Novel Orally Formulated Mixed Micelles Optimize Vitamin K Absorption Under Bile-Deficient Conditions

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Vitamin K prophylaxis is offered to newborns worldwide to prevent life-threatening vitamin K deficiency bleedings in infancy.[1] Intramuscular injection is an efficacious treatment option, but overall effectiveness is hampered by increasing rates of parental refusal.[2] Oral administration is well tolerated, but is associated with prophylactic failures in newborns with unrecognized cholestatic liver diseases (1:2,500 live births).[3] The absence of intraluminal bile precludes mixed micelle formation, a prerequisite for the absorption of the extremely hydrophobic vitamin K. A drug formulation containing vitamin K–loaded mixed micelles could in theory be used to overcome this issue. However, introduction of the mixed micellar product Konakion MM (mixed micelles) failed to measurably improve the efficacy of oral vitamin K prophylaxis.[4] Recent in vitro studies provided a plausible explanation: exposure of Konakion MM to simulated gastric fluids destroys the formulation.[5] In this study, we provide in vivo evidence on how and why current prophylactic regimens fail under cholestatic conditions and demonstrate how gastro-resistant mixed micelles could be used to overcome the limitations of current prophylactic vitamin K formulations.

Methods

Gastro-resistant MMs (Table 1) were developed through PEGylation, by replacing part of the phospholipid content by 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol) 2000 (DSPE-PEG; a PEGylated phospholipid), or via improved electrostatic repulsion with taurocholic acid (TA), either with or without PEGylation via d-α-tocopheryl polyethylene glycol 1000 (TPGS; PEGylated vitamin E). Formulation colloidal stability was studied in vitro and evaluated in vivo for the ability to facilitate vitamin K absorption in sham and cholestatic rat models. Influence of gastric passage was studied using simultaneous proton pump inhibitor therapy and direct intraduodenal administration. Descriptive pharmacokinetic metrics were calculated using noncompartmental analysis. Further details of the methods are available in the Supplementary Materials.

Results

Gastric Passage Decreases Vitamin K Absorption

Vitamin K absorption from an oil-based formulation improved substantially in sham-operated rats under fed conditions, illustrating bile-dependent vitamin K absorption (area under the curve $(AUC)_{0-5\ h}$ [coefficient of variation (%CV)] 0.09 [741] vs 9.34 [51] μg-h/mL, fasted vs fed; Figure 1A and 1C). Interestingly, administration of Konakion MM in fasted sham rats mimicked the absorption of the fed state ($AUC_{0-5\ h}$ [%CV] 7.77 [241] μg-h/mL; Figure 1A).

As expected, vitamin K absorption from the oil-based formulation was negligible in cholestatic rats ($AUC_{0-5\ h}$ [%CV] 0.004 [50] μg-h/mL; Figure 1A and 1C). Under these conditions, vitamin K absorption from Konakion MM was strongly reduced as well, but also strikingly variable ($AUC_{0-5\ h}$ [%CV] of 0.02 [23,062] μg-h/mL; Figure 1A). We assessed whether exposure to low gastric pH could explain the erratic absorption from Konakion MM. A group of cholestatic rats was treated with proton pump inhibitors (omeprazole), resulting in a significant increase in the extent of vitamin K absorption ($AUC_{0-5\ h}$ [%CV] 1.71 [304] μg-h/mL; $P < .0001$; Figure 1A and 1C). Vitamin K absorption from Konakion MM could be improved further

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Abbreviations used in this paper: **AUC, area under the curve; %CV, coefficient of variation; DSPE-PEG, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol) 2000; MM, mixed micelle; PEG, polyethylene glycol; TA, taurocholic acid; TPGS, d-α-tocopheryl polyethylene glycol 1000.**

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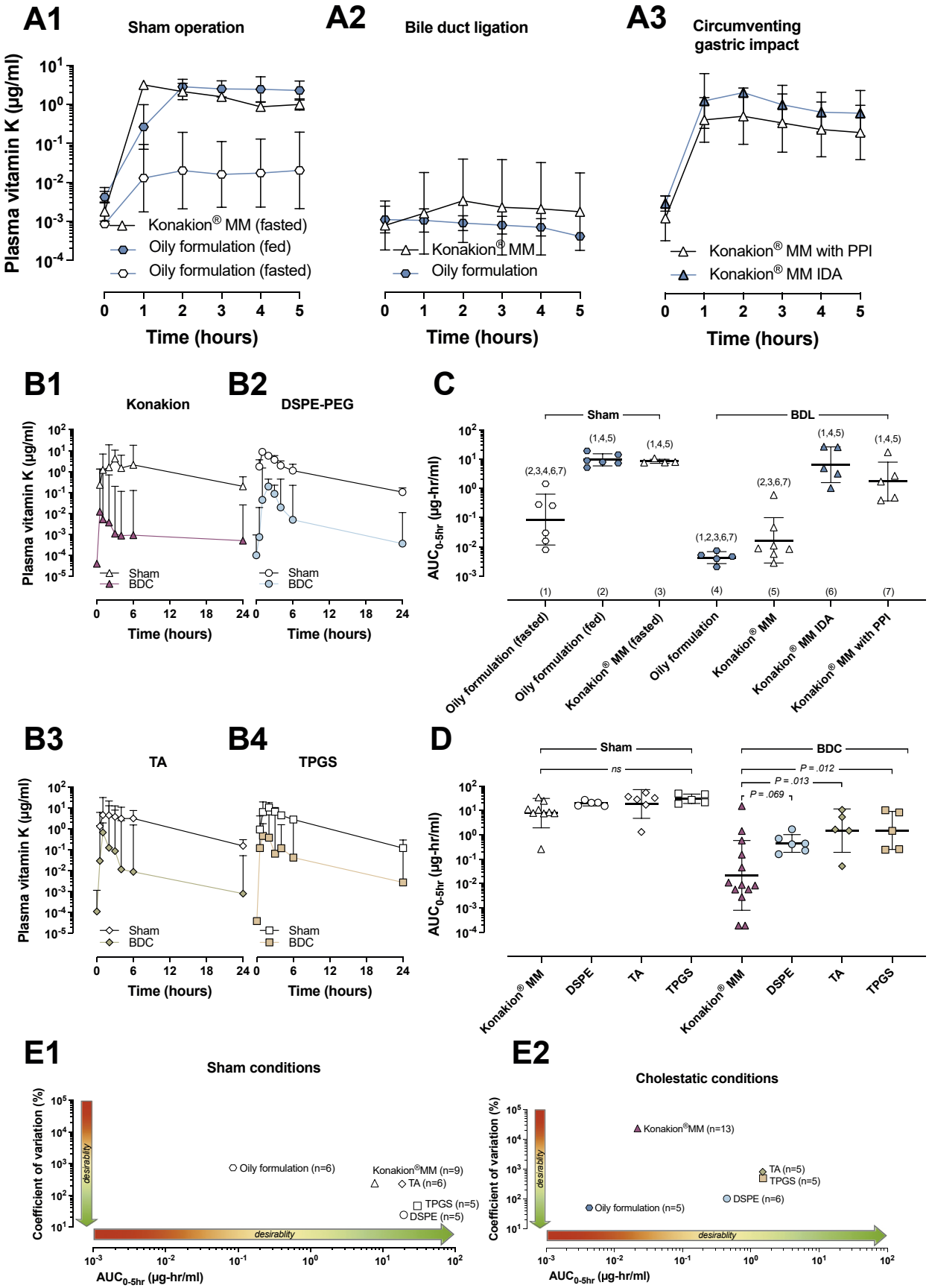

Table 1. Composition of the Formulations Used Throughout This Study

Component	Formulation			
	Konakion MM[a]	TPGS	DSPE-PEG	TA
Vitamin K_1 (mg/mL)	2.5	2.5	2.5	2.5
Phosphatidyl-choline (mmol/L)	24.6	12.5	12.5	25.0
Glycocholic acid (mmol/L)	29.3	-	30.0	-
Taurocholic acid (mmol/L)	-	30.0	-	30.0
DSPE-PEG 2000 (mmol/L)	-	-	12.5	-
TPGS (mmol/L)	-	12.5	-	-

[a]Concentrations adapted from Amédée-Manesme et al.[6]

when the stomach was bypassed using direct intraduodenal administration ($AUC_{0-5\ h}$ [%CV] 6.39 [248] μg-h/mL; $P < .0001$; Figure 1A and 1C).

Development of Gastro-resistant MMs

The MMs found in Konakion MM are composed of phosphatidylcholine (a phospholipid) and glycocholic acid (Table 1), a bile acid with a pKa of ~4.5. We hypothesized that vitamin K absorption could be improved by using a bile acid with a lower pKa, because colloidal stability is negatively impacted at pH<pKa due to a loss of charge. Taurocholic acid has a pKa of ~1.5, the capability for electrostatic repulsion would, therefore, remain preserved at gastric pH. Previously, we showed that colloidal stability may also be improved via steric stabilization via PEGylation (eg, with DSPE-PEG or TPGS) of the MMs.[5] Sensitivity of the different formulations to changes in pH was studied between pH 6.0 and 1.0. Konakion MM exhibited a rapid increase in particle size toward, and aggregated below, the pKa of glycocholic acid. In contrast, all stabilized mixed micellar formulations varied only slightly in particle size over the studied pH range (Supplementary Figure 1).

Gastro-resistant Formulations Improve Vitamin K Absorption

The AUCs of the gastro-resistant formulations and Konakion MM were comparable in sham-operated rats (Figure 1B and 1D). However, under cholestatic conditions, gastro-resistant formulations greatly improved vitamin K absorption compared with Konakion MM. The PEGylated DSPE formulation improved the $AUC_{0-5\ h}$ by 20-fold compared with Konakion MM ($AUC_{0-5\ h}$ [%CV] 0.45 [101] vs 0.02 [23,062] μg-h/mL; $P = .069$). The TA formulation, in which colloidal stability was improved by incorporation of TA instead of glycocholic acid, improved $AUC_{0-5\ h}$ (%CV) up to 70-fold to 1.49 (806) μg-h/mL ($P = .013$). Similar results were obtained when both protective concepts were combined (TPGS formulation): $AUC_{0-5\ h}$ (%CV) 1.51 (493) μg-h/mL ($P = .012$; Figure 1B and 1D).

When AUCs and variability (%CV) were evaluated together, electrostatic repulsion with taurocholic acid increases the extent of vitamin K absorption, whereas PEGylation decreases the variability (Figure 1E). An overview of the pharmacokinetic metrics calculated using non-compartmental analyses are reported in Supplementary Table 1.

Discussion

Diagnosis of infantile cholestatic liver disease takes place at a later age than vitamin K deficiency bleedings,[7] it is, therefore, critical that vitamin K prophylaxis provides both reliable and adequate vitamin K absorption irrespective of any underlying (undiagnosed) malabsorption syndrome. Current oral prophylactic regimens fail to do so, leaving the ones associated with the highest risk unprotected and thereby limiting overall prophylactic efficacy. Our data indicate that gastro-resistant MMs allow adequate and reliable oral vitamin K absorption under cholestatic conditions.

The poor oral absorption from Konakion MM in cholestatic rats aligns with clinical observations in infants with forms of biliary obstruction.[4] Our data provide an explanation why: unstabilized MMs will aggregate during gastric passage, once aggregated vitamin K will not be sufficiently resolubilized upon a subsequent pH increase. Gastro-resistant MMs have the potential to improve the overall efficacy of oral prophylaxis by protecting the needy: infants awaiting a diagnosis of cholestatic liver disease. Despite clear physiological differences between our animal model and newborns, we expect these findings to be predictive for human vitamin K absorption as well because the physicochemical principles responsible for the strongly reduced absorption remain the same. Although promising, the true

Figure 1. (*A1*) Vitamin K absorption in sham-operated rats after intragastric administration of Konakion MM or an oil-based vitamin K formulation under fed and fasted conditions. (*A2*) As panel *A1* but in bile duct–ligated (BDL) rats. (*A3*) Vitamin K absorption from Konakion MM in BDL rats after direct intraduodenal (IDA) administration vs intragastric administration in combination with proton pump inhibitor (PPI). (*B*) Lin-log plasma vs time curves after intragastric administration of a single 1-mg dose of vitamin K from (*B1*) Konakion MM, (*B2*) DSPE-PEG, (*B3*) TA, and (*B4*) TPGS formulations to sham or bile duct cannulated (BDC) rats. Symbols in *A* and *B* depict geometric mean ± geometric SD. (*C*) Column scatter plot of $AUC_{0-5\ h}$ data from panels *A1–A3*. Symbols depict the individual values within a group; numbers on top of the error bar indicate a SD of at least $P \leq .05$ to the corresponding group number on the x-axis. (*D*) Column scatter plot of $AUC_{0-5\ h}$ data from panels *B1–B4*. Symbols depict the individual values within a group; statistical differences are determined against the reference group Konakion MM under both experimental conditions. (*E*) XY scatter plot of the mean geometric $AUC_{0-5\ h}$ data against the corresponding geometric coefficient of variation in sham (*E1*) and cholestatic rats (*E2*).

efficacy of the suggested approaches is, however, to be demonstrated in clinical trials.

In conclusion, current prophylactic regimens do not ensure effective vitamin K absorption under cholestatic conditions. In contrast, gastro-resistant MMs offer a promising formulation strategy to overcome the limitations of current prophylactic regimens.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2021.05.056.

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Conflicts of interest
T.R. and H.V. are employees of Tiofarma BV. All other authors disclose no conflicts.

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Supplementary Materials and Methods

Materials

Konakion MM 10 mg/mL is a product of Cheplapharm Arzneimittel GmbH (Greifswald, Germany) and vitamin K_1 in arachis oil 10 mg/g was produced by Tiofarma BV (Oud-Beijerland, the Netherlands). Vitamin K_1 was purchased from DSM Nutritional Products Ltd (Bramsche, Germany), deuterated vitamin K_1 ring-D(4) from Buchem BV (Apeldoorn, the Netherlands). Phosphatidylcholine and DSPE-PEG (PEG 2 kDa) were kindly provided by Lipoid GmbH (Ludwigshafen, Germany). Vitamin E TPGS (PEG 1 kDa) was purchased from Gustav Parmentier GmbH (Frankfurt am Main, Germany) and TA from Prodotti Chimici Alimentari SPA (Basaluzzo, Italy). Zinc powder (-140+325 mesh, 99.9%) was obtained from AlfaAesar GmbH (Karlsruhe, Germany). Acetonitrile, dichloromethane, ethanol, methanol, and n-hexane were from Biosolve Ltd (Valkenswaard, the Netherlands). Vitamin K_2 (MK-4), glycocholic acid, and all other unnamed chemicals and reagents were obtained from Sigma-Aldrich (Zwijndrecht, the Netherlands). Used isoflurane/oxygen gas was from Abbott Laboratories Ltd, (Chicago, Illinois), buprenorphine HCl from Indivior Europe Ltd (Dublin, Ireland), heparin from Leo Pharma BV (Breda, the Netherlands), silicone cannulae from Degania Silicone Inc. (Cumberland), omeprazole and bupivacaine from AstraZeneca BV (Zoetermeer, the Netherlands), carprofen from Ast Farma BV (Oudewater, the Netherlands), and rodent jackets from either UNO BV (Zoetermeer, the Netherlands) or SAI Infusion Technologies (Lake Villa). The latter also supplied swivels and polyurethane tubing. Intravenous fluids (ie, 0.9% NaCl, 0.45% NaCl + 2.5% glucose, 50% dextrose, and 8.4% sodium bicarbonate) were obtained from Fresenius Kabi AG (Hamburg, Germany); polymethylmethacrylate gastric cannulae for chronic implantation were kindly provided by AstraZeneca R&D (Mölndal, Sweden). Bile duct–ligated (BDL) rats received a vitamin K–deficient diet from Arie Blok BV (Woerden, the Netherlands). Specials Diets Services (Witham, UK) supplied the standard rodent chow (AIN-93G) and vitamin K–deficient diet for bile duct–cannulated (BDC) rats.

Animals

Male Wistar rats (HsdCpb:WU; 275–375 g; n = 90) were obtained from Envigo RMS BV (Horst, the Netherlands) and were randomly allocated to 1 of each 15 experimental groups using a computer-based random order generator. Animals were only excluded if the animal died prematurely. Group size was determined using Lehr's formula based on data from pilot studies. Animals were allowed to acclimatize for at least 7 days under controlled environmental conditions (temperature 22℃ ± 2℃, relative humidity 40%–60%, light 06:00–18:00h). Animals were housed in groups of 3 in type IV cages, sawdust was provided as bedding, and paper towels were provided as enrichment. Animals had free access to rodent chow and fresh tap water, were deprived of food overnight, and were given vitamin K_1 (1 mg = 400 μL) via oral gavage under fasted conditions using a 16-gauge stainless steel feeding needle, unless stated otherwise. All interventions were carried out during the light cycle and were unblinded to the investigators. Protocols were approved by the local Animal Welfare Body and carried out and reported in compliance with the Dutch Act on Animals used for scientific purposes and Animal Research: Reporting of In Vivo Experiments guidelines.

Surgical Procedures

All surgical procedures were performed on a heated operating table, under general anesthesia using a combination of 2%–5% isoflurane/oxygen gas and 0.05 mg/kg subcutaneous buprenorphine. Sham operations consisted of a control laparotomy for the same duration as the BDL or BDC operation.

BDL Rats

The right jugular vein was cannulated with a 12.0-cm silicone cannula. Through a small, v-shaped incision the cannula was inserted 4.2 cm in the direction of the heart. After checking for intact flow, the system was flushed using heparin in physiological saline (100 IU/mL). The cannula was tunneled subcutaneously to the back, puncturing the skin between the shoulder blades. Through a ±2-cm midline incision the abdomen was opened and the liver gently retracted, thereby exposing the common bile duct. Using a 6-0 silk ligature, the common bile duct was ligated. Subsequently the muscular part of the stomach was exposed. For intragastric administration, a polymethylmethacrylate cannula was placed in the stomach, which was fixed in place using a purse-string suture. The wings of the cannula were sutured to the abdominal wall. A small incision, parallel to the midline incision was used to exteriorize the fistula. For duodenal administration, a 15.0-cm silicone cannula was inserted in the stomach and positioned 1.0–1.5 cm distal from the pylorus. The proximal end of the cannula was tunneled subcutaneously to the back, and exteriorized using the same puncture hole as used for the jugular vein cannula. The abdominal incision was closed in layers using a 4-0 Vicryl running suture. The jugular cannula and the duodenal cannula (if placed) were kept in place using a rodent infusion jacket. The jugular cannula was filled with a locking solution of 500 IU/mL heparin in 50% dextrose. After surgery animals were housed individually. BDL rats received vitamin K–deficient rodent chow directly on arrival.

Blood was withdrawn via the rodent infusion jacket. Each sample (0.45 mL) was replaced with an equal amount of warm heparinized physiological saline (25 IU/mL). The first blood sample was taken 2 days after surgery, just before administration of 1 mg vitamin K_1. Blood samples were subsequently taken every hour for 5 hours. The last sample was taken under general anesthesia followed by euthanasia by blood loss.

Animals allocated to the proton pump inhibitor group received 20 mg omeprazole on day 1 (8 AM and 6 PM) and on day 2 (8 AM) after BDL surgery. Omeprazole was dissolved in 1 mL 8.4% sodium bicarbonate. Konakion MM

(1 mg vitamin K_1) was administered 2 hours after the last omeprazole dose. Both omeprazole and vitamin K_1 were administered using the intragastric cannula. Intragastric pH was measured using a gastro-esophageal pH meter (Laborie, Enschede, the Netherlands).

BDC Rats

Three days before BDC surgery, animals were housed individually, switched to a vitamin K–deficient diet, and tap water was replaced with 0.45% NaCl + 2.5% glucose. Preoperatively 5.0 mg/kg subcutaneous carprofen was provided and 0.25% bupivacaine was infiltrated at the surgical site (±0.1 mL/cm). Via a midline incision (±2 cm) the abdomen was opened and the liver gently retracted. After exposing the common bile duct, a 6-0 silk ligature was placed in the distal region. A v-shaped incision was made just above the ligature and the catheter tip was inserted. Catheters were fashioned from intravascular polyurethane tubing (0.023" ID x 0.040" OD) with a 0.5-cm beveled tip (0.012" ID x 0.025" OD). Suture beads were placed at 0.5 and 5.0 cm from the tip. Interconnected sutures were placed on both sides of the bead, securing the catheter to the bile duct. The peritoneum and abdominal muscles were closed with a 4-0 Vicryl running suture; the skin was closed via a 6-0 Vicryl running subcuticular suture. Before skin closure, catheters were tunneled subcutaneously from the abdomen to the neck. All animals wore a rodent hardness with an integrated 36-cm tether, allowing the catheter to be directly connected to a dual pin swivel. The swivel was mounted just above the cage, facilitating continuous bile drainage. Vitamin K_1 was administered after a 9-day postsurgery washout and recovery period.

Vitamin K–deficient food was supplied ad libitum 3 hours postadministration. Approximately 200-μL blood samples were withdrawn from the lateral tail vein at alternating places from tip to base. The first sample was taken predose, followed by samples at 0.5, 1, 2, 3, 4, 6, and 24 hours. The last sample was taken under deep terminal anesthesia via cardiac puncture followed by cervical dislocation.

Vitamin K Analysis

All blood samples were collected in lithium heparin microtainer containers. After centrifugation, plasma was extracted and stored at -20°C until further analysis. Plasma samples from BDL rats were processed as described previously.[1] To 50 μL of BDC plasma samples, 125 μL internal standard (vitamin K_2 [MK-4], 50 ng/mL in ethanol), 1.0 mL milli-Q water, 2.0 mL ethanol, and 4.0 mL n-hexane were added. After vigorous vortexing, the upper organic layer was collected and evaporated to dryness and reconstituted in 125 μL ethanol (85%, vol/vol). Fluorometric High-Performance Liquid Chromatography analysis was based on the procedure as described by Marinova et al. with minor adjustments.[2]

Preparation of MMs

The formulation stabilized using DSPE-PEG was prepared as described previously.[3] TA formulations with and without TPGS were prepared by adding vitamin K_1 (20.8 mg/mL) and TPGS (104 mmol/L, if applicable) to a TA solution (250 mmol/L in 10 mmol/L citrate buffer pH 6.0) and stirring until a clear solution was obtained. Finally, phosphatidylcholine (104 or 208 mmol/L) was added in portions and stirred until a clear solution was obtained. The solution was diluted with a 10-mmol/L citrate buffer (pH = 6) to reach a final vitamin K_1 concentration of 2.5 mg/mL. Finally, the solution was passed over a 0.2-μm filter. Konakion MM was diluted 4 times with distilled water to a vitamin K_1 concentration of 2.5 mg/mL. An overview of used formulations is presented in Table 1.

In Vitro Gastric Stability

Formulations were evaluated for their colloidal stability as a function of pH over the range of 6.0–1.0 using a 10-mmol/L citrate buffer system; below pH 3.0 the pH was lowered further by small additions of 0.1–1mol/L HCl. Formulations from Table 1 were diluted 10 times in each buffer system. The pH was recorded and the particle size distribution was directly measured at each pH or after each step-wise pH change. Dynamic Light Scatterings (DLS) measurements were conducted at a 173 backscattering angle using the Zetasizer Nano S (Malvern Instruments Ltd, Malvern UK). The solvent viscosity and refractive index of water were used by the software.

Pharmacokinetic Analysis

Noncompartmental analysis was performed with WinNonlin (v.8.2; Certara, Princeton, New Jersey). The area under the plasma concentration-time curve (AUC_{0-t}) was calculated using the linear-log trapezoidal rule. When applicable, the AUC was extrapolated to infinity ($AUC_{0-\infty}$) by addition of the last quantifiable concentration divided by the terminal elimination rate constant (λ_z). The half-life ($t_{1/2}$) was determined over a time interval equal to at least 2 times the half-life, using at least 3 data points, not including the maximum plasma concentration (C_{max}). The half-life was determined when the adjusted Rsq value was 0.7 or higher. The $AUC_{0-\infty}$ was calculated for each individual animal when the extrapolated AUC was 30% or less using the group average for λ_z. Values below the limit of detection (LOQ) were replaced by 77.9 pg/mL (LOQ/2) and predose values by 0. Actual sampling times were used for all calculations. Data are presented as geometric mean and geometric coefficient of variation (%CV; calculated as: $100 \cdot \sqrt{(\exp(SD^2) - 1)}$, where SD is the standard deviation of the log scale data). The time to reach C_{max} (T_{max}) is reported as the median (range).

Statistical Analysis

Statistical analysis was performed with Prism8 (v.8.2.1; GraphPad Software, San Diego, California). Differences in absorption (AUC_{0-t}) between groups were assessed on the log transformed data via a 1-way analysis of variance, followed by a post-hoc Tukey (BDL data) or Dunnett (BDC data) multiple comparisons test with a single pooled variance. $P \leq .05$ was considered significant.

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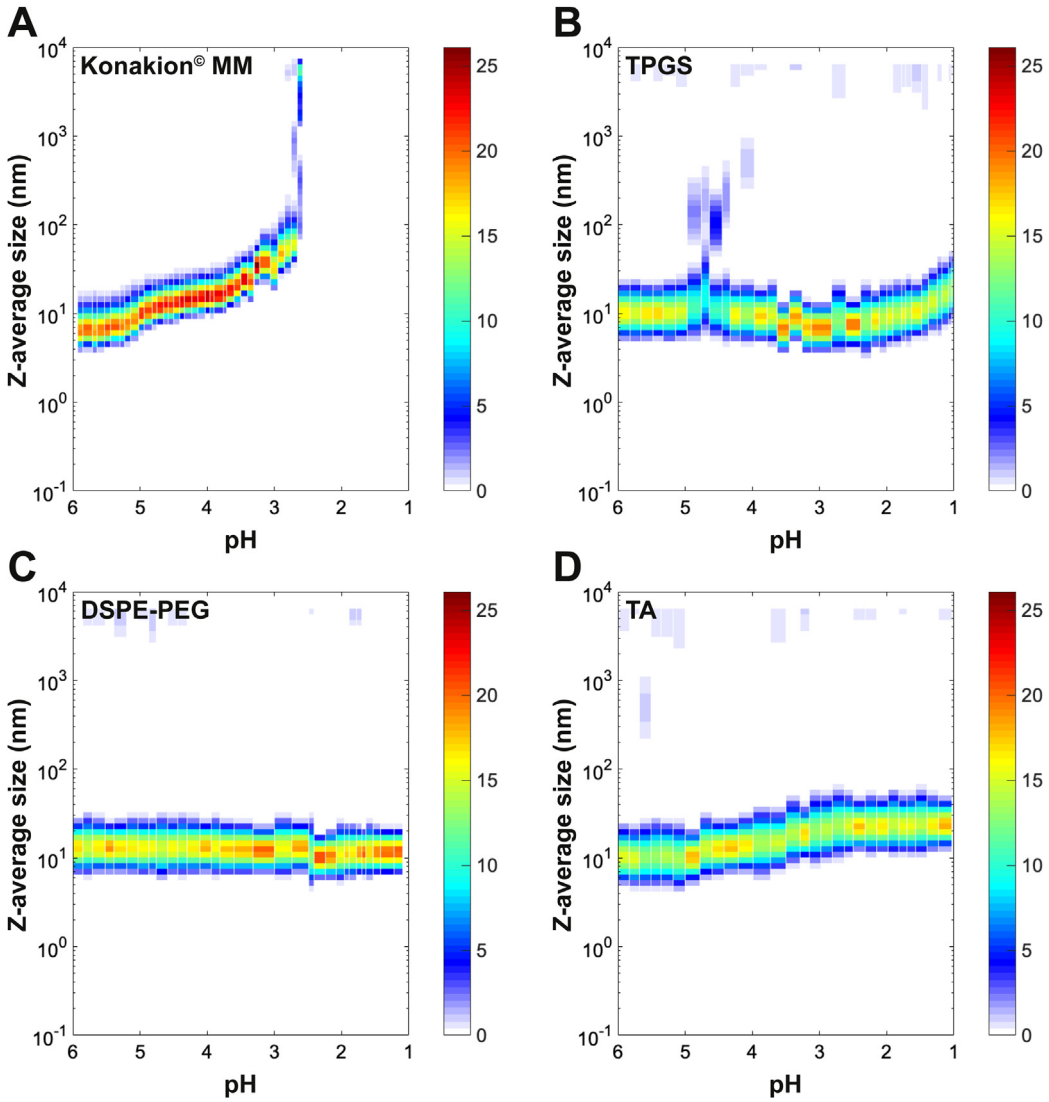

Supplmentary Figure 1. Influence of pH on the mean hydrodynamic particle size distribution of the Konakion MM (*A*), TPGS (*B*), DSPE-PEG (*C*), and TA (*D*) formulations between pH 6.0 and 1.0. The dilution factor was kept constant at 10 times for all measurements. Data are plotted as the intensity distribution on the z-axis; the color scale indicates the proportional intensity distribution (%).

Supplementary Table 1. Main Pharmacokinetic Metrics After Administration of a Single 1-mg Dose of Vitamin K From Oil-Based or Mm Formulations to Sham-Operated or Cholestatic Rats

		Pharmacokinetic metric					
		T_{max} (h)	C_{max} (μg/mL)	$AUC_{0-5\ h}$ (μg-h/mL)	$AUC_{0-24\ h}$ (μg-h/mL)	$AUC_{0-\infty h}$ (μg-h/mL)	$t_{1/2}$ (h)
Formulation	Status (n)	median (*min–max*)	geometric mean (*%CV*)	geometric mean (*%CV*)	geometric mean (*%CV*)	geometric mean (*%CV*)	arithmetic mean (SD; n)
Konakion MM	Sham (4)	1.1 (0.9–2.0)	3.50 (1.96 x 10^1)	8.37 (1.63 x 10^1)	-	-	6.3 (5.1; 4)
	BDL (7)	1.0 (0.0–3.3)	0.01 (1.47 x 10^3)	0.02 (4.79 x 10^2)	-	-	1.8 (1.2; 5)
	BDL + PPI (5)	2.0 (1.0–2.1)	0.63 (2.79 x 10^2)	1.71 (3.04 x 10^2)	-	-	2.7 (0.9; 5)
	BDL + IDA (5)	2.0 (1.0–5.1)	3.01 (4.81 x 10^2)	6.39 (2.48 x 10^2)	-	-	2.3 (0.5; 4)
Oily	Sham + fasted (6)	3.5 (0.0–5.1)	0.03 (7.59 x 10^2)	0.09 (7.41 x 10^2)	-	-	1.9 (0.6; 2)
	Sham + fed (6)	2.0 (2.0–5.0)	3.31 (6.39 x 10^1)	9.37 (5.08 x 10^1)	-	-	3.8 (NE; 1)
	BDL (5)	0.0 (0.0–1.1)	0.01 (8.28 x 10^2)	0.00 (5.02 x 10^2)	-	-	3.6 (3.9; 5)
Konakion MM	Sham (5)	4.0 (2.0–6.1)	4.32 (6.14 x 10^2)	7.77 (2.41 x 10^2)[a]	22.93 (1.11 x 10^3)	23.29 (1.13 x 10^3)	3.6 (0.8; 2)
TPGS	Sham (5)	1.1 (1.0–2.0)	10.78 (6.07 x 10^1)	30.24 (4.62 x 10^1)	50.26 (3.41 x 10^1)	51.06 (3.52 x 10^1)	4.0 (1.0; 5)
DSPE-PEG	Sham (5)	1.0 (1.0–3.0)	8.97 (3.29 x 10^1)	19.54 (2.41 x 10^1)	26.27 (3.81 x 10^1)	26.44 (3.86 x 10^1)	3.3 (2.0; 5)
TA	Sham (6)	1.5 (1.0–6.0)	8.54 (1.74 x 10^2)	18.58 (2.29 x 10^2)	46.16 (7.73 x 10^1)	47.84 (7.00 x 10^1)	3.7 (0.8; 4)
Konakion MM	BDC (6)	0.5 (0.5–1.0)	0.02 (3.58 x 10^7)	0.02 (2.31 x 10^4)[b]	0.07 (1.17 x 10^6)	0.07 (8.23 x 10^5)	7.5 (5.6; 3)
TPGS	BDC (5)	2.0 (1.0–4.0)	0.91 (3.62 x 10^2)	1.51 (4.93 x 10^2)	2.41 (5.81 x 10^2)	2.48 (6.00 x 10^2)	6.3 (4.8; 4)
DSPE-PEG	BDC (6)	2.0 (1.0–3.0)	0.20 (9.04 x 10^1)	0.45 (1.01 x 10^2)	0.65 (1.36 x 10^2)	0.67 (1.41 x 10^2)	7.1 (7.5; 6)
TA	BDC (5)	1.0 (1.0–3.0)	1.11 (2.34 x 10^2)	1.49 (8.06 x 10^2)	2.04 (1.63 x 10^3)	2.06 (1.66 x 10^3)	3.6 (1.7; 4)

%CV, geometric coefficient of variation; AUC_{0-5h}, area under the concentration-time curve from time zero to 5 h; $AUC_{0-24\ h}$, area under the concentration-time curve from time zero to 24 hs; $AUC_{0-\infty h}$, AUC from time zero extrapolated to infinity; BDL, bile duct ligation; BDC, bile duct cannulation; C_{max}, maximum plasma concentration; IDA, intraduodenal administration; NE, not estimable; PPI, proton pump inhibitor therapy; T_{max}, time to reach C_{max}; $t_{1/2}$, terminal elimination half-life.
[a]Supplemented with $AUC_{0-5\ h}$ data from BDL study, n = 9.
[b]Supplemented with $AUC_{0-5\ h}$ data from BDL study, n = 13.