Rationale and applications of lipids as prodrug carriers

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Abstract

Lipidic prodrugs, also called drug–lipid conjugates, have the drug covalently bound to a lipid moiety, such as a fatty acid, a diglyceride or a phosphoglyceride. Drug–lipid conjugates have been prepared in order to take advantage of the metabolic pathways of lipid biochemistry, allowing organs to be targeted or delivery problems to be overcome. Endogenous proteins taking up fatty acids from the blood stream can be targeted to deliver the drug to the heart or liver. For glycerides, the major advantage is the modification of the pharmacokinetic behavior of the drug. In this case, one or two fatty acids of a triglyceride are replaced by a carboxylic drug. Lipid conjugates exhibit some physico–chemical and absorption characteristics similar to those of natural lipids. Non-steroidal, anti-inflammatory drugs such as acetylsalicylic acid, indomethacin, naproxen and ibuprofen were linked covalently to glycerides to reduce their ulcerogenicity. Mimicking the absorption process of dietary fats, lipid conjugates have also been used to target the lymphatic route (e.g., L-Dopa, melphalan, chlorambucil and GABA). Based on their lipophilicity and resemblance to lipids in biological membranes, lipid conjugates of phenytoin were prepared to increase intestinal absorption, whereas glycerides or modified glycerides of L-Dopa, glycine, GABA, thiorphan and *N*-benzyloxycarbonylglycine were designed to promote brain penetration. In phospholipid conjugates, antiviral and antineoplasic nucleosides were attached to the phosphate moiety. After presenting the biochemical pathways of lipids, the review discusses the advantages and drawbacks of lipidic prodrugs, keeping in mind the potential pharmacological activity of the fatty acid itself. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Fatty acids; Phospholipids; Glycerides; Prodrugs; Drug delivery

1. Introduction

As proposed by Albert (1958), a prodrug is defined as an inactive pharmacological derivative of an active parent compound, which undergoes a spontaneous or enzymatic transformation resulting in the release of the free drug. The prodrug approach aims at modifying the physico–chemical properties of a drug by covalently attaching a transport moiety, thus improving its access to pharmacological targets (Fig. 1). Prodrugs have been designed for several purposes, e.g., to overcome pharmaceutical and/or pharmacological problems such as incomplete absorption, poor systemic bioavailability, too rapid absorption or too rapid excretion, toxicity, poor site-specificity, as well as formulations problems (Testa, 1995; Friis and Bundgaard, 1996; Testa and Caldwell, 1996). The characteristics of a prodrug are (a) a covalent linkage between the drug and the transport moiety, (b) in vivo cleavage of this bond, (c) lack of intrinsic activity, (d) lack of toxicity and (e) optimal kinetics of release to ensure effective drug levels at the site of action (Wermuth et al., 1996).

Lipidic prodrugs are chemical entities with two distinct parts: a drug covalently bound to a lipid moiety, i.e., a fatty acid, a glyceride or a phospholipid (Fig. 2). In this review, we describe the different lipid prodrugs prepared so far and examine whether these lipid prodrugs merit further consideration. After presenting the pathways of lipid biochemistry, the aim of this review is to focus on the advantages and drawbacks of a lipidic prodrug approach in terms of drug delivery and pharmacology. Three different lipid carriers are presented: fatty acids, glycerides and

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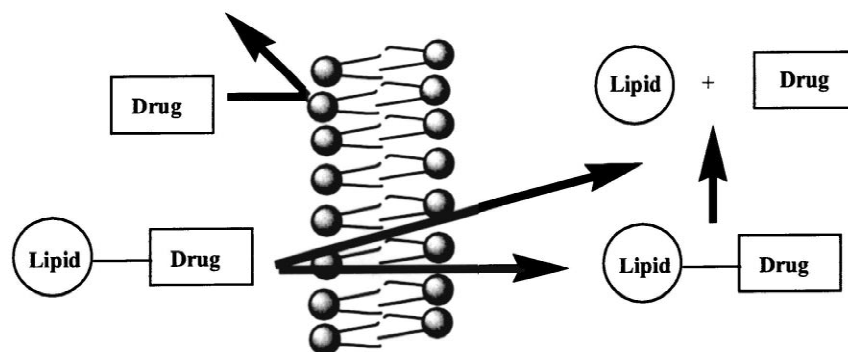*E-mail address:* lambert@cmfa.ucl.ac.be (D.M. Lambert).

Fig. 1. Illustration of the lipidic prodrug approach. A drug which is not able to cross biological membranes might be covalently coupled to a lipid carrier used as a transient transport moiety. The drug–lipid conjugate crosses the membranes to release after biotransormation the drug in the cell.

Lipidic prodrugs

1. Drug attached to a fatty acid.

with X= O, NH
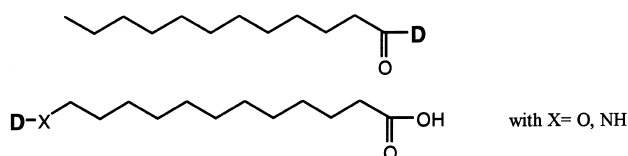
2. Drug attached to a glyceride.
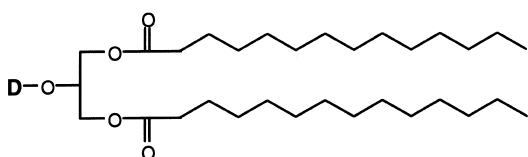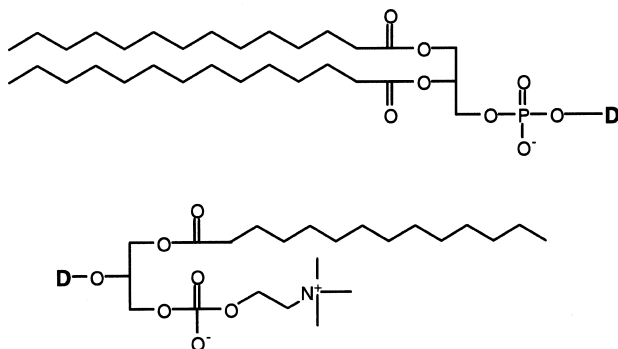

3. Drug attached to a phospholipid.

Fig. 2. Types of lipidic carriers: fatty acids, glycerides and phospholipids. In the case of fatty acids, the drug is attached directly to the carboxylate or to a modified ω-atom. Drug–glycerides conjugates are represented here by a 1,3-diglyceride where the drug is in position-2. Phopholipid prodrugs consist either in drugs linked to the phosphate group or to the glycerol backbone, in this case, the drug replaces a fatty acid.

phospholipids. The chemistry and the procedures to prepare drug glycerides and phospholipids conjugates can be found elsewhere (Lambert et al., 1995a).

2. Absorption and biochemical fate of lipids

Drug–lipid conjugates have been prepared in order to take advantage of the metabolic pathways of lipids (Fig. 3), assuming that this approach may allow organ targeting and/or control of drug delivery problems. To understand the potential benefit of a lipid prodrug approach, we present a brief but essential summary of the metabolic pathways of the lipids of interest, i.e., fatty acids, triglycerides and phospholipids. Also, according to these pathways and to the enzymes involved, a tentative follow-up of these various lipids is presented (Fig. 4) either after oral administration (the natural route for dietary lipids) or after parenteral administration.

Triglycerides are the major constituents of dietary fats. Their absorption involves hydrolysis by lipases to monoglycerides and free fatty acids. Several lipases are present in the gastrointestinal tract. Lingual lipase is secreted by the lingual serous glands of the tongue in mammals. A second lipase was described in the stomach, but it was unclear for a long time whether this lipase is of gastric origin or is simply a swallowed lingual lipase. The cloning (Bodmer et al., 1987) and crystallization (Canaan et al., 1999) of the human gastric lipase (EC 3.1.1.3) solved the controversy. The lingual and gastric lipases belong to the group of pre-duodenal lipases, which are acidic lipases stable and active at low pH (Carriere et al., 1998). Even if the contribution of these two lipases to the hydrolysis of the dietary fats is low, they produce an amount of monoglycerides and fatty acids sufficient for effective emulsification, preparing the intestinal hydrolysis of lipids. Most triglycerides reach the duodenum as an emulsion of droplets of small diameter (<0.5 μM). Three enzymes
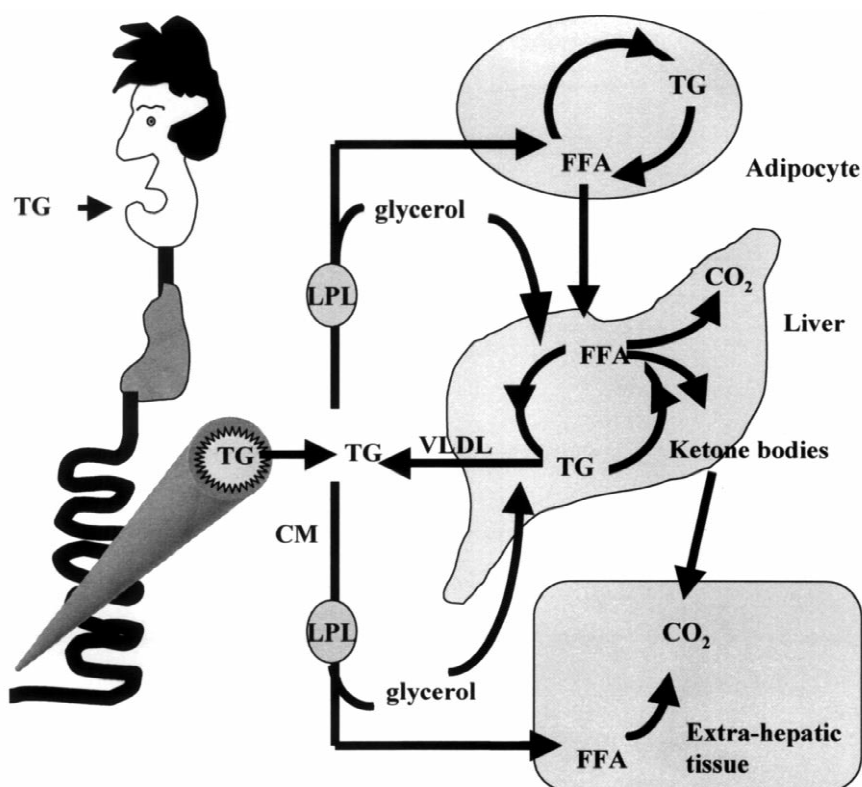
Fig. 3. Metabolic pathways of lipids. Most of dietary triglycerides (TG) are degraded by pancreatic lipase to give 2-monoglycerides which are subsequently re-esterified in the enterocytes. De novo triglycerides reach the blood as chilomycrons (CM), there the lipoprotein lipase (LPL) hydrolyses them in free fatty acids (FFA) and glycerol. Free fatty acids are taken up by different tissues, e.g., liver, adipocytes and extra-hepatic tissues such as muscles and heart. Liver and adipocytes may use the free fatty acids for the de novo synthesis of triglycerides secreted in the blood as very low density lipoproteins (VLDL) but as other tissues, they may also catabolize them for energy production.

participate in the intestinal catabolism of lipids: pancreatic lipase, phospholipase A2 and a nonspecific esterase.

The pancreatic lipases are the most important enzymes degrading triglycerides. Their activity requires the presence of a cofactor called colipase and the presence of bile salts (van Tilbeurgh et al., 1999). The pancreatic lipases represent distinct lipases with different sequence homology and catalytic properties (Carriere et al., 1998); they hydrolyze esters specifically at positions-1 and -3 of the glycerol backbone. Hydrolysis in position-2 is very slow and occurs after chemical or enzymatic isomerization. This ''resistance'' to hydrolysis in position-2 has been used in prodrug design. Indeed, most lipidic prodrugs have the drug attached at position-2 by an ester bond if the drug bears a carboxylic acid, or via a spacer if other functions are present.

After being released by lipases, monoglycerides and fatty acids enter the enterocytes, but their further fate depends of the nature of the remaining fatty acid. The short-chain fatty acids ($<C_{10}$) pass through the cells into the blood without re-esterification, where they subsequently circulate after binding to serum albumin. The medium-chain (C_{10}–C_{12}) acids are mainly oxidized. Their plasma concentrations are very low and little re-esterification occurs. Long-chain fatty acids ($>C_{12}$) are used for the re-esterification of monoglycerides into triglycerides. The de novo triglycerides are then incorporated into chylomicrons and are excreted into the lymph. The latter is transported via the thoracic duct before entering the circulation, thus bypassing the liver. Chylomicrons are made mainly of triglycerides (70–90%), phospholipids (4–8%), esterified cholesterol (4%), free cholesterol (3%) and proteins (2%). In plasma, triglycerides are hydrolyzed by the lipoprotein lipase into fatty acids and glycerol with a half-life of about 20 min.

Four main organs participate to the metabolism of lipids (Fig. 3). The small intestine, as described above, has an essential role in their absorption. The key organ is the liver, which synthesizes lipids, uses them for energy through β-oxidation, and to synthesize fatty acids and glycerides. The adipose tissue to a small extent is also able to perform these reactions. Its influence on global lipid metabolism was largely underestimated, but is now better recognized. In extra-hepatic and extra-adipose tissues, glycerides and fatty acids are used for energy production, e.g., in muscles and heart.
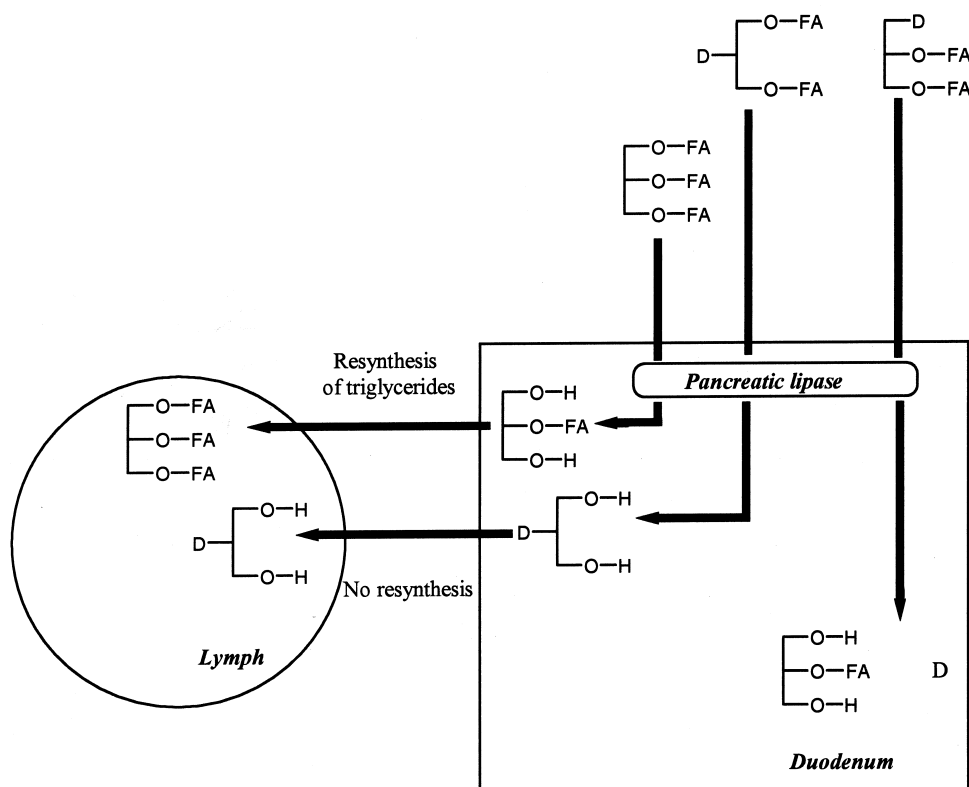
Fig. 4. Metabolic fate of triglycerides, 1-drug diglycerides and 2-drug diglycerides after oral administration. Natural triglycerides are cleaved by pancreatic lipase in positions-1 and -3 to give the 2-monoglycerides. In the enterocytes, if the remaining fatty acid chain is superior to C_{12}, re-esterification occurs and triglycerides reach the lymph. Drugs coupled to diglycerides in position-1 are cleaved by pancreatic lipase and released in the enterocytes. Drugs attached in position-2 reach the lymph as 2-drug pseudomonoglyceride, no re-esterification occurs. D=drug, FA=fatty acid residue.

3. The use of fatty acids in prodrug design

A variety of fatty acid prodrugs have been prepared and examined. Fatty acids have been used to couple drugs (a) directly to the carboxylate group or (b) to the modified ω-position in order to keep the free carboxylate (Fig. 2).

3.1. Prodrugs where the drug is attached to the carboxylate of the fatty acid.

A first approach has been to couple a drug containing an alcohol or an amino function with the fatty acid, yielding an ester or an amide (Alvarez and Stella, 1989; Geurts et al., 1998; Takayama et al., 1998). As a result, the lipophilicity of the prodrug compared to the drug is dramatically increased. The prodrug is expected to have a different pharmacokinetic profile, for example a long lasting effect. This approach has been successfully applied to neuroleptics using mid- to long-chain fatty acids. Flupentixol decanoate, for instance, is marketed as Fluanxol Depôt® (Jorgensen et al., 1982) and prepared for intramuscular administration dissolved in a vegetal oil. However, these prodrugs do not take advantage of fatty acid biochemistry since they do not bear the free carboxylate essential for binding to the fatty acid binding site

of albumin or for recognition by the fatty acid binding protein, a transporter present in cellular membranes.

3.2. Prodrugs having the drug attached to the ω-position of a modified fatty acid

Imaging techniques such as position emission tomography and gamma cameras use fatty acids as tissue markers for the heart (Keriel et al., 1990). Using [^{11}C]-labeled fatty acids, these techniques monitor the uptake of fatty acids by tissues (Tamaki et al., 1995) to probe myocardial fatty acid metabolism (Beckurts et al., 1985). An alternative is to label fatty acid analogues with radioactive iodine atoms. In this case, the fatty acid must be modified in the ω-position, where iodine reporter groups have been introduced, e.g., [16-^{123}I]-iodohexadecenoic acid (Bontemps et al., 1985) and iodophenylpentadecanoate (Reske, 1985) while keeping the free carboxylate to mimic the fatty acid structure.

In order to investigate new tools for selective drug delivery to a convenient site or even to a specific cellular compartment, we have used a similar approach to deliver drugs or contrast agents to hepatocytes (Gallez et al., 1993; Charbon et al., 1996), taking advantage of the fact that fatty acids present a high affinity for liver tissues. The efficient cellular uptake of fatty acids follows the dissociation of their complex with serum albumin and their

interaction and uptake by the fatty acid binding protein (FABP$_{PM}$), a transporter located at the sinusoidal pole of hepatocytes. Once inside the cells, the fatty acids are bound to the cytosolic fatty acid binding protein (FABPc).

Using a tritiated benzoyl residue bound by an amide bond to aminododecanoic acid as model of a fatty acid prodrug, we studied its uptake in isolated hepatocytes, focusing on the asialoglycoprotein receptor, as a second transporter. This protein recognizes endogenous neoglycoproteins and macromolecules bearing polygalactose residues (e.g., galactosylated albumin). Since fatty acids can bind to galactosylated albumin, we compared the fate of the fatty acid prodrug conjugated either to native albumin or to galactosylated albumin (Fig. 5). Native albumin can deliver the fatty acid prodrug to the cytosol, whereas the modified albumin can be taken up by endocytosis into lysosomes. No significant difference was observed in the binding properties of the fatty acid prodrug to albumin or galactosylated albumin and the fate of the fatty acid prodrug was the same in the two cases. Incorporation into hepatocytes was essentially dependent on the FABP$_{PM}$ transport system when the fatty acid prodrug was bound to albumin or to galactosylated albumin. The uptake was inhibited by phloretin, an inhibitor of sodium dependent transport, and was increased when less prodrug was bound. Moreover, the carrier system did not influence the intracellular fate of the fatty acid prodrug and, the radioactivity was recovered mostly in the cytosol and microsomes. Finally, the tritiated benzoyl residue was released in part from the prodrug indicating a cleavage of the amide bond. Even if this study failed to obtain different subcellular localizations by means of a mixed prodrug-carrier system, the derivatization of fatty acids with some hindered groups in the ω-position maintained recognition by the FABP$_{PM}$ transporter. The fatty acid prodrug approach described here

thus seems to preserve some properties of fatty acids, namely binding to cytoplasmic FABP, binding to albumin at the site of natural fatty acids, recognition and uptake through FABP$_{PM}$ system, β-oxidation, and accumulation into liver and heart.

4. The use of a triglyceride for a prodrug approach

4.1. Introduction

The triglyceride prodrug approach aims to take advantage of the metabolic fate of triglycerides to improve some drawbacks of the drug. It is first a pancreatic lipase driven drug delivery but it offers much more, having new physico–chemical properties and a different fate following breakdown and activation. Drugs bearing a carboxylate function are good candidates for coupling to a diglyceride via an ester bond. Drugs without this function may be also coupled to the glyceride via a spacer such as succinic acid. However, this spacer may influence the release of the drug.

Numerous objectives have been pursued with this approach, as classified here to present its advantages and limitations. Following the historical development of these prodrugs, we examine here four different objectives: (1) enteral delivery of the drug attached to the glyceride, (2) targeting to the lymphatic system of drugs attached to glycerides, (3) central nervous system targeting and (4) liver targeting.

Recent evidence indicates that metabolic coupling to a lipid can be achieved for xenobiotic carboxylic acids, which may be incorporated into glycerides in vitro and in vivo (Fears, 1985; Dodds, 1991, 1995; Mayer et al., 1994). Thus, 4-phenoxybenzoic acid was rapidly esterified to form pseudoglycerides found in the liver and adipose
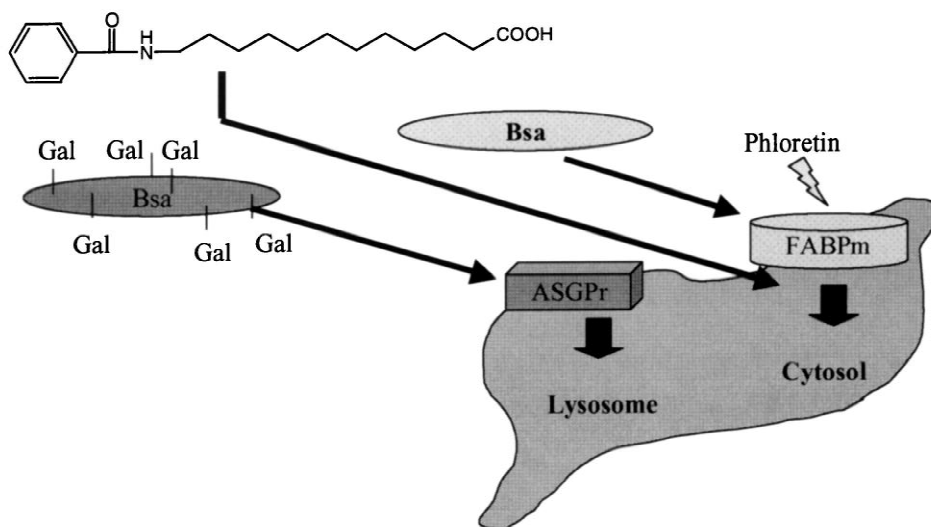

Fig. 5. Targeting of drug to the hepatocytes by fatty acids. Influence of the carrier (albumin or galactosylated albumin) on the fate of the fatty acids and their analogues. BSA=bovine serum albumin; Gal BSA=poly-galactosylated bovine serum albumin; ASGPr=asialoglycoprotein receptor; FABPm=fatty acid binding protein.

tissue of rats (Moorhouse et al., 1991; Haselden et al., 1998a,b). Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen or fenprofen (Dodds et al., 1995) have been shown to be incorporated into triglycerides in vitro by rat liver slices or adipocytes. However, these data are too fragmentary to conclude that the triglyceride prodrug approach is just a copy of what Mother Nature does. Are these endogenous lipid conjugates pharmacologically active effect or toxic? What is their kinetics and storage? These questions remain open and merit further attention to delineate the usefulness of the glyceride prodrug approach.

4.2. Enteral delivery of the drug attached to a glyceride: a lipase driven process

The first studies dealing with glycerides in which a fatty acid was replaced by a drug date from the late 1970s. At that time, reduction of the gastrointestinal side-effects of NSAIDs was already a major concern. Taking into account that the lipases present in the mouth and stomach are not very efficient, acetylsalicylic acid was coupled to a glyceride to reduce its gastric concentrations and, thus, to reduce gastric irritation. Acetylsalicylic acid was directly connected via an ester bond to the diglyceride backbone. Investigating a series of 1,3-diacyl-2-acetylsalicylateglycerides with varying length of the fatty acid chain (C_2–C_{16}), Paris et al. (1979) and Carter et al. (1980) showed that these prodrugs retained the potency of aspirin but elicited less gastric damage. It was postulated that like natural triglycerides, only a negligible fraction of the modified glyceride underwent breakdown to release the free drug in the stomach. This was confirmed (Kumar and Billimora, 1978) using a radiolabeled aspirin derivative, 1,3-dipalmitoyl-2-(2′-acetoxy-[^{14}C]carboxybenzoyl)-glycerol. The modified glyceride was found to be stable in the stomach, but to hydrolyze in the small intestine to give the corresponding 2-aspirin-glycerol which was absorbed to yield therapeutic concentrations of aspirin in the plasma. Around 20% went to the lymph as 2-aspirin-triglyceride.

This prodrug approach was applied to several other NSAIDs (Fig. 6), namely a cyclic derivative of aspirin (Paris et al., 1980a), indomethacin (Paris et al., 1980b), ketoprofen, diclofenac, ibuprofen, desmethylnaproxen and naproxen (Paris and Cimon, 1982; Cullen, 1984). In these studies, the authors defined a '' therapeutic index'' expressed as the ratio between the ulcerogenic dose (UD_{50}) and the effective dose (ED_{25}). The therapeutic index of the modified glycerides compared to the parent drug increased from three-fold for indomethacin to 80-fold for aspirin. It has been assumed that in the case of aspirin the direct local ulcerogenic action of the drug was suppressed while the systemic effect of the inhibition of cyclooxygenase was not affected. Hence, this prodrug approach was not fully successful for anti-inflammatory drugs with pronounced gastric side-effects due to cyclooxygenase inhibition.

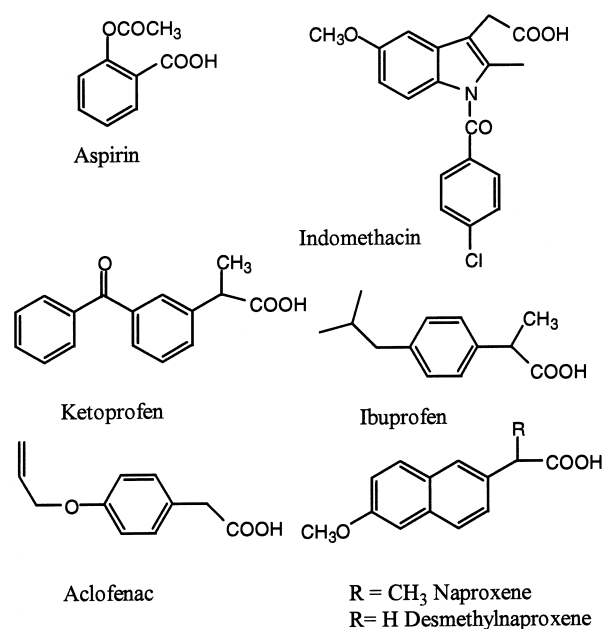Most of these examples concern modified glycerides

Fig. 6. Examples of drugs conjugated to diglycerides for enteral delivery of the drugs.

where the anti-inflammatory drug replaced a fatty acid chain in position-2. However, as the aim of this approach was to reduce the release of the free drug in the stomach, cleavage by pancreatic lipase was not critical. Paris and Cimon (1982) synthesized a glyceride with indomethacin in position-1, the two remaining alcohol functions of the glycerol being esterified by decanoyl residues. This prodrug was found to be six times less active than the corresponding analogue in position-2. An additional problem with 1-monosubstituted pseudoglycerides is their preparation which requires the synthesis of unstable 1,2-diglycerides.

A peptide drug was also coupled to a diglyceride in an attempt to reduce its enzymatic degradation in the intestine (Delie et al., 1994). The pentapeptide renin inhibitor Iva-Phe-Nle-Sta-Ala-Sta-acetyl was attached to 1,3-dipalmitoylglycerol. After digestion by pancreatic lipase, the cleaved prodrug was as effective in inhibiting renin than the peptide itself. Pseudoglyceride formation protected the peptide from degradation by gastric and intestinal fluids and by α-chymotrypsin in vitro, supporting the use of glycerolipidic prodrugs for the oral administration of peptides.

4.3. Targeting to the lymphatic system of drugs attached to glycerides

Another use of the glyceride prodrug approach is to target the lymphatic route (Fig. 7). If the fatty acid chains are long enough, the structural similarity between pseudoglycerides and natural triglycerides will allow the pro-
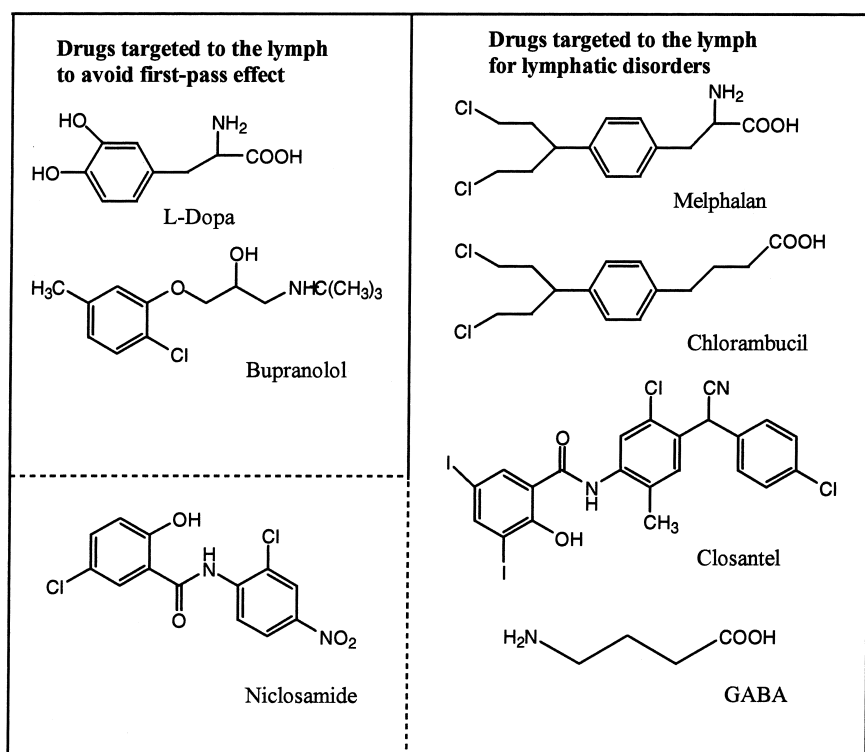
Fig. 7. Lymph targeting of drugs conjugated to diglycerides. On the left, are presented the drugs escaping from first pass metabolism (L-Dopa, bupranolol and niclosamide) and on the right, the drugs which target lymphatic disorders (chlorambucil, melphalan, closantel, GABA and niclosamide).

drug to bypass the liver by absorption via the lymphatic system and, thus, to avoid fist-pass metabolism. With this objective, triglycerides were prepared from alpha-blockers (Mantelli et al., 1985) or L-Dopa (Garzon-Aburbeh et al., 1986). In addition to a reduced first-pass metabolism, triglycerides have been used to target the lymphatic system itself for the treatment of lymphatic cancers or lymphatic filiariosis with melphalan (Deverre et al., 1992a; Loiseau et al., 1994), chlorambucil (Garzon-Aburbeh et al., 1983; Loiseau et al., 1994, 1997), closantel (Loiseau et al., 1997) and GABA (Deverre et al., 1989, 1992b). The amino acid GABA, better known as a major inhibitory neurotransmitter in mammals, represents a therapeutic agent in infection by *Molinema dessetae*, the pathogen responsible for lymphatic filiariosis. GABA itself showed antifilarial effects in vitro but a macrofilaricidal action in vivo was only observed after intraperitoneal injection of very high doses. The diglyceride prodrug of GABA, despite encouraging in vitro results (Deverre et al., 1989), was not found active in vivo after intraperitoneal or oral administration (Deverre et al., 1992b). In a recent study (Loiseau et al., 1997), new closantel and chlorambucil prodrugs expected to accumulate in the lymphatic system were prepared and evaluated on the filaria *Molinema dessetae*. A delayed effect and a decreased toxicity were observed with closantel prodrugs compared to the parent compound. The most active prodrug by the oral route was 1,3-dipalmitoyl-2-succinyl-glycerol-closantel. Chlorambucil prodrugs were only active in vitro (Loiseau et al., 1997).

In order to bypass the liver and to promote lymphatic transport, the antihelmintic agent niclosamide, which is very effective against infective larvae in vitro but not after oral administration, was conjugated to diglycerides including amide bio-isostere glycerides (el Kihel et al., 1994). The diamide prodrug 1,3-dihexadecanamido-2-[4-chloro(2-chloro-4-nitroanilinocarbonyl)phenyloxycarbonylpropanoyloxy]propane exhibited enhanced stability to gastrointestinal enzymes, a delayed action and oral activity against with *Molinema dessetae* infective larvae.

In these studies, few authors have analyzed lymph concentrations. Kumar and Billimora (1978) showed that approximately 20% of the total amount of an orally administered aspirin-glyceride reached the lymphatic system. Garzon-Aburbeh et al. (1983, 1986), studying 1,3-dipalmitoylpseudo-glycerides containing L-Dopa or chlorambucil, observed an increase in lymph concentrations from 0.2 and 3% for the parent drugs up to 20 and 26% for the triglyceride derivatives, respectively. Compared to the parent drug under the same conditions, a 40- to 60-fold increase in lymphatic concentrations was observed for a dipalmitoylpseudoglyceride of melphalan (Loiseau et al., 1994), an alkylating agent similar to chlorambucil. The plasma concentration of melphalan was two-fold higher than that observed after administration of

the free drug. A minor lymphotropism was observed in the case of a 2-nicotinic or 2-naproxen diglyceride, while the lymph incorporation for the monoglyceride was quite high (Sugihara et al., 1988a,b; Sugihara and Furuuchi, 1988).

In our laboratory, we investigated the influence of the fatty acid chain length on the lymphatic absorption of chlorambucil pseudoglycerides (unpublished results). A fatty acid chain length longer than 14 carbon atoms was found necessary for lymphotropism. The degree of unsaturation of the fatty acids (linoleoyl, linolenoyl, oleyl) was not a determining factor.

4.4. Central nervous system targeting of drugs attached to the glycerides

The use of glycerides to increase the brain penetration of drugs began with a report by Jacob et al. (1985) who synthesized GABA-glycerides. A dramatic increase in the brain penetration of GABA was observed and the diglyceride reached the brain easily with a penetration index (brain penetration index BPI=the ratio between cerebral and hepatic concentrations×100) 127 times higher than the parent compound. The introduction of unsaturated fatty acid chains was found to be favorable for activity, despite a lower lipophilicity (Jacob et al., 1987). No mechanistic explanation was offered to support this observation. Using an elegant design, the same group evaluated a glyceride prodrug approach to deliver simultaneously GABA and vigabatrin (γ-vinylGABA), an inhibitor of GABA transaminase (Jacob et al., 1990). An asymmetrically disubstituted pseudoglyceride was synthesized with the three hydroxyl functions esterified by linolenoic acid,

GABA and γ-vinyl-GABA, yielding a double prodrug. The activity of this pseudoglyceride was some 300 times greater than that of the GABA transaminase inhibitor.

A L-Dopa diglyceride was also prepared to improve the central effects of the drug (Garzon-Aburbeh et al., 1986). Here too, increased brain levels were observed. In contrast to GABA, L-Dopa easily penetrates the CNS by an active process, using the L-neutral amino acid carrier system at the blood–brain barrier. In this case, the increased brain concentration could be attributed, at least partially, to a peripheral metabolic protection of the drug. A similar approach was followed for lipidic conjugates of the NSAID niflumic acid. The dipalmitoylglyceride prodrugs were prepared and found more active than niflumic acid in an experimental brain edema model (el Kihel et al., 1996).

Phenytoin, a widely used antiepileptic drug, suffers from erratic resorption in patients. It is in fact poorly soluble in water and organic solvents. Many pseudoglycerides containing phenytoin have been prepared to increase its oral availability. Phenytoin enters the brain probably by passive diffusion. The drug is also a good model compound, lacking a carboxylate handle. Different approaches were investigated including spacers or a retro-carboxyl linkage (Scriba, 1993a, 1994, 1999; Fig. 8). Lipid conjugates including 2-phenytoin-succinyl pseudoglycerides and retro-lipid mimics are degraded by pancreatic lipase, like natural glycerides (Scriba, 1993b). The pharmacological evaluation of these conjugates revealed an anticonvulsant effect similar to that of phenytoin (Scriba et al., 1995a). Moreover, the anticonvulsant activity of the pseudolipids correlated with the lipase-mediated release of phenytoin. An improved pharmacokinetic profile was often observed,
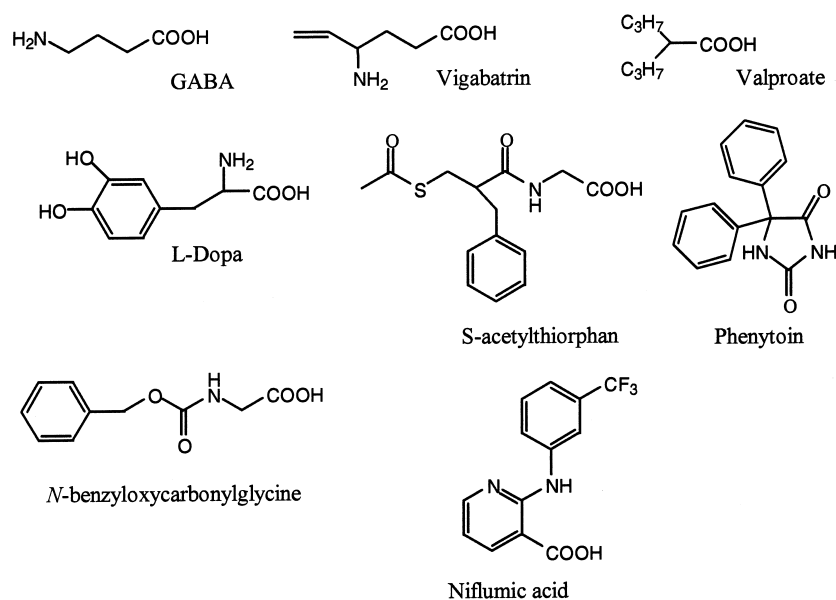

Fig. 8. Central nervous system-targeting of drugs coupled to diglycerides or modified diglycerides.

suggesting the interest of this approach for poorly absorbed drugs (Scriba et al., 1995b,c; Scriba and Lambert, 1997).

Another approach used in our laboratory to enhance brain penetration has been to synthesize amide bio-isostere pseudoglycerides (Mergen et al., 1991a; Poupaert and Lambert, 1992; Fig. 9). The 1,3-diaminopropan-2-ol backbone was acylated first by fatty acid residues in position-1 and 3 giving amides bio-isosteres of diglycerides. The drug was then attached to the remaining alcohol function by an ester bond. The rationale here was to retain the triglyceride structure, keeping in mind that amides exhibit an increased resistance to chemical and enzymatic hydrolysis. Valproate (Mergen et al., 1991b), the enkephalinase inhibitor acetylthiorphan (Lambert et al., 1993a, 1995b) and *N*-benzyloxycarbonylglycine (Mergen et al., 1991b, Lambert et al., 1996) were attached in the 2-position (Fig. 9). These bio-isosteric pseudoglycerides showed increased pharmacological activity. Non-natural side chains were also incorporated, e.g., 1,3-dicyclicdiamidopropan-2-ol, and exhibited intrinsic additional activity (Lambert et al., 1993b).

The case of *N*-benzyloxycarbonylglycine illustrates the potential of CNS targeting of amide bio-isosteres. This compound is a prodrug of glycine (Lambert et al., 1995c) displaying modest anticonvulsant activity after parenteral administration in mice (Lambert et al., 1994). It was incorporated both in amide bio-isosteres pseudoglycerides and in classical pseudoglycerides. In these prodrugs, both the amino group and the carboxylate were masked by a lipophilic residue, a benzyloxycarbonyl protecting group and a lipid moiety, respectively (Lambert, 1995d). Amide bio-isosteres and classical pseudoglycerides were found to be more active than the parent compound, but the amide

bio-isosteres displayed higher and longer activity compared to the corresponding esters (Lambert et al., 1996). Deprotected glycine pseudoglycerides exhibited an intermediary activity.

4.5. Liver targeting of drugs attached to a glyceride to the liver

The glyceride approach has been used in medical imaging to target the liver with contrast agents either for nuclear medicine or for magnetic resonance imaging. Pseudoglycerides containing ω-(3-amino-2,4,6-triiodophenyl)alkanoic acids (Weichert et al., 1986a,b; Schwendner et al., 1992; Bakan et al., 1996, 2000; Longino et al., 1996) or tetramethyl-pyrrolidinoxyle residues (Gallez et al., 1992) have been synthesized as potential hepatographic agents for medical imaging to visualize hepatic tumors. These studies evaluated the possibility of utilizing the fate of natural lipids, i.e., the uptake and subsequent metabolism of triglycerides associated with lipoproteins by the liver.

5. The use of phospholipids in a prodrug approach

As presented in Fig. 1, two classes of phospholipid prodrugs can be distinguished. In most cases, the drug is bound to the phosphate group, but recently the first phospholipid prodrug with a fatty acid moiety replaced by a drug was reported (Kurz and Scriba, 2000). The phospholipid approach has been applied to nucleoside agents, with the nucleoside linked to a monophosphate diacylglycerol, to a diphosphate diacylglycerol or to a triphosphate diacylglycerol. Most of the nucleosides so incorporated have antiviral or antineoplastic activity (Fig. 10), but they have poor absorption and poor pharmacokinetic behavior.

These include 5-fluorouracil, 5-fluoridine, 1-β-D-arabinofuranosylcytosine (ara-C), 1-β-D-arabinofuranosyluracil, neplanocin A, 3-azido-3′-deoxythimidine (AZT), 2′,3′-dideoxycytidiine and 2′,3′-dideoxythymidine (for a review, Lambert et al., 1995a).

Cytidine and 2′-deoxycytidine diphosphate diacylglycerols are naturally occurring liponucleotides which are precursors in the biosynthesis of phosphoglycerides such as phosphatidylinositol and phosphatidylglycerophosphate. All these reactions proceed with the concomitant release of cytidine 5-monophosphate or deoxycytidine 5′-monophosphate (van den Bosch, 1974). Thus, liponucleotides containing antineoplastic and antiviral nucleosides and nucleoside analogues have been designed with the objective of using these specific metabolic pathways to achieve the intracellular release of the nucleoside drug as the 5′-monophosphate, bypassing the first of the three phosphorylation steps. In fact, it has been demonstrated for ara-C and some antiviral dideoxy nucleosides that their
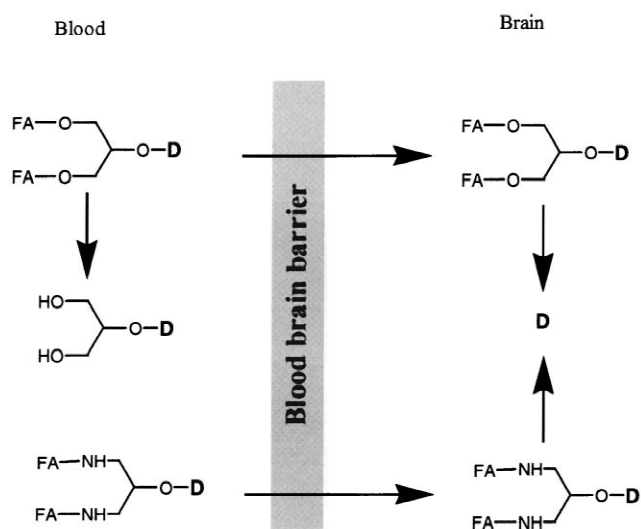
Fig. 9. Amide bio-isosteres of diglycerides as a central nervous system-targeting system.
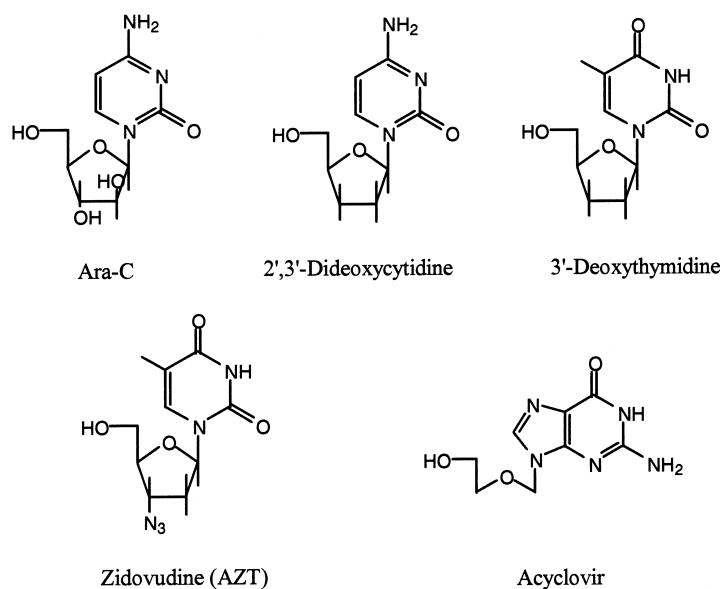
Fig. 10. Nucleosides and nucleosides analogues conjugated to phospholipids.

diphosphate diacylglycerols and triphosphate dia-clyglycerols are substrates of phosphoglyceride synthesizing enzymes releasing the nucleoside monophosphates in the process (van Wijk et al., 1992; Hostetler et al., 1993). High lymphatic levels of fluorouridine monophosphate diacylglycerols were seen after oral administration of fluorouridine monophosphate dimyristoylglycerol to rats, suggesting these compounds to be substrates of phospholipid-metabolizing enzymes in the gastrointestinal tract (Sakai et al., 1993).

The use of ara-C in various types of cancer is hampered by its rapid metabolism transforming it into the biologically ineffective 1-phospho-D-arabinofuranosyluracil. Phospholipid prodrugs of ara-C showed increased metabolic stability and higher activity against various cancer cell lines and leukemias, including kinase deficient cells (Matsushita et al., 1981). Correct configuration of the phospholipid is required for activity; the L-isomer of ara-C diphosphate dipalmitoylglycerol, which has the same configuration as the naturally occurring liponucleotides, exhibited a greater antineoplastic activity than the racemic mixture (Hong et al., 1984, 1988).

A comparison of the mono-, di- and triphosphate dimyristoylglycerol conjugates of AZT showed that their antiviral activity increased in the order monophosphate< triphosphate<diphosphate (van Wijk et al., 1994). Compared to the parent drug, acyclovir diphosphate dimyristoylglycerol showed considerable activity against the thymidine kinase-deficient DM21 strain and other acyclovir-resistant strains of the herpes simplex virus (Hostetler et al., 1993).

The second group of phospholipid prodrugs have one or two fatty acids replaced by a drug. Kurtz and Scriba (2000) very recently described the synthesis of valproate- or ibuprofen-containing phospholipids. The drug–phospholipid conjugates exhibited the same surface properties and aggregation behavior as the natural phospholipids. Upon incubation with porcine phospholipase A2, only the drug-phospholipids conjugates with a fatty acid in the sn-2 position were recognized and degraded by the enzyme (Kurz and Scriba, 2000).

6. Conclusion

We conclude this review by discussing whether lipidic prodrugs are true prodrugs. Among the criteria presented in the introduction, several have been met, but one question remains wide open, namely, is the lipidic pro-moiety devoid of pharmacological activity or toxicity? This indeed is far from certain, since lipids are increasingly implicated in pharmacology and biochemistry. Some examples are mentioned here just to alert the reader to potential effects of lipidic pro-moieties. Thus, it is known that fatty acids can modulate ion channels. Recent papers describe some new endogenous lipids in great details. Oleamide, a sleep inducer, seems to act at least partially at the serotonin receptor. Palmitoylethanolamide (Lambert and Di Marzo, 1999), an endogenous compound with analgesic and anti-inflammatory properties, has been described as an endocannabinoid, i.e., an endogenous compound acting at cannabinoid receptors. This compound has now entered clinical trials as an analgesic and anti-inflammatory agent, but its precise mechanism of action is still debated. Other endocannabinoids are also lipids, e.g., anandamide (arachidonoylethanolamide) and 2-arachidonoylglycerol (Bisogno et al., 1999), the endogenous ligands of the cannabinoid receptors which show some selectivity for the central CB_1 cannabinoid receptors. 2-Arachidonoylglycerol is in fact closely related to the glyceride

used as prodrugs, and if used as a drug carrier might have effects on its own. Tributyrate, a prodrug of butyric acid, is another example of a very active lipid (Chen and Breitman, 1994), now in phase I trials for patients with solid tumors (Conley et al., 1998).

Besides the potential intrinsic effects of the lipidic moieties, the lipidic approach seems to offer advantages. For instance, after oral administration of glyceride prodrugs, the reduction of the first pass effect together with a delayed and long-lasting effect due to the pancreatic lipase driven-enteral delivery may improve the pharmacokinetic profile of the drug. Some questions remain open regarding the metabolic fate of these lipidic prodrugs. Further studies are needed in two directions: First, to assess the potential for organ-targeting, it is essential to trace simultaneously the fate of both the drug and the lipid pro-moiety. This may be carried out by a double labeling technique, but the synthesis of such labeled prodrugs has not yet been achieved. Second, these lipidic entities need a formulation compatible with a convenient mode of administration. Some drug delivery problems may be solved using a lipidic prodrug approach but like for other prodrug systems, an adequate choice of both the drug and the lipid constituting the lipidic prodrug is essential.

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References

Albert, A., 1958. Chemical aspects of selective toxicity. Nature 182, 421–423.

Alvarez, F.J., Stella, V.J., 1989. Pancreatic lipase-catalyzed hydrolysis of esters of hydroxymethyl phenytoin dissolved in various metabolizable vehicles, dispersed in micellar systems, and in aqueous suspensions. Pharm. Res. 6, 555–563.

Bakan, D.A., Weichert, J.P., Longino, M.A., Counsell, R.E., 2000. Polyiodinated triglyceride lipid emulsions for use as hepatoselective contrast agents in CT: of physicochemical properties on biodistribution and imaging. Invest. Radiol. 35, 158–169.

Bakan, D.A., Longino, M.A., Weichert, J.P., Counsell, R.E., 1996. Physicochemical characterization of a synthetic lipid emulsion for hepatocyte-selective delivery of lipophilic compounds: application to polyiodinated triglycerides as contrast agents for computed tomography. J Pharm. Sci. 85, 908–914.

Beckurts, T.E., Shreeve, W.W., Schieren, R., Feinendegen, L.E., 1985. Kinetics of different [123]I- and ^{14}C-labelled fatty acids in normal and diabetic rat myocardium in vivo. Nucl. Med. Commun. 6, 415–424.

Bisogno, T., Melck, D., De Petrocellis, L., Di Marzo, V., 1999. Phosphatidic acid as the biosynthetic precursor of the endocannabinoid 2-arachidonoylglycerol in intact mouse neuroblastoma cells stimulated with ionomycin. J. Neurochem. 72, 2113–2119.

Bodmer, M.W., Angal, S., Yarranton, G.T., Harris, T.J., Lyons, A., King, D.J., Pieroni, G., Riviere, C., Verger, R., Lowe, P.A., 1987. Molecular

cloning of a human gastric lipase and expression of the enzyme in yeast. Biochim. Biophys. Acta 909, 237–244.

Bontemps, L., Demaison, L., Keriel, C., Pernin, C., Mathieu, J.P., Marti-Batlle, D., Vidal, M., Fagret, D., Comet, M., Cuchet, P., 1985. Kinetics of [16-^{123}I]iodohexadecenoic acid metabolism in the rat myocardium, influence of glucose concentration in the perfusate and comparison with [1-^{14}C]palmitate. Eur. Heart. J. 6, 91–96.

Canaan, S., Roussel, A., Verger, R., Cambillau, C., 1999. Gastric lipase: crystal structure and activity. Biochim Biophys. Acta 1441, 197–204.

Carriere, F., Withers-Martinez, C., van Tilbeurgh, H., Roussel, A., Cambillau, C., Verger, R., 1998. Structural basis for the substrate selectivity of pancreatic lipases and some related proteins. Biochim. Biophys. Acta 1376, 417–432.

Carter, G.W., Young, P.R., Swett, L.R., Paris, G.Y., 1980. Pharmacological studies in the rat with [2-(1,3-didecanoyloxy)-propyl]2-acetyloxybenzoate (A-45474): an aspirin pro-drug with negligible gastric irritation. Agents Actions 10, 240–245.

Charbon, V., Latour, I., Lambert, D.M., Buc-Calderon, P., Neuvens, L., De Keyzer, J.L., Gallez, B., 1996. Targeting of drug to the hepatocytes by fatty acids. Influence of the carrier (albumin or galactosylated albumin) on the fate of the fatty acids and their analogues. Pharm. Res. 13, 27–31.

Chen, Z.X., Breitman, T.R., 1994. Tributyrin: a prodrug of butyric acid for potential clinical application in differentiation therapy. Cancer Res. 1994, 3494–3499.

Conley, B.A., Egorin, M.J., Tait, N., Rosen, D.M., Sausville, E.A., Dover, G., Fram, R.J., Van Echo, D.A., 1998. Phase I study of the orally administered butyrate prodrug, tributyrin, in patients with solid tumors. Clin. Cancer Res. 4, 629–634.

Cullen, E., 1984. Novel anti-inflammatory agents. J. Pharm. Sci. 73, 579–589.

Delie, F., Couvreur, P., Nisato, D., Michel, J.B., Puisieux, F., Letourneux, Y., 1994. Synthesis and in vitro study of a diglyceride prodrug of a peptide. Pharm. Res. 11, 1082–1087.

Deverre, J.R., Loiseau, P., Couvreur, P., Letourneux, Y., Gayral, P., Benoit, J.P., 1989. In vitro evaluation of filaricidal activity of GABA and 1,3-dipalmitoyl-2-(4-aminobutyryl)glycerol HCl: a diglyceride prodrug. J Pharm. Pharmacol. 41, 191–193.

Deverre, J.R., Loiseau, P., Puisieux, F., Gayral, P., Letourneux, Y., Couvreur, P., Benoit, J.P., 1992a. Synthesis of the orally macrofilaricidal and stable glycerolipidic prodrug of melphalan,1,3-dipalmitoyl-2-(4′[bis(2″-chloroethyl)amino]phenylalaninoyl)glycerol. Arzneimittelforschung 42, 1153–1156.

Deverre, J.R., Loiseau, P., Gayral, P., Letourneux, Y., Couvreur, P., Benoit, J.P., 1992b. In vitro and in vivo evaluation of macrofilaricidal activity of GABA and 1,3-dipalmitoyl-2-(4-aminobutyryl)glycerol HCl: a diglyceride prodrug. Pharm. Acta Helv. 67, 349–352.

Dodds, P.F., 1991. Incorporation of xenobiotic carboxylic acids into lipids. Life Sci. 49, 629–649.

Dodds, P.F., 1995. Xenobiotic lipids: the inclusion of xenobiotic compounds in pathways of lipid biosynthesis. Prog. Lipid Res. 34, 219–247.

Dodds, P.F., Chou, S.C., Ranasinghe, A., Coleman, R.A., 1995. Metabolism of fenbufen by cultured 3T3-L1 adipocytes: synthesis and metabolism of xenobiotic glycerolipids. J. Lipid Res. 36, 2493–2503.

el Kihel, L., Loiseau, P.M., Bourass, J., Gayral, P., Letourneux, Y., 1994. Synthesis and orally macrofilaricidal evaluation of niclosamide lymphotropic prodrugs. Arzneimittelforschung 44, 1259–1264.

el Kihel, L., Bourass, J., Richomme, P., Petit, J.Y., Letourneux, Y., 1996. Synthesis and evaluation of the anti-inflammatory effects of niflumic acid lipophilic prodrugs in brain edema. Arzneimittelforschung 46, 1040–1044.

Fears, R., 1985. Lipophilic xenobiotic conjugates: the pharmacological and toxicological consequences of the participation of drugs and other foreign compounds as substrates in lipid biosynthesis. Prog Lipid Res. 24, 177–195.

Friis, G.J., Bundgaard, H., 1996. Design and applications of prodrugs. In:

Krogsgaard-Larsen, P., Liljefors, T., Madsen, U. (Eds.), A Textbook of Drug Design and Development. Harwoord Academic Publishers, pp. 351–385.

Gallez, B., Demeure, R., Debuyst, R., Leonard, D., Dejehet, F., Dumont, P., 1992. Evaluation of nonionic nitroxyl lipids as potential organ-specific contrast agents for magnetic resonance imaging. Magn. Reson. Imaging 10, 445–455.

Gallez, B., Debuyst, R., Demeure, R., Dejehet, F., Grandin, C., Van Beers, B., Taper, H., Pringot, J., Dumont, P., 1993. Evaluation of a nitroxyl fatty acid as liver contrast agent for magnetic resonance imaging. Magn. Reson. Med. 30, 592–599.

Garzon-Aburbeh, A., Poupaert, J.H., Claesen, M., Dumont, P., Atassi, G., 1983. 1,3-dipalmitoylglycerol ester of chlorambucil as a lymphotropic, orally administrable antineoplastic agent. J. Med. Chem. 26, 1200–1203.

Garzon-Aburbeh, A., Poupaert, J.H., Claesen, M., Dumont, P., 1986. A lymphotropic prodrug of L-Dopa: synthesis, pharmacological properties, and pharmacokinetic behavior of 1,3-dihexadecanoyl-2-[(S)-2-amino-3-(3,4-dihydroxyphenyl)propanoyl]propane-1,2,3-triol. J. Med. Chem. 29, 687–691.

Geurts, M., Poupaert, J.H., Scriba, G.K.E., Lambert, D.M., 1998. N-(benzyl-oxycarbonyl)glycine esters and amides as new anticonvulsants. J. Med. Chem. 41, 24–30.

Haselden, J.N., Dodds, P.F., Hutson, D.H., 1998a. The metabolism of the xenobiotic triacylglycerols, rac-1- and sn-2-(3-phenoxybenzoyl)-dipalmitoylglycerol, following intravenous administration to the rat. Biochem. Pharmacol. 56, 1599–1606.

Haselden, J.N., Hutson, D.H., Dodds, P.F., 1998b. The metabolism of 3-phenoxybenzoic acid-containing xenobiotic triacylglycerols in vitro by pancreatic, hormone-sensitive and lipoprotein lipases. Biochem. Pharmacol. 56, 1591–1598.

Hong, C.I., An, S.H., Buchheit, D.J., Nechaev, A., Kirisits, A.J., West, C.R., Ryu, E.K., MacCoss, M., 1984. 1-beta-D-arabinofuranosylcytosine-phospholipid conjugates as prodrugs of Ara-C. Cancer Drug Deliv. 1, 181–190.

Hong, C.I., An, S.H., Schliselfeld, L., Buchheit, D.J., Nechaev, A., Kirisits, A.J., West, C.R., 1988. Nucleoside conjugates. 10. Synthesis and antitumor activity of 1-beta-D-arabinofuranosylcytosine 5′-diphosphate-1,2-dipalmitins. J. Med Chem. 31, 1793–1798.

Hostetler, K.Y., Parker, S., Sridhar, C.N., Martin, M.J., Li, J.L., Stuhmiller, L.M., van Wijk, G.M., van den Bosch, H., Gardner, M.F., Aldern, K.A., Richman, D.D., 1993. Acyclovir diphosphate dimyristoylglycerol: a phospholipid prodrug with activity against acyclovir-resistant herpes simplex virus. Proc. Natl. Acad. Sci. USA 90, 11835–11839.

Jacob, J.N., Shashoua, V.E., Campbell, A., Baldessarini, R.J., 1985. γ-aminobutyric acid esters. 2. Synthesis, brain uptake, and pharmacological properties of lipid esters of γ-aminobutyric acid. J. Med. Chem. 28, 106–110.

Jacob, J.N., Hesse, J.W., Shashoua, V.E., 1987. γ-aminobutyric acid esters. 3. Synthesis, brain uptake, and pharmacological properties of C-18 glyceryl lipid esters of GABA with varying degree of unsaturation. J. Med. Chem. 30, 1573–1576.

Jacob, J.N., Hesse, J.W., Shashoua, V.E., 1990. Synthesis, brain uptake, and pharmacological properties of lipid esters of a glyceryl lipid containing GABA and the GABA-T inhibitor γ-vinyl-GABA. J. Med. Chem. 33, 733–736.

Jorgensen, A., Andersen, J., Bjorndal, N., Dencker, S.J., Lundin, L., Malm, U., 1982. Serum concentrations of cis(Z)-flupentixol and prolactin in chronic schizophrenic patients treated with flupentixol and cis(Z)-flupentixol decanoate. Psychopharmacology 77, 58–65.

Keriel, C.M., Humbert, T.G., Berard, C.R., Marti-Batlle, D.S., Le Bars, D., Mathieu, J.P., Luu-Duc, C., Comet, M., Cuchet, P.J., 1990. The intramyocardial fate of [1-¹⁴C] palmitate, iodopalmitate and iodophenylpentadecanoate in isolated rat hearts. A contribution to the choice of an iodinated fatty acid as a tracer of myocardial metabolism. J. Mol. Cell. Cardiol. 22, 1379–1392.

Kumar, R., Billimora, J.D., 1978. Gastric ulceration and the concentration of salicylate in plasma in rats after administration of ¹⁴C-labelled aspirin and its synthetic triglyceride, 1,3-dipalmitoyl-2(2′-acetoxy-[¹⁴C]carboxylbenzoyl glycerol. J. Pharm. Pharmacol. 30, 754–758.

Kurz, M., Scriba, G.K.E., 2000. Drug-phospholipid conjugates as potential prodrugs: synthesis, characterization, and degradation by pancreatic phospholipase A₂. Chem. Phys. Lipids (in press).

Lambert, D.M., Mergen, F., Poupaert, J.H., Dumont, P., 1993a. Analgesic potency of S-acetylthiorphan after intravenous administration in mice. Eur. J. Pharmacol. 243, 129–134.

Lambert, D.M., Neuvens, L., Mergen, F., Gallez, B., Poupaert, J.H., Ghysel-Burton, J., Maloteaux, J.M., Dumont, P., 1993b. 1,3-Diacylaminopropan-2-ols and corresponding 2-acyl derivatives as drug carriers: unexpected pharmacological properties. J. Pharm. Pharmacol. 45, 186–191.

Lambert, D.M., Poupaert, J.H., Maloteaux, J.M., Dumont, P., 1994. Anticonvulsant activities of N-benzyloxycarbonylglycine after parenteral administration. Neuroreport 5, 777–780.

Lambert, D.M., Scriba, G.K.E., Gallez, B., Poupaert, J.H., Dumont, P., 1995a. Glyceride conjugate as drug carriers. Curr. Med. Chem. 1, 376–391.

Lambert, D.M., Mergen, F., Berens, C.F., Poupaert, J.H., Dumont, P., 1995b. Synthesis and pharmacological properties of 2-[S-acetylthiorphan]-1,3- diacylaminopropan-2-ol derivatives as chimeric lipid drug carriers containing an enkephalinase inhibitor. Pharm. Res. 12, 187–191.

Lambert, D.M., Gallez, B., Poupaert, J.H., 1995c. Synthesis and distribution of N-benzyloxycarbonyl-[¹⁴C]-glycine, a lipophilic derivative of glycine. J. Labelled Comp. Radiopharm. 37, 397–406.

Lambert, D.M., 1995d. Des dérivés simples de la glycine aux systèmes lipidiques transporteurs de glycine. Essai de vectorisation d'un acide aminé neutre de petite taille vers le système nerveux central. Bulletin et Mémoires de l'Académie royale de Médecine de Belgique 150, 294–300.

Lambert, D.M., Scriba, G.K.E., Poupaert, J.H., Dumont, P., 1996. Anticonvulsant activity of ester- and amide-type lipid conjugates of glycine and N-benzyloxycarbonylglycine. Eur. J. Pharm. Sci. 4, 159–166.

Lambert, D.M., Di Marzo, V., 1999. The palmitoylethanolamide and oleamide enigmas: are these two fatty acid amides cannabimimetic? Current Med. Chem. 6, 739–755.

Loiseau, P.M., Bourass, J., Letourneux, Y., 1997. Lymphotropic antifilarial agents derived from closantel and chlorambucil. Int. J. Parasitol. 27, 443–447.

Loiseau, P.M., Deverre, J.R., el Kihel, L., Gayral, P., Letourneux, Y., 1994. Study of lymphotropic targeting and macrofilaricidal activity of a melphalan prodrug on the *Molinema dessetae* model. J. Chemother. 6, 230–237.

Longino, M.A., Bakan, D.A., Weichert, J.P., Counsell, R.E., 1996. Formulation of polyiodinated triglyceride analogues in a chylomicron remnant-like liver-selective delivery vehicle. Pharm. Res. 13, 875–879.

Mantelli, S., Speiser, P., Hauser, H., 1985. Phase behaviour of a diglyceride prodrug: spontaneous formation of unilamellar vesicles. Chem. Phys. Lipids. 37, 329–343.

Matsushita, T., Ryu, E.K., Hong, C.I., MacCoss, M., et al., 1981. Phospholipid derivatives of nucleoside analogs as prodrugs with enhanced catabolic stability. Cancer Res. 41, 2707–2713.

Mayer, J.M., Roy-de Vos, M., Audergon, Ch., Testa, B., Etter, J.Cl., 1994. Interactions of anti-inflammatory 2-arylpropionates (profens) with the metabolism of fatty acids – in vitro studies. Intern. J. Tissue Reactions 16, 59–72.

Mergen, F., Lambert, D.M., Poupaert, J.H., Bidaine, A., Dumont, P., 1991a. Synthesis of 1,3-diacylamino-2-propanols and corresponding 2 acyl derivatives as amide isosteres of natural lipids. Chem. Phys. Lipids 59, 267–272.

Mergen, F., Lambert, D.M., Goncalves Saraiva, J.C., Poupaert, J.H., Dumont, P., 1991b. Antiepileptic activity of 1,3-dihexadecanoylamino-

2-valproyl-propan-2-ol, a prodrug of valproic acid endowed with a tropism for the CNS. J. Pharm. Pharmacol. 43, 815–816.

Moorhouse, K.G., Dodds, P.F., Hutson, D.H., 1991. Xenobiotic triacylglycerol formation in isolated hepatocytes. Biochem. Pharmacol. 41, 1179–1185.

Paris, G.Y., Garmaise, D.L., Cimon, D.G., Swett, L., Carter, G.W., Young, P., 1979. Glycerides as prodrugs. 1. Synthesis and antiinflammatory activity of 1,3-bis(alkanoyl)-2-(O-acetylsalicyloyl)glycerides (aspirin triglycerides). J. Med. Chem. 22, 683–687.

Paris, G.Y., Garmaise, D.L., Cimon, D.G., Swett, L., Carter, G.W., Young, P., 1980a. Glycerides as prodrugs. 2. 1,3-Dialkanoyl-2-(2-methyl-4-oxo-1,3-benzodioxan-2-yl)glycerides (cyclic aspirin triglycerides) as antiinflammatory agents. J. Med. Chem. 23, 79–82.

Paris, G.Y., Garmaise, D.L., Cimon, D.G., Swett, L., Carter, G.W., Young, P., 1980b. Glycerides as prodrugs. 3. Synthesis and antiinflammatory activity of [1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetyl]glycerides (indomethacin glycerides). J. Med. Chem. 23, 9–13.

Paris, G.Y., Cimon, D.G., 1982. Glycerides as prodrugs. 4. Synthesis and antiinflammatory activity of 1,3-dialkananoyl-2-arylalkanoylglycerides. Eur. J. Med. Chem. 17, 193–195.

Poupaert, J.H., Lambert, D.M., 1992. Design de vecteurs micromoléculaires de médicaments. Nouvelles de la Science et des Technologies 10, 45–50.

Reske, S.N., 1985. ^{123}I-phenylpentadecanoic acid as a tracer of cardiac free fatty acid metabolism. Experimental and clinical results. Eur. Heart. J. 6, 39–47.

Sakai, A., Mori, N., Shuto, S., Suzuki, T., 1993. Deacylation-reacylation cycle: a possible absorption mechanism for the novel lymphotropic antitumor agent dipalmitoyl-phosphatidylfluorouridine in rats. J. Pharm. Sci. 82, 575–578.

Schwendner, S.W., Weichert, J.P., Longino, M.A., Gross, M.D., Counsell, R.E., 1992. Potential organ or tumor imaging agents. 32. A triglyceride ester of p-iodophenyl pentadecanoic acid as a potential hepatic imaging agent. Int. J. Rad. Appl. Instrum. B 19, 639–650.

Scriba, G.K.E., 1993a. Phenytoin-lipid conjugates as potential prodrugs of phenytoin. Arch. Pharm. 326, 477–481.

Scriba, G.K.E., 1993b. Phenytoin-lipid conjugates: chemical, plasma esterase-mediated, and pancreatic lipase-mediated hydrolysis in vitro. Pharm. Res. 10, 1181–1186.

Scriba, G.K.E., 1994. Synthesis and in vitro evaluation of 4-(2-glyceryl)butyric acid: a glyceride mimic for drug delivery via drug–lipid conjugates. Arch. Pharm. 327, 347–348.

Scriba, G.K.E., Lambert, D.M., Poupaert, J.H., 1995a. Anticonvulsant activity of phenytoin–lipid conjugates, a new class of phenytoin prodrugs. J. Pharm. Pharmacol. 47, 197–203.

Scriba, G.K.E., Lambert, D.M., Poupaert, J.H., 1995b. Bioavailability and anticonvulsant activity of a monoglyceride-derived prodrug of phenytoin after oral administration to rats. J. Pharm. Sci. 84, 300–302.

Scriba, G.K.E., Lambert, D.M., Poupaert, J.H., 1995c. Bioavailability of phenytoin following oral administration of phenytoin–lipid conjugates to rats. J. Pharm. Pharmacol. 47, 945–948.

Scriba, G.K.E., Lambert, D.M., 1997. Bioavailibility and anticonvulsant activity of phenytoin-bis-hydroxybutyrate after oral administration to rats. Pharm. Res. 14, 251–253.

Scriba, G.K.E., 1999. Pancreatic lipase drug delivery with glyceride-containing prodrugs. Pharm. Unserer Zeit. 28, 87–94.

Sugihara, J., Furuuchi, S., Nakano, K., Harigaya, S., 1988a. Studies on intestinal lymphatic absorption of drugs. I. Lymphatic absorption of alkyl ester derivatives and alpha-monoglyceride derivatives of drugs. J. Pharmacobiodyn. 11, 369–376.

Sugihara, J., Furuuchi, S., Ando, H., Takashima, K., Harigaya, S., 1998b. Studies on intestinal lymphatic absorption of drugs. II. Glyceride prodrugs for improving lymphatic absorption of naproxen and nicotinic acid. J. Pharmacobiodyn. 11, 555–562.

Sugihara, J., Furuuchi, S., 1988. Lymphatic absorption of hypolipidemic compound,1-O-[p-(myristyloxy)-alpha-methylcinnamoyl]glycerol (L-K-903). J. Pharmacobiodyn. 11, 121–130.

Takayama, H., Watanabe, A., Hosokawa, M., Chiba, K., Satoh, T., Aimi, N., 1998. Synthesis of a new class of camptothecin derivatives, the long-chain fatty acid esters of 10-hydroxycamptothecin, as a potent prodrug candidate, and their in vitro metabolic conversion by carboxylesterases. Bioorg. Med. Chem. Lett. 8, 415–418.

Tamaki, N., Fujibayashi, Y., Magata, Y., Yonekura, Y., Konishi, J., 1995. Radionuclide assessment of myocardial fatty acid metabolism by PET and SPECT. J. Nucl. Cardiol. 2, 256–266.

Testa, B., 1995. Drug metabolism. In: Wolff, M.E. (Ed.), 5th Edition. Burger's Medicinal Chemistry and Drug Discovery, Vol. 1. Wiley-Interscience, New York, pp. 129–180.

Testa, B., Caldwell, J., 1996. Prodrugs revisited: the ad hoc approach as a complement to ligand design. Med. Res. Rev. 16, 233–241.

van den Bosch, H., 1974. Phosphoglyceride metabolism. Annu. Rev. Biochem. 43, 243–277.

van Tilbeurgh, H., Bezzine, S., Cambillau, C., Verger, R., Carriere, F., 1999. Colipase: structure and interaction with pancreatic lipase. Biochim. Biophys. Acta 1441, 173–184.

van Wijk, G.M., Hostetler, K.Y., van den Bosch, H., 1992. Antiviral nucleoside diphosphate diglycerides: improved synthesis and facilitated purification. J. Lipid Res. 33, 1211–1219.

van Wijk, G.M., Hostetler, K.Y., Kroneman, E., Richman, D.D., Sridhar, C.N., Kumar, R., van den Bosch, H., 1994. Synthesis and antiviral activity of 3′-azido-3′-deoxythymidine triphosphate distearoylglycerol: a novel phospholipid conjugate of the anti-HIV agent AZT. Chem. Phys. Lipids 70, 213–222.

Weichert, J.P., Longino, M.A., Schwender, S.W., Counsell, R.E., 1986a. Potential tumor- or organ-imaging agents. 26. Polyiodinated 2-substituted triacylglycerols as hepatropic agents. J. Med. Chem. 29, 1674–1682.

Weichert, J.P., Groziak, M.P., Longino, M.A., Schwendner, S.W., Counsell, R.E., 1986b. Potential tumor- or organ-imaging agents. 27. Polyiodinated 1,3-disubstituted and 1,2,3-trisubstituted triacylglycerols. J. Med. Chem. 29, 2457–2465.

Weichert, J.P., Longino, M.A., Bakan, D.A., Spigarelli, M.G., Chou, T.S., Schwendner, S.W., Counsell, R.E., 1995. Polyiodinated triglyceride analogs as potential computed tomography imaging agents for the liver. J. Med. Chem. 38, 636–646.

Weichert, J.P., Lee, F.T., Longino, M.A., Bakan, D.A., Spigarelli, M.G., Francis, I.R., Counsell, R.E., 1996. Computed tomography scanning of hepatic tumors with polyiodinated triglycerides. Acad. Radiol. 3, S229–S231.

Wermuth, C.G, Gaignault, J.C., Marchandeau, C., 1996. Designing prodrugs and bioprecursors. I. Carrier prodrugs. In: Wermuth, C.G. (Ed.). The Practice of Medicinal Chemistry. Academic Press, pp. 671–696.