Effects of Anesthesia and Different Lymphatic Fluid Collection Methods on the Lymphatic Transport of Orally Administered Drugs: A Comparative Study in Rats

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Abstract

With the continuous progress of new drug development technology, the proportion of lipophilic drug candidates among new chemical entities is increasing. These compounds exhibit poor water solubility, which affects the oral bioavailability of drugs. The absorption of oral dosage forms in the body primarily involves intestinal vascular and intestinal lymphatic absorption into the bloodstream. The investigation of drug absorption, distribution, and transportation through the lymphatic circulation has gained significant attention in the mechanistic study of bioavailability. Vitamin D_3 [1] and Halofantrine hydrochloride [2, 3], highly lipophilic compounds, are significantly absorbed into the bloodstream through the lymphatic transport pathway. This poster compared the lymphatic transport of vitamin D_3 via mesenteric lymph cannulation under anesthetized and conscious rats. The anesthetized group exhibited approximately 70% reduction in lymph flow rate compared to the conscious group, and the results were consistent with the total recovery of the compounds. Therefore, anesthesia did not affect the absorption pathway of the drug. In conclusion, the anesthetized rat model is more suitable for early drug screening experiments, and the conscious animal model is more suitable for targeted studies. However, to measure lymphatic transport more accurately and completely, conscious rat models are still needed. The study also compared the effects of two different methods of lymph collection, mesenteric lymph duct cannulation and thoracic lymph duct cannulation, on the lymphatic transport of halofantrine hydrochloride following oral administration in conscious rats. The experimental results show that different lymphatic fluid collection methods have an impact on the corresponding PK parameters, and the appropriate surgical model should be selected according to the specific research needs.

Method

Vitamin D_3 (cholecalciferol), Vitamin D_3-d_6, Halofantrine hydrochloride and Oleic acid were purchased from Sigma (St. Louis, MO, USA). All chemicals were of HPLC analytical grade. Male SD rats (8 weeks, weighing between 290-340g) were acclimatized for at least 3 days prior to the study. All surgical and experimental procedures were reviewed and approved by the Animal Experimentation Ethics Committee. The mesenteric lymph duct was cannulated using the method of Warshaw [4], with some modification. Similarly, the thoracic duct was cannulated using the method of Bollman [5], with some modification. The animals in the anesthetized group were cannulated 1 cm below the pylorus for the intraduodenal infusion. An indwelling cannula was also placed in the animals' right jugular vein for systemic blood sampling. The experimental design is shown in Table 1. Effect of anesthesia on animals' lymphatic transport rate as shown in Figures 1, 2 and Table 2. The experimental results showed slight differences in the evaluation of PK parameters of the drug in the lymphatic circulation using different surgical models. (As shown in Table 3).

Table 1 Design of lymphatic transport model in anesthetized and conscious rats

Group No.		Anesthesia (Y/N)	Administration route	Drug	Surgery Cannulation	Dosage (mg/kg)	Samples	
							Plasma (Y/N)	Lymph (Y/N)
A	1	Y	Duodenum cannulation	Vitamin D_3	Mesenteric lymph duct	5	N	Y
	2	N	PO			5	N	Y
B	3	N	PO	Halofantrine hydrochloride	Thoracic duct	7.5	Y	Y
	4	N	PO		Mesenteric lymph duct	7.5	Y	Y
	5	N	PO		Portal vein	7.5	Y	N
	6	N	PO		None	7.5	Y	N

Results

Table 2 Vitamin D_3 pharmacokinetic parameters obtained in the two experimental models

Model	Total Recovered Compound (μg)	Cumulative Vitamin D_3 transported via the lymph (% of dose)	Lymph flow rate (μL/6h)
Anesthetized	37.1 ± 31.0	2.5 ± 2.1	3597.3 ± 1563.9
Conscious	340.9 ± 179.9	23.5 ± 11.0	13643.7 ± 4.9

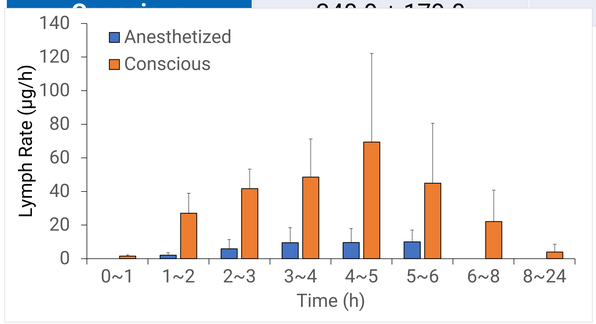

Figure 1 Lymphatic transport of Vitamin D_3 (μg/h) in anesthetized (blue) and conscious (red) models.

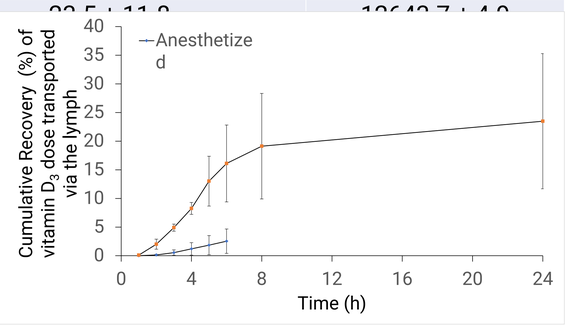

Figure 2 Cumulative recovery of vitamin D3 (% of dose) in the lymph of anesthetized (blue) and conscious (orange) models.

The experimental results showed that the bioavailability of lipophilic compounds is the sum of the fraction absorbed through the portal blood plus the fraction absorbed through the lymphatic system. Different rat models were used to compare the effects of anesthesia on drug absorption. The results showed that the lymphatic flow rate of conscious animals was significantly higher than that of anesthetized animals (about three times). This might be due to the decreased gastrointestinal tract (GIT) function in anesthetized rats, but anesthesia did not hinder their lymphatic absorption. The use of an anesthetized rat model in the experiments simplifies the surgical process, speeds up the operation, and saves the postoperative recovery time. Furthermore, compared to the conscious rat model, the anesthetized rat model is easier to maintain in terms of the patency rate of lymphatic cannulation and reducing the occurrence of postoperative decannulation. Therefore, for rapid screening of drugs with oral lymphatic absorption, anesthetic animal models can be the preferred choice, while for exploring the absorption characteristics of specific molecules, conscious animal models may be more appropriate.

Table 3 Halofantrine hydrochloride pharmacokinetic parameters (plasma) in various models

PK parameter	Thoracic duct	Mesenteric-duct	Portal vein	Non-surgical
C_{max} (ng/mL)	161.5 ± 43.2	182.8 ± 49.0	452.5 ± 226.9	383.3 ± 191.6
T_{max} (h)	20.0 ± 8.00	16.0 ± 9.2	8.0 ± 0.0	12.0 ± 8.0
$T_{1/2}$ (h)	36.7 ± 17.9	30.5 ± 2.7	64.1 ± 60.1	66.7 ± 19.0
$AUC_{0\text{-}inf}$ (ng.h/mL)	11194.5 ± 5968	7082.7 ± 1908.2	21246.8 ± 10794.1	18814.8 ± 5248.7
$MRT_{0\text{-}inf}$ (h)	61.8 ± 24.5	45.6 ± 6.7	77.6 ± 49.6	79.6 ± 14.7
CL (mL/min/kg)	3.8 ± 1.5	2.3 ± 1.11	N/A	N/A
Hepatic Extraction ratio	N/A	N/A	0.12 ± 0.11	N/A
Total Compound Recovered Amount (μg)	531402.2 ± 107797.8	261916.8 ± 86024.8	N/A	N/A
Cumulative Compound Transported Via the Lymph (% of dose)	23.5 ± 4.8	11.1 ± 3.4	N/A	N/A

The experimental results of different lymph collection locations showed that the mesenteric lymph cannula mainly collected drugs absorbed by the lower body and intestinal lymph, while the thoracic duct mainly collected drugs collected by the capillary lymph vessels in the interstitial space, accounting for about three-quarters of the lymphatic circulation in the body (as shown in Table 3).

Therefore, different experimental models are slightly different in the evaluation of PK parameters of drugs in lymphatic circulation. In the process of experiment, more accurate feedback on experimental results can be obtained by selecting a matching experimental model combined with different experimental purposes.

Conclusion

Using different experimental models may result in slight differences in the pharmacokinetic evaluation of orally administered drugs transported via the body's lymphatic system. Therefore, it's necessary to select an appropriate surgical model based on specific research objectives to accurately reflect the body's lymphatic absorption of drugs. In addition to investigating the absorption kinetics of orally administered compounds into the bloodstream through the intestine, it is sometimes crucial to consider their pathways of lymphatic absorption, particularly for compounds with high lipophilicity. Mechanistic studies on the absorption, distribution, and transportation processes of oral drugs through lymphatic pathways can provide new opportunities for optimizing the oral bioavailability of drugs in the discovery phase.

References

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