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Rapid communication

Tween 20 increases intestinal transport of doxorubicin *in vitro* but not *in vivo*

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ABSTRACT

The chemotherapeutic drug substance doxorubicin has been reported to be a substrate of P-gp, which induces a barrier for oral administration and leads to a bioavailability of 3% in male Sprague Dawley rats. Literature studies have reported increased transport of P-pg substrates, like digoxin, when co-administered with P-gp inhibitors (non-ionic surfactants) *in vitro* and *in vivo* . The aim of the present study was thus to investigate if different non-ionic surfactants would have a similar effect on the *in vitro* and *in vivo* absorption of doxorubicin. This was investigated *in vitro* in Caco-2 cells and by oral co-administration of doxorubicin together with tween 20 to male Sprague Dawley rats. 200 μM (0.025%) tween 20 increased the intestinal absorptive permeability of doxorubicin *in vitro* by 48 ± 4% from 8.8×10^{-6} cm/s to 13.0×10^{-6} cm/s. Further, the efflux ratio was reduced from 2.2 ± 0.06 to 1.2 ± 0.03 (n = 3–7). *In vivo*, co-administration of doxorubicin and 0–25% tween 20 did not yield detectable doxorubicin plasma concentrations, probably due to extensive intestinal metabolism. In conclusion, the present study demonstrated that non-ionic surfactants increased the transport of doxorubicin *in vitro*, most likely by inhibition of the efflux activity. However, this effect was not transferable to the *in vivo* situation.

Doxorubicin is a chemotherapeutic agent widely used in treatment of various cancers types *e.g.*, leukaemia, lymphomas and breast cancers (Minotti et al., 2004). Intravenous injection is the most common route of administration of the compound. However, intravenous administration results in an undesired systemic exposure profile with a high initial plasma concentration followed by a fast decay below the minimal therapeutic level (Kim et al., 2013). Choi et al. (2011a) reported that P-gp mediated efflux is one of the main barriers for oral absorption of doxorubicin leading to a very low bioavailability of 3.04 ± 0.74% in male Sprague Dawley rats. Non-ionic surfactants have been reported as inhibitors of intestinal P-gp (Lo, 2003; Rege et al., 2002). Bromberg and Alakhov (2003) investigated the effects of pluronic L61 L92 on doxorubicin transport in Caco-2 cells and reported that the surfactants decreased the active efflux of doxorubicin by 2.4–3.2 fold (Bromberg and Alakhov, 2003). For the well-known P-gp substrate digoxin, tween 80 was reported to improve the intestinal absorption *in vitro* and *in vivo* (Cornaire et al., 2004). Zhang et al. (2003) concluded that this effect was most likely caused by inhibition of P-gp in the gastro intestinal tract and that similar enhancement might be evident for other P-gp substrates. The aim of the present study was therefore to investigate if non-ionic surfactants affect the efflux ratio of doxorubicin *in vitro* and subsequently if this could be translated into an improved oral bioavailability *in vivo*.

All reagents and materials were obtained from Sigma–Aldrich (St. Louis, MO, USA). Transport experiments were performed on Caco-2 cells grown on 0.4 μm-pore polycarbonate Transwell filters for 14–15 days as previously described (Nohr et al., 2014). Surfactants (200 or 400 μM) were added to the apical compartment to simulate the *in vivo* situation together with ^{3}H-doxorubicin (American Radiolabeled Chemicals, St. Louis, MO, USA). The pharmacokinetic study was conducted on male Sprague Dawley rats from Charles River (Sulzfeld, Germany). The protocol was approved by the local ethical committee. The experiments were performed in agreement with the NIH guidelines for the Care and Use of Laboratory Animals and the Danish law regulating experiments on animals, EC Directive 2010/63/EU. Rats (weighting 285–364 g on the day of dosing) were fasted at least for 16–20 h before initiation of the experiment. One group was dosed intravenously with 2 mg/kg of doxorubicin (1 mL/kg) in the tail

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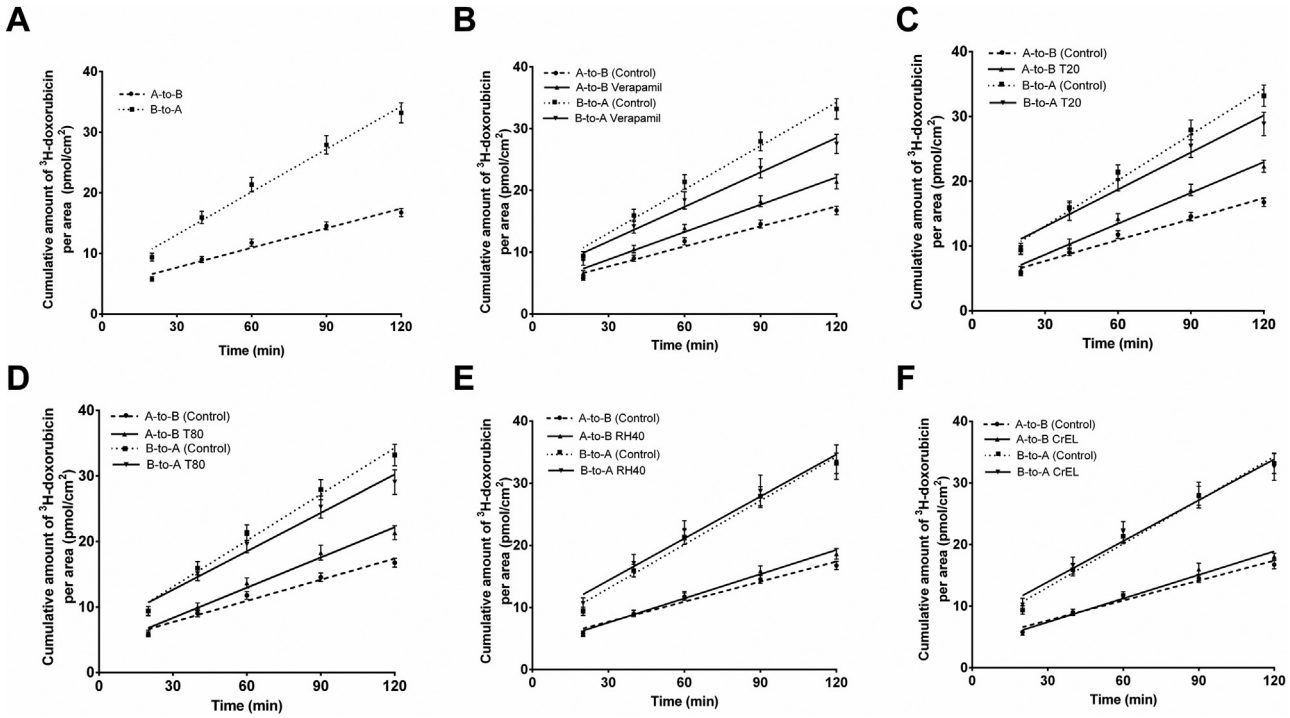

Fig. 1. Transepithelial transport curves of ^{3}H-doxorubicin (204.1 nM, 1 μCi/mL) across Caco-2 monolayers grown for 14–15 days. (A) Absorptive (A-to-B) and secretory (B-to-A) transport of the control group (B) in absence or presence of 100 μM verapamil (C) 200 μM tween 20 (T20) (D) 200 μM tween 80 (T80) (E) 400 μM cremophor RH40 (RH40) (F) 400 μM cremophor EL (CrEL). Experiments were performed in HBSS (pH 7.4) buffer in both compartments at 37 °C. Tweens and cremophors were only present in the apical compartments. Verapamil was added to both compartments as a positive control. Results for the control group were optained from 7 cell passages ($n = 7$) and 4 cell passages for treated groups ($n = 4$). Data points are displayed as mean ± SEM.

Table 1
Transepithelial electrical resistance (TEER) ($\Omega\,cm^2$) of Caco-2 cell monolayers, apparent permeability coefficients (P_{app}), efflux ratio and% recovery of ^{3}H-doxorubicin (204.1 nM, 1 μCi/mL) and ^{14}C-mannitol (18.2 μM, 1 μCi/mL) for the absorptive (A-to-B) and secretory (B-to-A) transport direction in absence (control) or presence of surfactants and verapamil. TEER was measured before and after transport experiments. Transport experiments were performed in HBSS (pH 7.4) buffer in both compartments at 37 °C. Tweens (200 μM) and cremophors (400 μM) were only present in the apical compartments. Verapamil (100 μM) was added to both compartments as a positive control. Results for the control group were obtained from 4 to 7 cell passages ($n = 4$–7) and 3 to 4 cell passages for treated groups ($n = 3$–4). Data points are displayed as mean ± SEM. Stars marks statistical significance in an ordinary one-way ANOVA followed by Tukey–Kramer test. Level of significance: $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

^{3}H-doxorubicin					
	P_{app} ($\times 10^{-6}$ cm/s)		Efflux ratio	Recovery (%)	
	A-to-B	B-to-A		A-to-B	B-to-A
Control	8.80 ± 0.3	19.2 ± 0.8	2.2 ± 0.06	84.8 ± 2.1	82.5 ± 2.5
Verapamil	12.1 ± 0.7***	15.2 ± 0.7*	1.3 ± 0.05****	87.6 ± 2.5	84.4 ± 3.7
Tween 20	13.0 ± 0.4****	15.6 ± 0.8	1.2 ± 0.03****	87.1 ± 2.2	90.1 ± 4.5
Tween 80	12.6 ± 0.6****	16.0 ± 1.1	1.3 ± 0.05****	90.0 ± 2.8	87.9 ± 4.3
Cremophor RH40	10.6 ± 0.4*	18.4 ± 1.6	1.7 ± 0.09****	90.2 ± 4.4	87.3 ± 4.0
Cremophor EL	10.4 ± 0.4	18.1 ± 1.1	1.7 ± 0.07****	90.5 ± 2.0	89.0 ± 3.9

^{14}C-mannitol					
	P_{app} ($\times 10^{-6}$ cm/s)		Efflux ratio	Recovery (%)	
	A-to-B	B-to-A		A-to-B	B-to-A
Control	0.49 ± 0.02	0.70 ± 0.09	1.3 ± 0.10	99.8 ± 1.4	93.8 ± 3.9
Verapamil	0.58 ± 0.03	0.51 ± 0.06	0.9 ± 0.07	102.5 ± 1.0	95.6 ± 3.5
Tween 20	0.64 ± 0.11	0.71 ± 0.07	1.2 ± 0.10	99.8 ± 1.5	101.2 ± 1.0
Tween 80	0.69 ± 0.09	0.63 ± 0.08	0.9 ± 0.04	100.3 ± 2.0	100.2 ± 1.0
Cremophor RH40	0.60 ± 0.08	0.67 ± 0.06	1.3 ± 0.03	101.4 ± 3.2	97.1 ± 3.0
Cremophor EL	0.53 ± 0.08	0.67 ± 0.08	1.3 ± 0.04	99.8 ± 2.1	102.4 ± 0.7

TEER				
	A-to-B before	A-to-B after	B-to-A before	B-to-A after
Control	455 ± 29	88 ± 19 ****	470 ± 27	148 ± 10 ****
Verapamil	488 ± 45	124 ± 13 ****	443 ± 25	148 ± 24 ****
Tween 20	467 ± 19	276 ± 14 ***	453 ± 36	263 ± 16 ***
Tween 80	482 ± 33	258 ± 8 ****	435 ± 43	284 ± 11 **
Cremophor RH40	472 ± 25	237 ± 14 ****	469 ± 28	188 ± 16 ****
Cremophor EL	462 ± 34	293 ± 25 **	412 ± 38	248 ± 9**