EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES 32 (2007) 367–374

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/ejps

The effect of general anesthesia on the intestinal lymphatic transport of lipophilic drugs: Comparison between anesthetized and freely moving conscious rat models

Arik Dahan[a], *Avivit Mendelman*[b], *Sofia Amsili*[b], *Nathan Ezov*[b], *Amnon Hoffman*[a,*]

[a] *Department of Pharmaceutics, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel*
[b] *Harlan Biotech Israel Ltd., Rehovot, Israel*

ARTICLE INFO

Article history:
Received 7 June 2007
Received in revised form
17 September 2007
Accepted 19 September 2007
Published on line 26 September 2007

Keywords:
Lymphatic transport
Lipophilic drugs
Oral bioavailability
Anesthetization
Vitamin D_3

ABSTRACT

The purpose of this study was to evaluate the impact of general anesthesia on the lymphatic transport of orally administered drugs. Vitamin D_3 (0.5 mg/kg), a model lipophilic molecule with significant lymphatic transport, was administered to anesthetized rats in close proximity to the lymphatic cannulation procedure. The lymphatic and non-lymphatic absorption of the vitamin in this experimental model was compared to lymph-duct cannulated freely moving conscious rats. The amounts of vitamin D_3 transported via the lymph in the anesthetized animals throughout the time frame of this experimental model (8 h) were 25% lower as compared to the conscious animals, but showed similar absorption kinetics. However, the duration of the anesthesia is limited and thus failed to produce the complete picture of the absorption process. The cumulative percent of the vitamin dose that was recovered in the lymph as well as the vitamin plasma AUC values were both 25% lower in the anesthetized animals as compared to the conscious animals. Hence, the anesthesia did not influence the proportion of the vitamin fraction absorbed via the different pathways. The lymph flow rate was significantly decreased by the anesthesia (threefold), however, higher lymph vitamin concentrations in these animals led to lower differences in the vitamin lymphatic transport (25%) between the models. In conclusion, the anesthetized rat model is suitable for approximating the lymphatic transport. However, the conscious rat model is still required in order to have a more precise and complete measurement of lymphatic transport.

1. Introduction

As a consequence of modern drug discovery techniques (i.e., advances in *in vitro* screening methods, the introduction of combinatorial chemistry), the number of poorly water-soluble drug candidates is constantly increasing. To date, more than 40% of new active chemical entities are lipophilic and exhibit poor water solubility (Lipinski et al., 2001). These molecules suffer from low oral bioavailability, and thus fail to proceed to the advanced stages of research and development, despite their favorable pharmacological activity (Martinez and Amidon, 2002; Amidon et al., 1995). One approach to improve the oral bioavailability of these molecules is their targeting to the intestinal lymphatic system (Porter et al., 2007; Nordskog et al., 2001).

The majority of orally administered drugs gain access to the systemic circulation by direct absorption into the portal blood. However, highly lipophilic compounds may reach

* *Corresponding author at*: Department of Pharmaceutics, School of Pharmacy, The Hebrew University of Jerusalem, P.O. Box 12065, Jerusalem 91120, Israel. Tel.: +972 2 6757014; fax: +972 2 6757246.
E-mail address: ahoffman@cc.huji.ac.il (A. Hoffman).
0928-0987/$ – see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.ejps.2007.09.005

the systemic blood circulation via the intestinal lymphatic system (Dahan and Hoffman, 2006a). This alternative absorption pathway from the gastrointestinal tract (GIT) has been shown to be significant contributor for the overall bioavailability of a number of highly lipophilic drugs, including fat soluble vitamins (Kuksis, 1987), halofantrine (Karpf et al., 2004; Holm et al., 2003; Khoo et al., 2001), probucol (Palin and Wilson, 1984), ontazolast (Hauss et al., 1998), seocalcitol (Grove et al., 2006), mepitiostane (Ichihashi et al., 1992) and others. In addition to increased overall bioavailability of lipophilic molecules, lymphatic transport of a drug provides further advantages, including avoidance of hepatic first pass metabolism, a potential to target the drug into the lymphatic system for the case of specific disease states known to spread via the lymphatics, and improved plasma profile of the drug. For these reasons, studies of the absorption of drugs via the intestinal lymphatic system have received increasing attention in recent years (O'Driscoll, 2002; Porter and Charman, 2001). However, the understanding of the precise processes involved in this transport pathway is still limited, mainly due to significant difficulties in conducting research in this field: the *in vivo* evaluation of lymphatic transport of potential molecules is time consuming and of limited capacity.

A number of animal models have been proposed for the investigation of intestinal lymphatic drug transport (Edwards et al., 2001). The most common animal used for such experiments is the laboratory rat (Boyd et al., 2004), which allows comparison with the substantial data accumulated in the literature regarding the biochemistry of lipid absorption and intracellular processing. Different surgical methods have been proposed; all based on an indwelling cannula in a lymphatic vessel, mainly the mesenteric lymph duct, providing the ability to continuously collect the lymph fluid. The freely moving conscious rat model seems to be the superior experimental method in this field.

Placing a cannula in a frail lymphatic vessel demands a high level of surgical skill and the percentage of success is very limited. The surgical recovery period is often associated with high mortality rate. Thus, even following a successful completion of this complicated surgical procedure, animals still drop out of the experiment. Problems such as dislodgement of the cannula, clot formation and poor lymph flow rate tend to appear after surgery (throughout the recovering period) and further decrease the success rate of this model. These circumstances make intestinal lymphatic transport investigation very difficult, and the screening of a large amount of candidate molecules, a need that often arises in the industry, is practically impossible. Employment of an anesthetized, rather than conscious, rat experimental model facilitates the procedure and expedites it as it saves the time required for the post-surgery recovery period. Moreover, it is easier to maintain the viability of lymphatic cannula in anesthetized, rather than conscious, animals: the lack of movements prevents problems such as dislodgement or blockade of the cannula, and no drop-out occurs following the completion of the surgical procedures. Hence, the yield of this model is much higher, and in fact approaches 100%. For these reasons, the utilization of an anesthetized animal experimental model in the investigation of intestinal lymphatic transport has increased recently (Trevaskis et al., 2005, 2006; Griffin and O'driscoll, 2006). General anesthesia, however, may lead to major physiological alterations, and the implication of the results may be seriously hampered. This issue was addressed previously but the reports were contradictory. While in one case the lymphatic transport was enhanced as a result of the anesthesia (Charman et al., 1986), the other report showed either similar or reduced lymphatic transport kinetics in comparison to conscious rats (Porter et al., 1996b). Hence, the question as to what is the price that one pays while conducting lymphatic transport experiments under general anesthesia is still ambiguous.

The purpose of this study was to evaluate the impact of general anesthesia and the lack of surgical recovery period on the lymphatic transport of orally administered drugs, by comparing the two extensively used methods in this field. Vitamin D_3, a model lipophilic molecule with significant lymphatic transport (Dahan and Hoffman, 2005; Liu et al., 1995), was administered to rats in two experimental models: (1) freely moving conscious rats, including overnight surgery recovery period, and (2) anesthetized animals, with the drug administration in close proximity to the ending of the surgical procedures. The advantages and drawbacks of the two experimental models were explored, as well as their ability to provide reliable results. This data set will help the researcher in this field to choose the suitable experimental model with awareness to the consequence advantages and disadvantages. In addition, it will simplify to evaluate and interpret the published *in vivo* database accumulated in the literature that was obtained in both anesthetized and freely moving conscious rat experimental models.

2. Materials and methods

2.1. Materials

Vitamin D_3 (cholecalciferol), vitamin E (tocopherol), L-α-lysophosphatidylcholine, oleic acid, sodium taurocholate and peanut oil were purchased from Sigma Chemical Co. (St. Louis, MO). Normal saline solution was obtained from Teva Medical (Ashdod, Israel). Ethanol, methanol, water and *n*-hexane (J.T. Baker, Deventer, Holland) were HPLC grade. All other chemicals were of analytical reagent grade.

2.2. Surgical procedures

All surgical and experimental procedures were reviewed and approved by the Animal Experimentation Ethics Committee of the Hebrew University Hadassah Medical School Jerusalem. Male Wistar rats (Harlan, Israel), 300–325 g in weight, were used for all surgical procedures.

One hour before the mesenteric lymph duct cannulation, the rats were given 1 ml of peanut oil by oral gavage, for visualization of the lymphatics. Animals were anesthetized for the period of surgery by intra-peritoneal injection of 1 ml/kg of ketamine–xylazine solution (9%:1%, respectively) and placed on a heated surface maintained at 37 °C (Harvard Apparatus Inc., Holliston, MA). The mesenteric lymph duct was cannulated by the method of Warshaw (Warshaw, 1972), with some modification. A polyethylene tubing (PE-50, Intramedic®, Bec-