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In vitro investigation on the impact of Solutol HS 15 on the uptake of colchicine into rat hepatocytes

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Abstract

In the current investigation, the impact of the surface-active formulation ingredient Solutol HS 15 on the uptake of colchicine into freshly isolated rat hepatocytes was investigated using a centrifugal filtration technique through a silicone oil layer. Colchicine is taken up into the cells by an active transport mechanism. When conducting the experiment at 37 °C, it was found that at concentrations below its critical micellar concentration (CMC) of 0.021% (0.0003 and 0.003%, w/v), Solutol HS 15 did not impact the uptake of colchicine. By contrast, at a Solutol HS 15 concentration above its CMC (0.03%, w/v), the amount of colchicine taken up into the cells as well as its uptake velocity were significantly decreased. However, in control experiments performed at 4 °C, a temperature at which active transport processes should be significantly slowed down, Solutol HS 15 at 0.03% did not affect colchicine uptake and/or its association with the cells. The described findings might be rationalized by inhibition of colchicine transport either due to direct interaction at the transport site or due to alterations of membrane properties in the presence of Solutol HS 15 at concentrations above its CMC. Moreover, a strong molecular interaction between Solutol HS 15 and colchicine as well as an incorporation of colchicine into micelles formed by Solutol HS 15, this way resulting in a limited contact of colchicine with the cells, cannot be excluded as contributors to the observed effect.

Keywords: Colchicine; Solutol HS 15; Uptake; Hepatocytes; Micelles

1. Introduction

Solutol HS 15, the main component of which is the polyethylene glycol 660 ester of 12-hydroxy stearic acid (Ruchatz and Schuch, 1998), is an amphiphilic formulation ingredient that has been approved in a parenteral Phytonadion formulation for human use on the Canadian market. However, as the surfactant has been shown to impact transport and metabolism of different compounds in vitro, its use as excipient in pharmaceutical preparations has to be considered with care. Solutol HS 15 reportedly reverses multidrug resistance (MDR)-like activity in tumor cells as well as in natural killer cells in vitro (Coon et al., 1991, Chong et al., 1993, Buckingham et al., 1995). In addition, in previous experiments in our laboratory, Solutol HS 15 was found to reduce the intrinsic clearance (Cl_{int}) of the cytochrome P450 3A (CYP 3A) substrate colchicine

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in rat hepatocytes. This observation was significant already at concentrations that had no toxic impact on cell integrity as shown by measuring the lactate dehydrogenase release into the cell culture medium as well as by determining the adenosine triphosphate content in the cells (Bravo González et al., 2001).

As colchicine is taken up into hepatocytes via active transport (Wierzba et al., 1989), interactions with this transport process in the presence of Solutol HS 15, leading to a reduced amount of colchicine entering the cells, may have been responsible for the observed changes in Cl_{int}. The aim of the current investigation was, therefore, to study in vitro the impact of Solutol HS 15 on the uptake of colchicine into rat hepatocytes.

2. Materials and methods

2.1. Materials

Cold colchicine was purchased from Fluka (Buchs, Switzerland) and [^{3}H]colchicine from ARC (St. Louis, USA). Solutol HS 15 was obtained from BASF (Ludwigshafen, Germany). All other materials used were of analytical grade or will be described separately in this section.

2.2. Uptake of colchicine into rat hepatocytes

Hepatocytes were isolated from the in situ perfusion of a whole liver from male Wistar rats (BRL, Füllinsdorf, Switzerland) as described previously (Seglen, 1976; Bittner et al., 2003). The uptake of colchicine into the cells was determined using a centrifugal filtration technique through a silicone oil layer (Schwab et al., 1997). Briefly, 10 μl of a stock solution composed of 2.5 mM cold colchicine and 1500 nCi ^{3}H-labeled colchicine (76.5 Ci/mmol) were pipetted into 500 μl of hepatocytes in suspension. These samples were incubated either in the presence or in the absence of Solutol HS 15 at different concentrations (0.0003, 0.003, and 0.03%, w/v). All stock solutions were prepared in the uptake buffer. This buffer medium was composed of 118 mM NaCl, 4.69 mM KCl, 2.54 mM $CaCl_2$, 1.18 mM KH_2PO_4, 1.1 mM $MgCl_2$, 15 mM $NaHCO_3$, 5.5 mM D-glucose and 19 mM HEPES. The pH of the buffer was adjusted to 7.4 and gassed with oxygen for 30 min. In order to study an involvement of active transport processes, the experiment was run at 37 as well as at 4 °C, a temperature at which active transport processes should be heavily reduced or abolished. In a control experiment cells were killed by alternate storage at 37 and 4 °C, respectively. 6, 16, 26, and 36 s after starting the incubation, 100 μl of the incubations were pipetted into 0.4-ml Eppendorf cups filled with 10 μl KOH overlaid with 150 μl silicone oil (Wacker Silikonoel AR20 Salben IG03818 and Wacker Silikonoel AR200 IG03827, Wacker-Chemie GmbH, Burkhausen, Germany). After centrifugation (45 s at 2000 × g) the hepatocytes together with the amount of colchicine that was either taken up or associated with the cell surface membrane were located within the KOH layer. The silicon oil layer separated this KOH layer from colchicine present in the extracellular medium. The Eppendorf cups were placed into fluid nitrogen, cut above the KOH layer, the tips were transferred into liquid scintillation vials and measured for total radioactivity (Liquid Scintillation Counter, LS 6000TA, Beckman Instruments, Irvine, CA, USA).

The uptake velocity was calculated by linear regression analysis using the data points recorded after 6, 16, 26, and 36 s of incubation (Enzfitter 1.05, Elsevier-Biosoft, Cambridge, UK). Statistical analysis was done by Student's t-test. The level of significance was $P = 0.05$.

3. Results

The present study was undertaken in order to determine the effect of Solutol HS 15 on the uptake of colchicine into rat hepatocytes. The alkaloid is known to be taken up into the cells by active transport (Wierzba et al., 1989). Fig. 1 shows the impact of different concentrations of Solutol HS 15 on the amount of colchicine taken up into rat hepatocytes within 36 s at 37 °C. At surfactant concentrations below its CMC of 0.021% (Buszello et al., 2000) no significant surfactant effect was observed. By contrast, at 0.03% Solutol HS 15 present in the incubation medium the amount of colchicine taken up into the cells was significantly reduced. This observation was also reflected in the uptake velocity of colchicine that

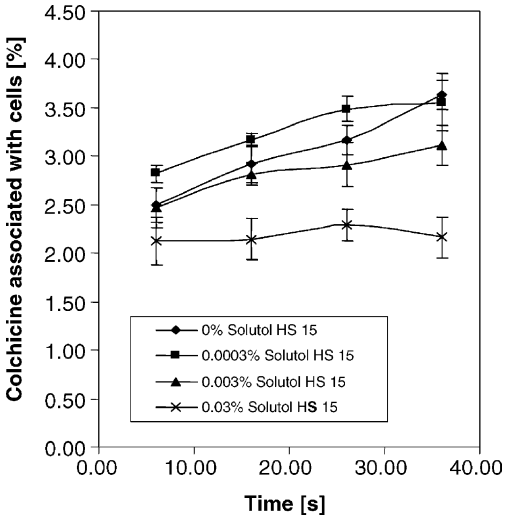

Fig. 1. Impact of different concentrations of Solutol HS 15 on the amount of colchicine taken up into rat hepatocytes within 36 s at 37 °C, mean ± S.E.M. of triplicate measurements in three independents cell preparations.

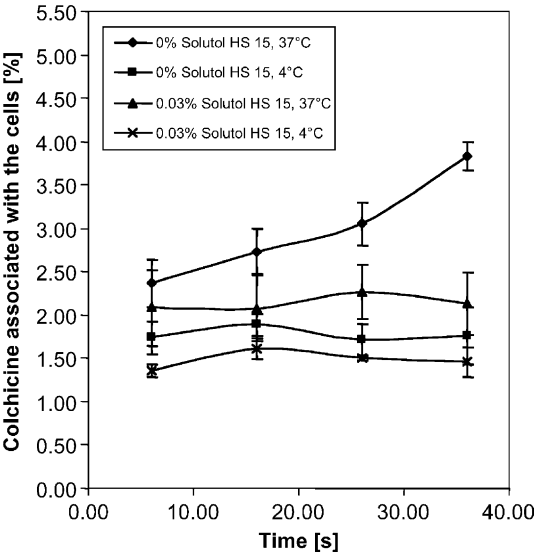

Fig. 3. Impact of 0.03% Solutol HS 15 on the amount of colchicine taken up into rat hepatocytes within 36 s at 37 and 4 °C, mean ± S.E.M. of triplicate measurements in three independents cell preparations.

was significantly reduced only at 0.03% Solutol HS 15 present in the incubations (Fig. 2).

It has been demonstrated that at low temperature transporter mediated uptake of colchicine is inactive (Wierzba et al., 1989). Thus, in an additional experiment the incubations were performed in the presence and in the absence of 0.03% Solutol HS 15 both at 4 and 37 °C. In contrast to the experiments run at 37 °C without Solutol, in the studies at 4 °C, the amount of colchicine that was associated with the cells within a period of 36 s did not increase over time. Moreover, at 4 °C, Solutol HS 15 did not affect colchicine association with the cells (Figs. 3 and 4). The same was true when measuring the association of colchicine with death cells (data not shown).

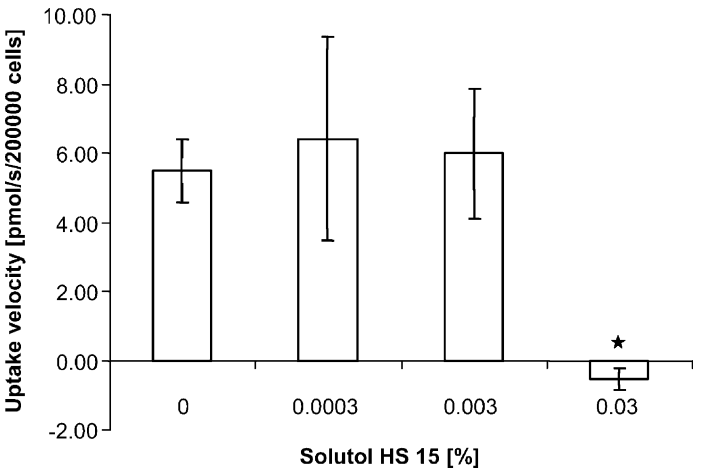

Fig. 2. Uptake velocity of colchicine into rat hepatocytes measured over a period of 36 s in the presence and in the absence of different concentrations of Solutol HS 15, mean ± S.E.M. of triplicate measurements in three independents cell preparations.

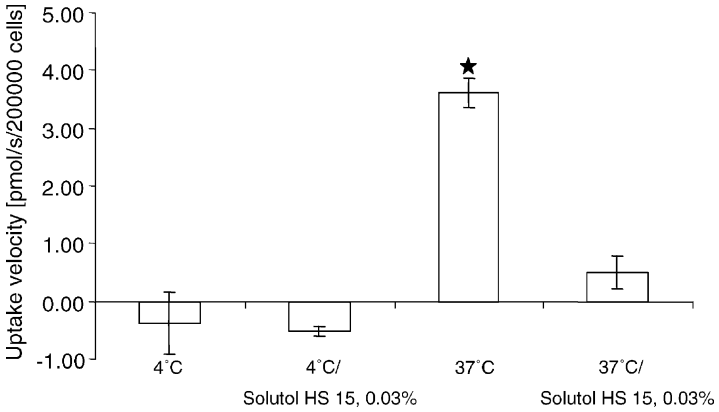

Fig. 4. Uptake velocity of colchicine into rat hepatocytes at 37 and 4 °C measured over a period of 36 s in the presence and in the absence of 0.03% Solutol HS 15, mean ± S.E.M. of triplicate measurements in three independents cell preparations.

4. Discussion

The data presented here show a significant impact of Solutol HS 15 on the amount of colchicine taken up into rat hepatocytes as well as on the velocity of this process exclusively at a surfactant concentration above its CMC and in experiments performed at 37 °C. However, in experiments performed at 4 °C, a temperature at which active transport processes should be heavily reduced or abolished, Solutol HS 15 did not affect colchicine association with the hepatocytes. Likely explanations for the observed findings are an incorporation of the alkaloid into micellar structures as well as a strong molecular interaction between the alkaloid and the surfactant, reducing the amount of colchicine entering the cells as well as a Solutol HS 15-induced alteration of cell membrane properties, leading to a destabilization of the colchicine transport, or a direct interaction at the transport site.

In a previous study, the impact of Solutol HS 15 on the pharmacokinetic fate of intravenously co-dosed colchicine was investigated in male Wistar rats. The initial plasma concentration of Solutol HS 15 was calculated to be above its CMC. In the presence of Solutol HS 15, the maximum plasma concentration (c_{max}) of colchicine was significantly increased, its plasma clearance (Cl) was significantly decreased and the excretion of parent colchicine into urine was enhanced several fold as compared to an aqueous solution. Moreover, there was a trend to a decrease in the volume of distribution (V_d). The terminal half-life of colchicine was not altered in the presence of the surfactant. In vitro, in rat hepatocytes, the intrinsic clearance of colchicine was markedly reduced in the presence of Solutol HS 15. This effect was significant already at concentrations below its CMC (Bittner et al., 2003). At these concentrations, the surfactant did not disrupt cell integrity as demonstrated in different cytotoxicity assays (Bravo González et al., 2001). The blood:plasma concentration ratio (λ), the erythrocyte:plasma concentration ratio (K_e) as well as the free fraction of colchicine in rat plasma (FF) were not affected in the presence of the surfactant at concentrations above and below is CMC. Based on these data, an inhibition of the hepatic metabolism of colchicine was suggested to be one of the major reasons for the altered pharmacokinetic profile of the alkaloid (Bittner et al., 2003).

Taking into consideration the results from our in vitro uptake experiment, a reduction of the amount of colchicine entering the liver at early time points might be a contributor to the altered pharmacokinetic profile of colchicine in the presence of Solutol HS 15 in the rat. A partial prevention of colchicine uptake into the hepatocytes together with the postulated inhibition of CYP 3A mediated metabolism in the presence of Solutol HS 15, would have increased the amount of colchicine present in the central compartment. As the pharmacokinetic behavior of Solutol HS 15 has not yet been investigated in detail, an exhaustive explanation of the observed in vivo effect is not feasible at the moment.

5. Conclusions

Our data demonstrate that the use of Solutol HS 15 in pharmaceutical preparations should be considered with care as the surfactant can significantly alter the pharmacokinetic profile of co-dosed compounds. Especially for compounds that are prone to active transport processes, in vitro experiments in the presence and in the absence of Solutol HS 15 might be required in order to estimate potential drug-formulation interactions in the in vivo situation.

References

Bittner, B., Bravo González, R.C., Walter, I., Kapps, M., Huwyler, J., 2003. Impact of Solutol HS 15 on the pharmacokinetic behavior of colchicine upon intravenous administration to male Wistar rats. Biopharm. Drug Dispos. 24, 173–181.

Bravo González, R.C., Mountfield, R.J., Walter, I., Delobel, F., Bittner, B., 2001. Impact of Solutol HS 15 on the clearance of midazolam in rat hepatocytes. Drug Metab. Rev. 33, 183.

Buckingham, L.E., Balasubramanian, M., Emanuele, R.M., Clodfelter, K.E., Coon, J.S., 1995. Comparison of Solutol HS 15, Cremophor EL and novel ethoxylated fatty acid surfactants as multidrug resistance modification agents. Int. J. Cancer 62, 436–442.

Buszello, K., Harnisch, S., Müller, R.H., Müller, B.W., 2000. The influence of alkali fatty acids on the properties and the stability of parenteral O/W emulsions modified with Solutol HS 15. Eur. J. Pharm. Biopharm. 49, 143–149.

Chong, A.S.F., Markham, P.N., Gebel, H.M., Bines, S.D., Coon, J.S., 1993. Diverse multidrug-resistance-modification agents inhibit cytolytic activity of natural killer cells. Cancer Immunol. Immunother. 36, 133–139.

Coon, J.S., Knudson, W., Clodfelter, K., Lu, B., Weinstein, R.S., 1991. Solutol HS 15, nontoxic polyoxyethylene esters of 12-hydroxystearic acid, reverses multidrug resistance. Cancer Res. 51, 897–902.

Ruchatz, F., Schuch, H., 1998. Physicochemical properties of Solutol HS 15 and its solubisates. BASF ExAct 1, 6–7.

Schwab, D., Stieger, B., Hagenbuch, B., Meier, P.-J., Gerok, W., Kurz, G., 1997. Uptake of 3 alpha, 7 alpha, 12 alpha-trihydroxy-24-nor-5 beta- cholan-23-sulfonate into isolated rat hepatocytes by three transport systems. J. Lipid Res. 38, 935–948.

Seglen, P.O., 1976. Preparation of isolated rat liver cells. Methods Cell Biol. 13, 29–83.

Wierzba, K., Sugiyama, Y., Okudaira, K., Iga, T., Hanano, M., 1989. Uptake of colchicine, a microtubular system disrupting agent, by isolated rat hepatocytes. Pharm. Res. 6, 235–238.