moghimi and Patel, 1989). Macrophages are essential in the phagocytosis of the liposomal material. The release of pharmaceuticals from liposomes is often more rapid and is dependent on release-inducing (apo)proteins and lipoproteins in the circulation. Furthermore, in the muscles termination is provided for by macrophages and possibly other phagocytosing cells. The release of the encapsulated compound, however, is triggered by inducing compounds originating from serum ultrafiltrate and possibly material originating from the turnover of cells in the surroundings of the injection site. After depletion of the initial amount, the burst effect, the process which is dependent on new supply, slows down.

To summarise, it can be stated that at present the release-triggering factor of liposome-encapsulated drugs after intramuscular injection has not been definitely identified but a model can be described which conforms with the actual information.

5.2. Clinical considerations

The intramuscular route of drug administration is used either for single doses of drugs intended for rapid action or for sustained release systems intended for action over several weeks or months. Many drugs, used for rapid action, must be considered as being incompletely absorbed within the therapeutically relevant time. The second phase of absorption following intramuscular injections of lipophilic or highly concentrated drugs is mostly clinically not useful and has contributed to the conflicting clinical impressions of this route of administration. An important initial conclusion of this review is that absorption from aqueous solutions for injection is often slower, more variable and even more incomplete than often is assumed.

In the clinic, the main reason for keeping injection volumes small is to help to minimise or to reduce pain caused by hydrostatic pressure on the surrounding tissue. However, when the rapid and complete action of an injected lipophilic drug in an aqueous vehicle is essential, a deep intramuscular injection of a drug formulated in a large injection volume is desirable. It is possible to use up to 5 ml and sometimes even 10 ml injection volume in the gluteal region. Highly soluble drugs, however, can be formulated in small volumes.

Biopharmaceutical characteristics of injections, formulated as suspensions in water, are strongly dependent on the dose administered. An increase in dose generally will not result in proportionally increasing concentrations in the blood, nevertheless, via more sustained release, a longer residence time in the blood will be effected. Even lower concentrations are possible. This is contrary to what clinicians generally expect. When higher concentrations are essential simultaneous administration of multiple injections at different places is recommended.

The next important conclusion is that absorption from oily systems is often more rapid than generally is assumed. The idea that oily systems are always sustained release systems is erroneous. In particular, moderately lipophilic drugs with lower molecular weights are rapidly absorbed even when formulated in oily suspension. It is worthwhile to investigate the possibility of formulating clinically important, moderately lipophilic drugs in oil which are currently formulated in aqueous systems but are known to exhibit insufficiently reliable kinetic properties. Long-acting oily injections, in contrast, must contain highly lipophilic pharmaceuticals.

The clinically held belief that oil systems are always long-acting systems probably originates from the long residence times of the oily vehicle. Of the oils which are in use for injection, olive oil is absorbed most rapidly, remaining a few weeks,

whereas castor oil seems to remain almost indefinitely at the injection site (Tse and Welling, 1980).

Liposomal drug formulation still has limited applications for clinical use at the moment. Its drawbacks include the scaling-up of the production, shelf life and sterilisation; all these problems may be solved. Indications exist that at least the sterilisation of liposomal preparations is manageable in the future. However, for experimental use liposomes are very useful. They are very safe and can be used in experimental therapies such as liposomal amphotericin B for fungal infections (Janknecht et al., 1992) and for studying aspects of parenteral sustained release, intramuscularly and subcutaneously as well as intravenously. Moreover, they are useful for experimental drug targeting, especially in the cases of experimental treatment of neoplastic diseases. From the liposome studies discussed in this review, important clinical implications can be derived.

From the reviewed studies with chloroquine in liposomes, for example, an important and an unexpected conclusion could be drawn (Kadir et al., 1992b). From a kinetic point of view, there is no reason to formulate the slowly eliminated chloroquine in a sustained release system. Nevertheless, the *effectivity* of chloroquine appeared much higher and the *toxicity* much lower with the sustained release liposomal formulation than expected.

An explanation for the higher efficacy is that sustained absorption of chloroquine prolongs the period of availability for loading up the circulating erythrocytes and the newly formed erythrocytes, the so-called reticulocytes (Crommelin and Eling, personal communication). After the absorption phase the circulating chloroquine remains bound to the erythrocytes and is not readily available for redistribution to the reticulocytes, the latter remaining unprotected from the parasite. Perhaps, this is one of the problems in the emergence of worldwide resistance against chloroquine.

Unwanted (side) effects may also be related to the absorption phase. During the absorption of rapidly absorbed drugs, large amounts of drug and thus high concentrations pass through the portal system, liver and spleen after oral absorption and subsequently also through the heart and lungs after both oral and parenteral administration. Arterial concentrations are substantially elevated, even compared to venous concentrations during the absorption phase as follows from drug concentration studies in saliva (Zuidema and Ginneken, 1983a,b); the higher the concentration, the more rapid the absorption process occurs. In the cases of more lipophilic drugs, which show considerable saliva clearance, drug concentrations in saliva reflect the arterial concentrations, as far as no active transport over the saliva gland membranes is relevant. Measuring the concentrations of pharmaceuticals in saliva might be very useful for toxicity studies, especially for drugs which might be toxic in essential organs such as liver and heart.

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