ELSEVIER

International Journal of Pharmaceutics 231 (2002) 237–240

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Time-dependent loss of radioactivity counts associated with paracellular markers in the presence of cyclodextrin

I. Pezron [1], G.S. Tirucherai, A.K. Mitra *

Division of Pharmaceutical Sciences, School of Pharmacy, University of Missouri- Kansas City, 5005 Rockhill Road, Kansas City, MO 64110-2499, USA

Received 14 May 2001; received in revised form 6 September 2001; accepted 7 September 2001

Abstract

This communication reports an unexpected phenomenon observed during the counting of radiolabeled paracellular marker solutions in the presence of 2-hydroxypropyl-β-cyclodextrin (HPβCD). The results revealed time-dependent loss of ^{14}C-mannitol and ^{14}C-polyethlene glycol 4000 radioactivity counts with increased percentages of HPβCD. However, ^{14}C-diazepam, a transcellular marker, displayed a stable count. A hypothesis behind this phenomenon is being proposed, involving water transfer from aqueous droplets to the surfactant rich scintillation fluid. The remaining droplets, becoming more and more concentrated in cyclodextrin, entrap the hydrophilic markers and consequently exhibit an increasing quenching effect. This effect shows that careful monitoring of radiolabeled markers used in transport experiments is necessary, even with high quench resistant scintillation fluids, to prevent erroneous interpretation of the transport data.

Keywords: Cyclodextrin; Paracellular marker; Transport; Radioactivity; Liquid scintillation counting; Quenching

Cyclodextrins are cyclic oligosaccharides, which can form inclusion complexes with non-polar drugs (Rajewski and Stella, 1996). Their ability to enhance absorption of both lipophilic and hydrophilic drugs has been widely studied in the last decade (Loftsson and Järvinen, 1999; Uekama et al., 1998). The enhancement mechanisms are not fully understood and may involve increased drug aqueous solubility and stability (Uekama et al., 1998), reduction in the aqueous diffusion layer (Masson et al., 1999) and/or extraction of membrane components (Hovgaard and Bronsdted, 1995; Shao et al., 1992).

As a part of our study related to the delineation of enhancement mechanisms, we investigated the effect of 2-hydroxypropyl-β-cyclodextrin (HPβCD) on the transport of radiolabeled paracellular and transcellular markers across Caco-2 cell monolayers. Typical transport studies were performed with Caco-2 monolayers grown on Transwell filters (Corning-Costar). The apical chamber contained ^{14}C-mannitol, a paracellular

* Corresponding author. Tel.: +1-816-235-1615; fax: +1-816-235-5190.

E-mail address: mitraa@umkc.edu (A.K. Mitra).

[1] On leave from Département de Génie Chimique, Université de Technologie de Compiègne, BP 529, 60205 Compiègne cedex, France.

0378-5173/02/$ - see front matter
PII: S0378-5173(01)00883-3

marker, or ^{14}C-diazepam, a transcellular marker, dissolved in Dulbecco's modified phosphate buffer saline (DPBS) containing various concentrations of HPβCD ranging from 0 to 20% (by weight). The samples were analyzed by liquid scintillation using Scinti-Safe 30% (Fisher Scientific) in a Beckman LS6500 scintillation counter. The present paper reports unexpected behavior observed during the counting of the radiolabeled paracellular marker solutions in presence of cyclodextrin, independent of the transport experiments.

The measurement of the radioactivity in the donor solution at time 0, i.e. before the experiment started, was essential to accurately determine the fraction of marker transported as a function of time. For this purpose, 100 μl of the donor solutions were directly placed in the scintillation vials, to which 5 ml of scintillation liquid was added (all experiments were performed in triplicate). After mixing, the samples were analyzed by the scintillation counter. The results revealed surprisingly smaller activities of ^{14}C-mannitol with increased percentages of HPβCD although the same amount of mannitol was used to prepare the donor solutions (^{14}C-mannitol concentration 0.5 μCi/ml). The samples were homogenized and counted again. Further decrease in activity was noted, which continued for at least 10 days, as shown in Fig. 1a. No loss of activity was noticed for ^{14}C-mannitol in the absence of HPβCD, but up to 25% loss was observed with 20% HPβCD after 10 days. Additional mixing of the sample did not allow recovery of the initial sample activity. Remarkably, in the case of ^{14}C-diazepam, absolutely no change in activity was noticed (Fig. 1b).

Additional experiments were performed with different markers of varying hydrophilicity including ^{14}C-polyethylene glycol (PEG) 4000, ^{14}C-glycocholic acid sodium salt and ^{14}C-1-propanol. The same specific activity was used in the preparation of all marker samples (0.05 μCi). Activity loss was monitored for 10 days in buffer solutions containing 20% HPβCD, as shown in Fig. 2. The most important activity loss after 10 days was observed for ^{14}C-PEG 4000 (40% loss), followed by ^{14}C-mannitol (25% loss). Very little loss was observed for ^{14}C-glycocholic acid sodium salt and ^{14}C-1-propanol (less than 2%). No loss was observed in the case of ^{14}C-diazepam.

Measurement of radioactivity by liquid scintillation counting involves several energy transfer processes arising from radioactive disintegration, which produce visible light. The aqueous radioactive sample is mixed with the scintillation cocktail, which contains an aromatic organic solvent, an organic fluor scintillator and emulsifiers, to form a homogeneous and stable solution or microemulsion. As the beta particles produced by the radioactive molecules interact with the solvent, a fraction of the solvent molecules becomes excited. This energy is then transferred to the fluor scintillator molecules, which rapidly lose their energy

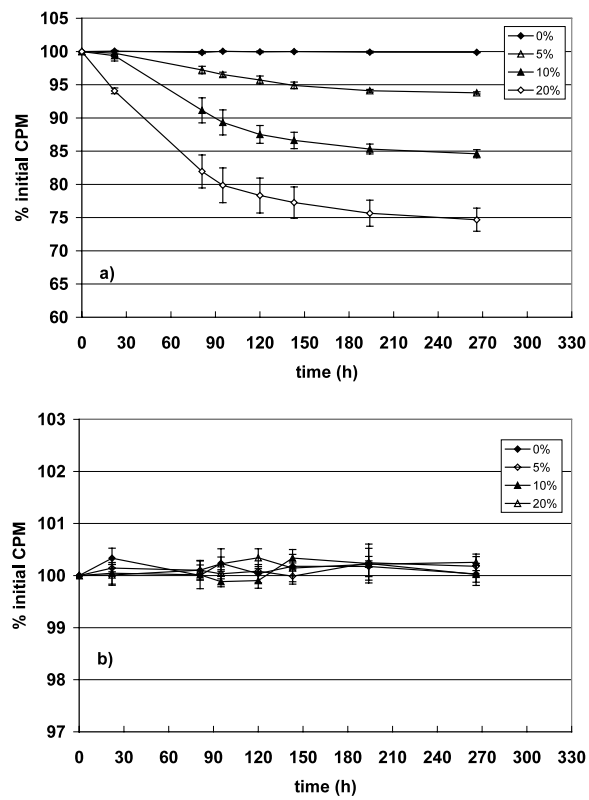

Fig. 1. Effect of various concentrations of HPβCD on the relative variations of the radioactivity counts per minute (CPM) for: (a) a paracellular marker ^{14}C-mannitol; (b) a transcellular marker ^{14}C-diazepam.

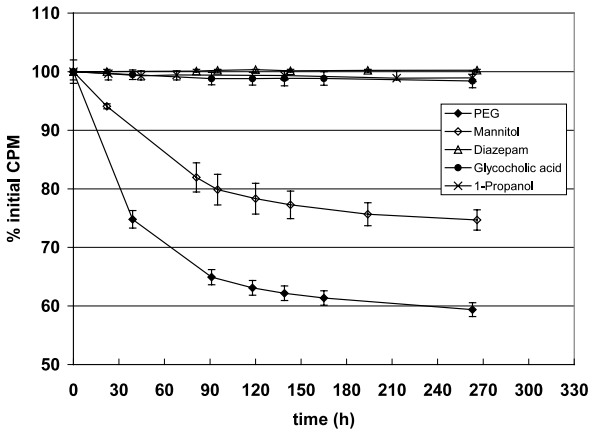

Fig. 2. Effect of 20% HPβCD on the relative variations of the radioactivity counts per minute (CPM) for various hydrophilic and lipophilic radiolabeled markers.

and produce fluorescence. The emission of light is detected by a photomultiplicator tube (L'Annunziata and Kessler, 1998). The counting rate obtained depends on the efficiency of the energy transfers. A reduction of the counting rate can arise from chemical quenching, caused by the interference between a chemical substance present in the sample and the beta particles, which prevents an efficient transfer of energy to the solvent molecules. To our knowledge, no quenching effects have been reported so far with cyclodextrins.

Unstable counting rate, decreasing with time, has been reported in studies in which a biphasic system is obtained with sample-cocktail mixtures. If the radioactive molecule is more soluble in the aqueous phase than the organic phase, and a phase separation occurs, decreasing counts per minute (CPM) can be observed (Thomson, 1998). Dilution of the sample with water or a suitable co-solvent has been suggested to ensure the formation of a homogeneous and stable phase. The effect of microstructure changes in micellar and microemulsion systems formed in the scintillation fluid upon the addition of water has also been studied (Boussaha and Ache, 1980; Lundsten, 1996). In some cases, crossing the border from isotropic to birefringent phases caused a deep decrease in counting rate of the sample (Lundsten, 1996). In other studies, counting efficiency has been used as an efficient probe to study structural changes in micellar solutions and microemulsions (Boussaha and Ache, 1980).

In the study reported here, the scintillation fluid used, Scinti-Safe 30%, as many other modern scintillation liquids, has been developed for the measurement of aqueous samples, and has a high quenching resistance (L'Annunziata and Kessler, 1998). It contains a high amount of non-ionic surfactants, ethoxylated alkyl phenol (around 40%), an organic solvent, phenylethylxylene (around 60%), and fluor scintillators (around 2%), which usually allow the formation of a stable and homogeneous microemulsion phase following mixing with aqueous samples. A significant decrease in counting rate observed in this study, and its irreversibility upon agitation of the sample, are rather unusual and intriguing. We noticed the same effect—even stronger in magnitude—with another commonly used scintillation liquid, Ultima Gold (Hewlett-Packard).

In an attempt to explain this phenomenon, mixtures of HPβCD solutions and scintillation liquid were visually observed in a transparent glass tube. After homogenization, the mixtures showed at first a significant turbidity, which started to fade after several hours. The presence of small droplets was noted by optical microscopy, probably a result of aqueous droplets of a water-in-oil emulsion. The solutions became clear after several days and a gel-like white precipitate was observed in the bottom of the tube, more particularly in the system containing 20% HPβCD. The system with no HPβCD (pure buffer) did not exhibit any turbidity and formed a clear solution with the scintillation fluid. The presence of HPβCD in the aqueous phase thus appears to prevent or delay the solubilization of water (or buffer) in the surfactant rich scintillation fluid. We propose that the phenomenon observed arise from the progressive, partial transfer of water molecules from the aqueous droplets to the surfactant rich phase. According to this hypothesis, the aqueous droplets become increasingly concentrated in cyclodextrin (aqueous

solubility of HPβCD is around 40% at room temperature). As water transfer occurs, cyclodextrin molecules eventually precipitate, entrapping the water-soluble radiolabeled markers. Quenching effect due to cyclodextrin molecules, preventing a smooth transfer of energy to the solvent molecules, will then increase with time and with increasing cyclodextrin concentrations. According to literature data based on octanol/water partition coefficients, the hydrophilicity of the neutral radiolabeled markers increases as follows: diazepam < propanol < < mannitol < PEG 4000 (Yee, 1997; McKarns et al., 1997). Glychocolic acid sodium salt is also a lipophilic compound (Fini et al., 1992). The hydrophilic paracellular markers as PEG 4000 and mannitol may preferably be solvated in the aqueous phase and be partially subjected to the quenching effect of HPβCD, whereas the more lipophilic compounds may partition predominantly into the liquid scintillation phase, where no quenching will occur. Complexation of a lipophilic component by HPβCD is also possible, but does not appear to play an important role in this process. Addition of water to the sample reduces this quenching activity due to dilution of cyclodextrin, but it was not totally suppressed.

Transport experiments using radiolabeled paracellular markers and cyclodextrins are commonly used in cell culture (Hovgaard and Bronsdted, 1995). The results presented here suggest strong time-dependent decrease of radioactivity counts for paracellular markers in the presence of cyclodextrin, which may lead to erroneous data interpretation of transport experiments. The possible effect of any absorption enhancer or excipient on the counting efficiency should thus be carefully examined before any conclusion is drawn. Since radioactivity measurements have been used to study structural changes in emulsion systems, radioactive paracellular markers can also act as sensitive probes to study physicochemical interactions involved in cyclodextrin/water systems.

Acknowledgements

Supported by a grant from Hoechst-Marion-Roussel and Phoenix Scientific Company.

References

Boussaha, A., Ache, H.J., 1980. Structural changes in micellar solutions and microemulsions and their relevance to emulsion counting. J. Phys. Chem. 84, 3249–3254.

Fini, A., Fazio, G., Roda, A., Bellini, A.M., Mencini, E., Guarneri, M., 1992. Basic cholane derivatives. XI. Comparison between acid and basic derivatives. J. Pharm. Sci. 81, 726–730.

Hovgaard, L., Bronsdted, H., 1995. Drug delivery studies in caco-2 monolayers. IV. Absorption enhancer effects of cyclodextrins. Pharm. Res. 12, 1328–1332.

L'Annunziata, M.F., Kessler, M.J., 1998. Radiotracer liquid scintillation analysis. In: L'Annunziata, M. (Ed.), Handbook of Radioactivity Analysis. Academic press, San Diego, pp. 209–329.

Loftsson, T., Järvinen, T., 1999. Cyclodextrins in ophtalmic drug delivery. Adv. Drug Deliv. Rev. 36, 59–79.

Lundsten, G., 1996. Microstructure of oil-surfactant-rich phase in relation to liquid scintillation counting efficiency. J. Colloid Interface Sci. 180, 22–26.

Masson, M., Loftsson, T., Masson, G., Stefansson, E., 1999. Cyclodextrins as permeation enhancers: some theoretical evaluations and in vitro testing. J. Control Rel. 59, 107–118.

McKarns, S.C., Hansch, C., Caldwell, W.S., Morgan, W.T., Moore, S.K., Doolittle, D.J., 1997. Correlation between hydrophobicity of short-chain aliphatic alcohols and their ability to alter plasma membrane integrity. Fund. Appl. Tox. 36, 62–70.

Rajewski, R.A., Stella, V.J., 1996. Pharmaceutical applications of cyclodextrins. II. In vivo drug delivery. J. Pharm. Sci. 86, 1142–1168.

Shao, Z., Krishnamoorthy, R., Mitra, A.K., 1992. Cyclodextrins as nasal absorption promoters of insulin: mechanistic evaluations. Pharm. Res. 9, 1157–1163.

Thomson, J., 1998. Sample preparation techniques for liquid scintillation analysis. In: L'Annunziata, M. (Ed.), Handbook of Radioactivity Analysis. Academic press, San Diego, pp. 407–452.

Uekama, K., Hirayama, F., Irie, T., 1998. Cyclodextrin drug carrier systems. Chem. Rev. 98, 2045–2076.

Yee, S., 1997. In vitro permeability across Caco-2 cells (colonic) can predict in vivo (small intestinal) absorption in man-fact or myth. Pharm. Res. 14, 763–766.