THE INHIBITION OF THE INTESTINAL ABSORPTION OF VITAMIN K BY A MEDIUM CHAIN CHLORINATED PARAFFIN (MCCP) IN AN IN-VITRO EVERTED RAT INTESTINAL SAC MODEL.


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Introduction

High oral doses of medium chain chlorinated paraffins (MCCPs) administered to female rats prior to pregnancy and during gestation cause increased peri-natal mortality due to internal haemorrhaging as a consequence of decreased concentrations of Vitamin K in maternal plasma and milk. Previous studies have shown that the effect on Vitamin K levels may be due to a reduction in Vitamin K absorption from the GI tract. This potential MOA has been explored by studying the effect of an MCCP on the absorption of Vitamin K in an in-vitro everted rat intestinal sac model.

Methods

Everted rat small intestinal sacs (2.5 cm) were validated by studying the transfer of glucose and digoxin across the intestinal wall. Once validated, sacs were incubated in a commercial feed-state simulated intestinal fluid (FeSSIF) containing 0.1 µM [14C]-Vitamin K in the presence or absence of MCCPs. At the end of the incubation period, the sacs were cut open, the serosal contents collected and the sacs weighed and digested. External media, serosal media and sac digests were assayed for their content of [14C]-Vitamin K using liquid scintillation counting. Results are Mean ± SD (n = 2-6).

Results

Validation of model. The intestinal sac methodology was validated by measuring the transfer of [3H]-digoxin (a P-gp transporter substrate) in the presence or absence of quinidine (a P-gp transporter inhibitor). In the presence of quinidine, the efflux of [3H]-digoxin was greatly reduced, increasing the concentration of digoxin within the sac, suggesting the P-gp transporter was functional (Fig 1). Viability was also assessed by measuring the uptake of [14C]-glucose into the serosal media (Fig 2). The viability of the intestinal sacs was therefore considered to be fit for purpose.

Validation with Vitamin K. An initial assessment of Vitamin K absorption was performed using TC199 as the external medium. In this study, with 100µM Vitamin K in the external medium, less than 25 pmol of Vitamin K was transferred from the external to serosal medium in 90 minutes. Vitamin K absorption is dependent on its incorporation into mixed micelles and requires the presence of bile salts and acids. When the experiment was repeated replacing TC199 medium with FeSSIF, approximately 7500 pmol of Vitamin K was transferred from the external medium to the serosal medium in 45 minutes.

Effect of MCCP on Vitamin K absorption. The absorption of Vitamin K was measured both with and without MCCPs in the external FeSSIF medium. The concentrations of Vitamin K (0.1 µM) and MCCPs (0.5 mM, 1 mM and 2 mM) were selected to be representative of those measured in the milk of female rats administered MCCPs prior to pregnancy and during gestation in the previous studies. Under control conditions, approximately 12 pmol Vitamin K was transferred to the serosal fluid in 90 minutes.

Conclusions

• A valid in-vitro system for studying the absorption of Vitamin K was established using everted rat intestinal sacs.

• High concentrations of MCCP in the external medium reduced the transfer of Vitamin K into the serosal medium, suggesting that MCCPs may inhibit the absorption of Vitamin K.

• These results support the hypothesis that the peri-natal mortality seen in rats receiving high oral doses of MCCPs is due to a reduction in maternal levels of Vitamin K, most likely due to an inhibition of its intestinal absorption during pregnancy.

• MCCPs are present in the milk of dams following exposure during pregnancy, suggesting that MCCPs may also have a similar effect on the intestinal absorption of Vitamin K in the suckling offspring, leading to further reductions in blood levels of Vitamin K in the offspring and a tendency to internal haemorrhaging.