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International Journal of Pharmaceutics 196 (2000) 187–191

international journal of pharmaceutics

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Direct detection of dissolution of ^{14}C-labeled compounds into an oil phase by the fat scintillation proximity method

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Abstract

Traditional analysis of the dissolution of lipophilic compounds from aqueous phase into oil is often hampered by the necessity to separate the receiver oil compartment from the aqueous phase. In order to avoid possible artefacts associated with additional separation methods, a procedure was developed to selectively detect the entry of a compound into the oil phase of a oil/water dispersion. When a combination of a primary and secondary scintillator was predissolved in the oil, and solid ^{14}C-tetrahydrolipstatin was added, increasing signals from the same container were measured upon prolonged incubation. The data are consistent with the hypothesis that ^{14}C-THL that has dissolved in the oil phase is essentially responsible for the measured signal. The obtained dissolution profiles of ^{14}C-THL into oil match with parallel experiments using classical procedures.

Keywords: Fat; Dissolution; Proximity; Scintillation

1. Introduction

Tetrahydrolipstatin (THL, orlistat) is designed to bind to gastric and pancreatic lipase locally in the GI-tract. One route by which THL can reach its target is by prior dissolution in dietary fat droplets (Borgström, 1988). Therefore, there is a need for measuring the dissolution profile of this highly lipid soluble drug (clogP > 9.0) in a dispersed oil phase.

In receptor bioanalytics, scintillation proximity (SP) measurement is a well established technique which is lacking the need to separate bound and unbound ligand (Bosworth and Towers, 1989). Primary and secondary scintillators convey the radioactive signal by luminescence and are present on the surface of a solid support to which receptor proteins can be conveniently coated. ^{14}C-labeled ligands can only induce scintillation of these compounds in direct proximity, i.e. after binding to the receptor. As the primary and secondary scintillators commonly used are lipophilic, we hypothesised to explore the possibility to detect dissolution of ^{14}C-THL into the fat particle by using a receiver fat phase with preincorporated scintillators. Only a small fraction of the ^{14}C-THL microcrystalline material that remains dispersed in the aqueous

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0378-5173/00/$ - see front matter
PII: S0378-5173(99)00418-4

phase would be in proximity with the scintillators and therefore induce a signal (mean pathlength of the ^{14}C radioisotope = 50 μm). A strong increase of the signal over time however, would occur, if the labeled drug dissolves in the oil phase.

2. Materials and methods

2.1. Materials

HiSafe scintillation cocktail, PPO (2,5 diphenyloxazole) and bis-MSB (1,4-bis(2-methylstyryl)benzene) were purchased from Packard (Groningen, The Netherlands). Olive oil was from Fluka (Buchs, Switzerland). ^{14}C-THL (spec. activity 1 mCi/mg) was synthesized by Hoffmann-La Roche (Basel, Switzerland). Lecithin (phospholipon 80) was from Nattermann RPR (Köln, Germany). MIFLOC potato mash powder was from Migros (Bischofszell, Switzerland). ^{3}H-triolein (835 MBq/mg) was purchased from Amersham (Little Chalfont, UK).

2.2. Linearity of the signal as a function of the amount of ^{14}C material

A total of 600 mg of PPO and 150 mg of bisMSB were dissolved in 100 ml olive oil in an amber container. A series of solutions of ^{14}C-THL, ranging from 27 400 to 2 220 000 dpm/ml in 0.6% PPO and 0.15% bisMSB in olive oil were prepared by stirring ^{14}C-THL into PPO-bisMSB olive oil. Emulsions were then prepared by vortexing 200 mg of the oil solution in a 10 ml glass tube containing 50 mg egg lecithin and 1500 μl buffer (pH 4.0, USP XXI). These emulsions were transferred into β-vials using an additional 1.5 ml buffer to rinse the tube and counted for ^{14}C-radioactivity in a Packard TriCarb Liquid Scintillation Analyzer. After counting, 1000 μl of the same sample were mixed with 15 ml HiSafe scintillation cocktail for determination of the true ^{14}C-radioactivity.

2.3. Dependence of the signal on PPO/bisMSB concentration

Serial dilutions of PPO/bisMSB in olive oil containing 1 mg/ml ^{14}C-THL were prepared by diluting a solution of 1 mg/ml ^{14}C-THL in olive oil containing 0.6% PPO and 0.15% bisMSB with a solution of 1 mg/ml ^{14}C-THL in olive oil. To obtain emulsions, 200 mg of these dilutions were processed as described above and samples were counted for β-radioactivity.

2.4. Dissolution of THL into PPO/bisMSB olive oil from an aqueous dispersion

A total of 4 mg of ^{14}C-THL microcrystalline powder was weighed into a β-vial, and 5 ml of buffer was added. Altogether 1 ml of PPO/bisMSB olive oil (0.6/0.15%) was carefully placed on top of the aqueous phase. The resulting preparation was directly counted for ^{14}C-radioactivity and subsequently incubated by mounting the β-vial in a rotating holder (Dianorm, 4 rpm, room temperature). At the indicated times, the β-vial was removed and immediately counted for ^{14}C-radioactivity in the oil phase. ^{14}C-radioactivity of 100% THL dissolved in the oil phase was determined by dissolving 4 mg of ^{14}C-THL in 1 ml of PPO/bisMSB olive oil (0.6/0.15%) and subsequent addition of 5 ml of buffer.

2.5. Dissolution of THL into PPO/bisMSB olive oil in gastric milieu

In order to disperse lipid droplets in a viscous matrix under acidic pH, olive oil containing 0.6% PPO and 0.15% bisMSB was mixed into 6.7% potato mash and this dispersion was incubated with 4 mg ^{14}C-THL microcrystalline powder while rotating. Potato mash powder (1.0 g) was added to 14.0 g warm (40°C) simulated gastric juice (USP XXI buffer, pH 4.0) and suspended to homogeneity. 0.50 g PPO/bisMSB olive oil (0.6/0.15%) was vortexed with 4.50 g of the potato mash vehicle. A total of 4 mg of ^{14}C-THL microcrystalline powder was weighed into a β-vial, and 4.0 ml of the dispersion of PPO/bisMSB olive oil was added. Incubation and measurements were performed as described above. In a separate experiment, ^{14}C-THL microcrystalline powder was incubated with the olive oil-potato mash dispersion under the same conditions as described above, except for the fact

that the olive oil was spiked with ^{3}H-triolein. A total of 200 μl samples were taken at the indicated time points and lipid droplets were isolated with

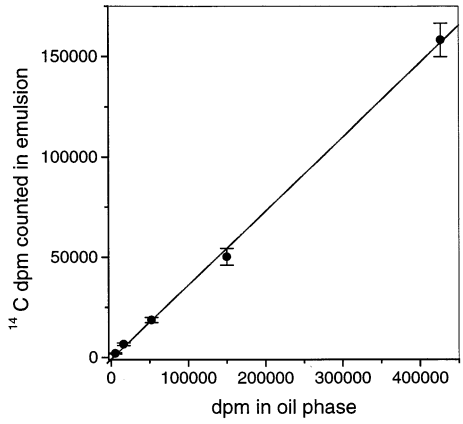

Fig. 1. Linearity of ^{14}C-induced luminescence from emulsified fat (proximity principle). Dilutions of ^{14}C-THL in 0.6% PPO and 0.15% bisMSB in olive oil were emulsified with lecithin in artificial gastric juice and counted directly for ^{14}C-radioactivity (y-axis). Subsequently, the same samples were mixed with scintillation cocktail and measured with classical β-scintigraphy (x-axis), ($n = 2$).

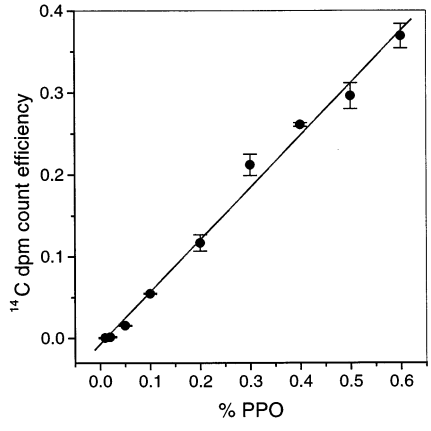

Fig. 2. Relationship of concentration of primary and secondary scintillators in the oil phase with count efficiency. Serial dilutions of PPO/bisMSB in olive oil containing 1 mg/ml ^{14}C-THL were prepared by diluting a solution of 1 mg/ml ^{14}C-THL in olive oil containing 0.6% PPO and 0.15% bisMSB with a solution of 1 mg/ml ^{14}C-THL in olive oil. To obtain emulsions, 200 mg of these dilutions were processed as described under Section 2 and samples were counted for β-radioactivity without the addition of scintillation cocktail ($n = 2$).

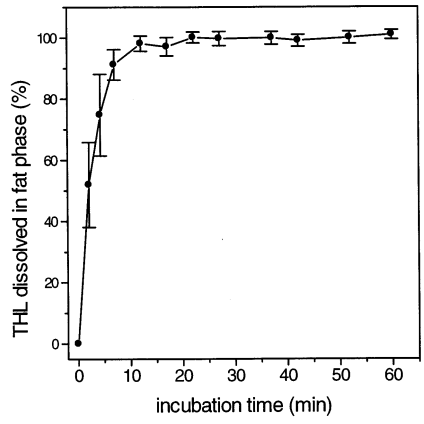

Fig. 3. Dissolution of ^{14}C-THL microcrystalline powder into olive oil as assessed by the fat proximity principle. ^{14}C-THL was dispersed in artificial gastric juice (buffer pH 4.0, USP XXI) in a β-vial. Olive oil (containing 0.6% PPO and 0.15% bisMSB) was added to the aqueous phase. The first data point was determined after addition of the oil phase. Subsequent data points were measured after incubation of the sample by mounting it in a rotating chamber at room temperature. The containers were removed at set time points and measured for ^{14}C-radioactivity in a β-counter ($n = 2$).

the use of glass micropipettes. After mixing in 5 ml HiSafe scintillation cocktail these samples were counted for ^{14}C- and ^{3}H-radioactivity. The theoretical ratio of totally applied ^{14}C- and ^{3}H-radioactivity was used to determine 100% dissolution of THL into the olive oil.

3. Results and discussion

Fig. 1 shows the relationship between true radioactivity and the signal obtained with the fat proximity principle. The relationship is linear ($r = 0.999$) and count efficiency is 36.7%. The loss of signal is presumably as a result of scattering by the emulsified preparation, and was constant over the concentration range.

Count efficiency is also linearly dependent upon the amount of primary and secondary scintillators (PPO and bisMSB) in the oil phase ($r = 0.992$). At 0.6% PPO, an efficiency of 37.4% was obtained that is comparable with the 36.7% efficiency from the first experiment, indicating that the method can be performed reproducibly (Fig. 2).

Fig. 3 represents the dissolution of ^{14}C-THL

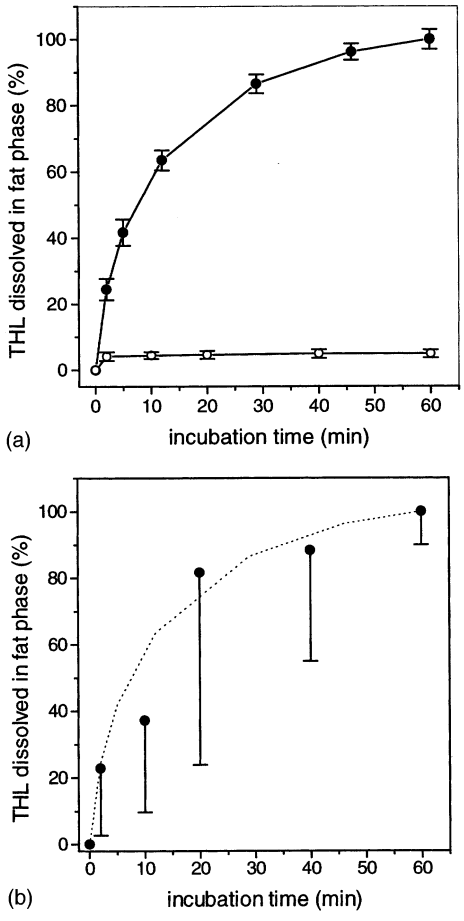

Fig. 4. (a) Dissolution of ^{14}C-THL microcrystalline powder into olive oil as assessed by the fat proximity principle. ^{14}C-THL was incubated with a dispersion of olive oil (containing 0.6% PPO and 0.15% bisMSB) in potato mash suspended in artificial gastric juice (buffer pH 4.0, USP XXI) in a β-vial mounted in a rotating chamber (filled circles) or without agitation (open circles) at room temperature. The containers were removed at set time points and measured for ^{14}C-radioactivity in a β-counter ($n=2$). (b) Dissolution of ^{14}C-THL microcrystalline powder into ^{3}H-triolein-labeled olive oil. ^{14}C-THL was incubated with a dispersion of ^{3}H-triolein-labeled olive oil in potato mash suspended in artificial gastric juice (buffer pH 4.0, USP XXI) in a β-vial mounted in a rotating chamber under the same conditions as the experiment presented in Fig. 4a (filled circles). Samples from the containers were removed at set time points and lipid droplets isolated from these samples were measured for ^{14}C- and ^{3}H-radioactivity in a β-counter ($n=2$). For comparison, the dissolution profile obtained with the scintillation proximity method is included (dashed line).

into a layer of PPO/bisMSB olive oil. The first measurement of ^{14}C-radioactivity was performed directly after the addition of the PPO/bisMSB olive oil. A rapid increase in ^{14}C-radioactivity could be detected, indicating that immediately after addition of PPO/bisMSB olive oil the ^{14}C-labeled drug starts to adsorb to and dissolve into the oil phase. Within approximately 15 min the total amount of the dispersed THL had dissolved in the oil phase.

The rate of dissolution of solid ^{14}C-THL, into a dispersion of PPO/bisMSB olive oil in potato mash-gastric juice (Fig. 4a) is comparable to the rate as determined by an alternative method using 'classical' scintillation ^{3}H/^{14}C spectrometry of isolated lipid droplets (Fig. 4b).

The experiments indicate that a steadily increasing luminescence signal is emerging from the same reaction vial over time, and reaches a plateau that corresponds excellently (> 99%) with the theoretical luminescence signal of the total amount of added ^{14}C-radioactivity. Pre-dissolution of the studied amount of ^{14}C-THL in the oil phase and addition of the blank aqueous phase yielded identical dpm values.

In contrast, if the sample was not agitated, a signal lower than 5% of the maximum was obtained (Fig. 4a). This might be explained by the pathlength of the ^{14}C-radioisotope and/or a low degree of transfer into the oil phase.

The kinetics of ^{14}C-THL dissolution are similar in the SP method and the droplet method: $t_{1/2}$ values of 8 and 11 min can be deducted from the experimental data. However, the SP method resulted in substantially much lower standard deviations (Fig. 4a, b).

The SP method can be especially useful for measuring dissolution of ^{14}C-labeled compounds into coarse fat emulsions, comparable to those existing in the stomach after ingestion of a mixed meal. These coarse emulsions had tSIE (transformed spectral index of the external standardvalues) of around 40 and yielded reproducible results. In contrast, sonication of fat emulsions resulted in a rapid and significant loss of signal (data not shown). The methodology however, can be used to compare transfer of a radiolabeled

compound from a range of solid and colloidal forms into crudely emulsified oil compartments or oil layers, simulating physiological conditions in the stomach.

Although in the current studies a ^{14}C-labeled compound was studied, it can be expected that other β-emitting isotopes can also be employed; ^{3}H and ^{125}I are especially recommended as a result of their short pathlengths.

It is concluded that this method offers a very simple and rapid procedure, lacking the necessity of additional separation steps, to measure dissolution of compounds into an oil compartment.

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