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RAPID COMMUNICATIONS

Intestinal Lymphatic Transport: an Overlooked Pathway for Understanding Absorption of Plant Secondary Compounds in Vertebrate Herbivores

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Abstract Herbivores employ numerous strategies to reduce their exposure to toxic plant secondary chemicals (PSCs). However, the physiological mechanisms of PSC absorption have not been extensively explored. In particular, the absorption of PSCs via intestinal lymphatic absorption has been largely overlooked in herbivores, even though this pathway is well recognized for pharmaceutical uptake. Here, we investigated for the first time whether PSCs might be absorbed by lymphatic transport. We fed woodrats (*Neotoma albigula*) diets with increasing concentrations of terpene-rich juniper (*Juniperus monosperma*) either with or without a compound that blocks intestinal lymphatic absorption (Pluronic L-81). Woodrats consuming diets that contained the intestinal lymphatic absorption blocker exhibited increased food intakes and maintained higher body masses on juniper diets. Our study represents the first demonstration that PSCs may be absorbed by intestinal lymphatic absorption. This absorption pathway has numerous implications for the metabolism and distribution of PSCs in the systemic circulation, given that compounds absorbed via lymphatic transport bypass first-pass hepatic metabolism. The area of lymphatic transport of PSCs represents an understudied physiological pathway in plant-herbivore interactions.

Keywords Herbivory · Lymphatic absorption · Plant-herbivore interactions · Plant secondary metabolites

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Introduction

Many plants defend themselves with noxious plant secondary compounds (PSCs) that deter feeding by herbivores (Dearing et al. 2005). In response, herbivores have evolved a number of strategies to overcome these challenges, such as altered feeding behaviors and physiological adaptations (Dearing et al. 2005). Regulation of absorption in the small intestine represents one route by which herbivores could limit systemic exposure to PSCs (Forbey et al. 2013). One overlooked route of absorption, and thus potential site of adaptation for herbivores, is intestinal lymphatic transport. Inside intestinal cells, lipid metabolites and lipophilic compounds are packaged into chylomicrons, which are then released from the basal membranes of intestinal cells via exocytosis and enter the lymphatic system (Porter et al. 2007). Upon entry to the lymphatic system, lipophilic compounds can be distributed in the systemic circulation over the course of several hours, thus bypassing first-pass metabolism by the liver (Busbee et al. 1985; Porter et al. 2007). It is highly appreciated that lipophilic pharmaceutical compounds can be absorbed via this route (Gershkovich and Hoffman 2005; Porter et al. 2007). Furthermore, a large number of PSCs are highly lipophilic, and some isolated flavonoids and flavonoid-glycosides can be absorbed by this pathway (Chen et al. 2010; Murota and Terao 2005). However, several recent review papers on the absorption of plant secondary compounds in herbivores overlook this potential absorption pathway, and instead focus on transcellular or paracellular absorption directly to the bloodstream (Forbey et al. 2013; McLean and Duncan 2006).

We conducted an initial investigation into whether intestinal lymphatic absorption might play a role in mediating plant-herbivore interactions. We used a commercial block copolymer surfactant, Pluronic L-81, to block formation of chylomicrons while feeding herbivorous woodrats diets with

lipophilic PSCs. We tested the hypothesis that the lymphatic system was a route of absorption for PSC and predicted that blocking intestinal lymphatic absorption would increase the tolerance of a toxic diet, thereby facilitating increased food intake and maintenance of body mass.

Methods

We conducted our study on a dietary generalist, *Neotoma albigula,* which cannot sustain body mass in the long term (>21 days) when fed diets with juniper concentrations of 50%. Juniper contains monoterpene defensive compounds that prevent *N. albigula* from consuming juniper in high amounts (Dearing et al. 2000). Wild woodrats were collected from Castle Valley, Utah, USA and transported to the University of Utah animal facility. Woodrats were housed in individual cages (48 × 27 × 20 cm) under a 12:12-h light:dark cycle, with 28 °C ambient temperature and 20% humidity, and were fed commercial rabbit chow (Harlan Teklad 2031) when not undergoing experiments detailed below.

We inhibited intestinal lymphatic absorption by adding the compound Pluronic L-81 (BASF Corporation, Wyandotte, MI, USA) to experimental diets. This compound effectively inhibits chylomicron formation in the gut, thus blocking intestinal lymphatic absorption. Inhibition by Pluronic L-81 is specific to intestinal lymphatic absorption, as transcellular or paracellular pathways are unaffected (Dahan and Hoffman 2005). Moreover, this inhibition is readily reversed within 5–7 h after Pluronic L-81 is no longer administered (Fujimoto et al. 1992).

First, we investigated whether Pluronic L-81 influenced diet choice. We first investigated whether woodrats would avoid or select Pluronic L-81 on a control, nontoxic diet. We placed 8 woodrats in individual cages with two feeder hoods. One hood contained a mixture of powdered rabbit chow (53% rabbit chow, 47% water, to mimic the water content of juniper). To the diet in the second hood we added 1% Pluronic L-81 (53% rabbit chow, 46% water). A diet containing 0.5% Pluronic L-81 reduces intestinal lymphatic absorption by 85% in laboratory rats (Tso et al. 1980). Diets were presented to animals for 3 days, and the position of each diet was alternated each day. Next, we conducted this choice experiment again, this time using juniper–containing diets: Control (50% juniper, 26.5 rabbit chow, 23.5% water) or Pluronic L-81 (50% juniper, 26.5% rabbit chow, 22.5% water, 1% Pluronic L-81), again for 3 days. Juniper foliage was ground as described elsewhere (Sorensen et al. 2004). The mass of wet food presented in each hood was measured daily, and subsamples were used to estimate the dry weight of the diet. The following day, leftover food was collected, dried overnight at 40 °C and weighed to measure intake. Food intake was calculated as the dry mass of food presented – dry mass of food recovered. For both trials, we calculated the percentage of food consumed from the Pluronic L-81 hood. We used a Student's t-test to determine whether this value differed significantly from 50%.

Next, we split the animals into two groups and fed them increasing concentrations of juniper (0%, 25%, 50%, and 75%, increasing every 3 days). This experiment was conducted 3 weeks after the choice trials described above. For the 3 weeks in between experiments, woodrats were fed control rabbit chow lacking Pluronic L-81. The control group ($N = 7$) was offered diets only containing juniper, rabbit chow, and water. The Pluronic L-81 group ($N = 6$) was given a treatment of 1% of Pluronic L-81 mixed into the food for the complete length of the experiment. The 8 animals used in the choice trials described above were split evenly between these two groups. Food intake was measured as described above. Body mass was also measured daily over the course of the experiment. Percent change in body mass was calculated as [(body mass on day X – body mass on day 0)/ body mass on day 0]. We used a repeated-measures ANOVA to compare food intake and body mass for days 4–11 of the experiment (during which the woodrats were being fed diets containing juniper). This interval represented the time when all animals were being fed juniper, but no animals had lost more than 10% of their original body mass (at which point animals were removed from the experiment; only 2 animals reached this point, 1 from each group). All experiments were approved under University of Utah Animal Care and Use Committee protocols 12–12,010.

Results

We found that when given the choice of similar diets (rabbit chow or 50% juniper) with or without the Pluronic L-81 inhibitor, *N. albigula* did not prefer or avoid diets containing Pluronic L-81. The percentage of food consumed from the hoods containing Pluronic L-81 did not significantly differ from 50% (Fig. 1a; $P > 0.3$ for both trials).

Blocking lymphatic transport significantly increased the ability of *N. albigula* to ingest a juniper diet. Woodrats fed the juniper diet containing Pluronic L-81 exhibited significantly higher food intake compared to those fed the juniper diet lacking the inhibitor (Fig. 1b, Pluronic L-81 effect: $F_{1,11} = 10.87$, $P = 0.007$; Time effect: $F_{7,5} = 12.96$, $P = 0.006$; Pluronic L-81 × Time effect: $F_{7,6} = 1.40$, $P = 0.37$). On the third day of the 50% juniper diet (day 9 of the experiment), the Pluronic L-81 group consumed 28% more juniper than the Control group. Additionally, woodrats given the diet containing Pluronic L-81 increased body mass on a juniper diet, and had significantly higher body masses than woodrats feeding on juniper but without the intestinal lymphatic absorption blocker (Fig. 1c; Pluronic L-81 effect: $F_{1,11} = 5.39$, $P = 0.04$; Time effect: $F_{7,5} = 14.12$, $P = 0.005$; Pluronic L-81 × Time effect: $F_{7,5} = 0.76$, $P = 0.64$).

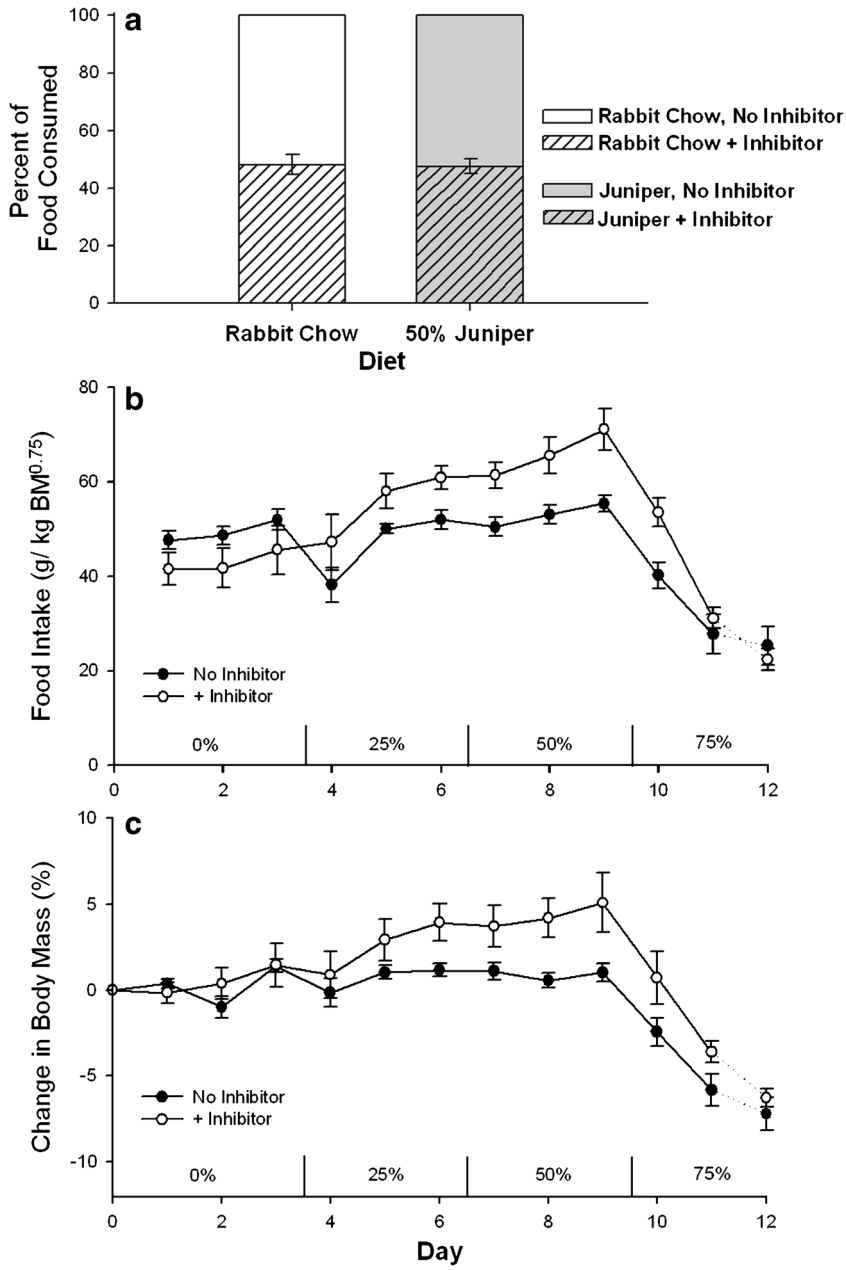

Fig. 1 Effects of Pluronic L-81 on performance (food intake and body mass) of *Neotoma albigula* fed a diet containing juniper. **a** Diet choice of control or Pluronic L-81 foods. **b** Food intake over the course of the experiment. **c** Percent change in body mass over the course of the experiment. For both (**b**) and (**c**) percentage juniper in the diet is noted on the x-axis. Also, two animals lost more than 10% of their original body mass, and so data from day 12 was not used in statistical analyses (denoted with *dotted lines*)

Discussion

Understanding the physiological underpinnings of herbivore tolerance to PSCs has implications for understanding the nature of these ecological interactions at larger scales. For example, herbivores can influence ecosystem structure (Martin and Maron 2012), and often their diet selection is dictated by PSCs (Dearing et al. 2000). Our results provide some of the first evidence that PSCs may be absorbed by intestinal lymphatic absorption. These results open up many areas of future research and suggest the possibility that specialist herbivores could regulate intestinal lymphatic absorption to enhance toxin tolerance.

Blocking intestinal lymphatic absorption significantly increased the performance of *N. albigula* feeding on diets of juniper, suggesting that at least some of the PSCs in juniper are absorbed through this pathway. Interestingly, these results are qualitatively similar to previous studies on a juniper-specialist woodrat species, *N. stephensi*. In that study, *N. stephensi* increased their food intake by 22% and body mass by 5.5% when feeding on a 50% juniper diet compared to a non-juniper diet (Sorensen et al. 2004). Similarly, *N. albigula* given the lymphatic absorption blocker increased food intake by 25.6% and body mass by 5.1% when feeding on a 50% juniper diet, compared to when feeding on the diet lacking juniper and PSCs (control: days 1–3, 50% juniper: days 7–

9). Thus, blocking intestinal lymphatic absorption in the generalist, *N. albigula*, effectively elicited the feeding behaviors observed in the specialist, *N. stephensi*. However, it should be noted that the juniper specialist *N. stephensi* are able to consume even higher levels of juniper (75% in the laboratory, >90% in the wild), while *N. albigula*, even with the lymphatic absorption blocker, began to decrease food intake and body mass on the 75% juniper diet. Other physiological adaptations, such as detoxification and/or elimination mechanisms may enable *N. stephensi* to feed on high doses of juniper.

Lymphatic absorption of PSCs may have implications for understanding the pharmacokinetics and pharmacodynamics of these compounds. Absorption pathways and rates can influence the systemic concentrations of PSCs, and thus the severity of their physiological consequences (Forbey et al. 2013). For example, compounds absorbed by intestinal lymphatic transport can evade metabolism by enterocyte-based detoxification enzymes (Trevaskis et al. 2006). Additionally, compounds absorbed via the transcellular or paracellular pathways enter the hepatic portal vein and undergo first-pass metabolism in the liver, thereby lowering the concentration entering systemic circulation, while compounds absorbed through lymphatic absorption bypass the liver, entering systemic circulation hours later at higher concentrations (Busbee et al. 1985; Porter et al. 2007). Last, lymphatic absorption can influence the distribution of PSCs in the body. The lymphatic system is the primary location of immune cells (Porter et al. 2007), and a number of PSCs can have stimulatory or inhibitory properties on immune cells (Provenza and Villalba 2010). Thus, lymphatic absorption may increase exposure of immune cells to PSCs over that of transcellular or paracellular pathways. The physiological consequences of PSCs absorbed via lymphatic absorption is a possible area of future research.

These results might also have applications to domestic herbivores. Juniper is widespread in the desert southwest, and there is interest in the use of small ruminants to control its expansion (Utsumi et al. 2013). Supplementing the diet with polyethylene glycol (PEG), which inhibits the action of tannins (a class of PSCs) increased the preference ratio of goats to consume more juniper (Utsumi et al. 2013). Pluronic L-81 may be another feed additive to increase the capacity of domestic herbivores to consume juniper and other PSC-rich plants. Though, it should be noted that PEG and Pluronic L-81 have structural similarities, and our study does not specifically test whether Pluronic L-81 may counteract the negative effects of tannins versus terpenes. Follow up studies using purified PSCs, coupled with the measurement of concentrations of PSCs in lymph, serum, and excretory pathways (urine and bile) will enhance our understanding of the role of intestinal lymphatic transport in PSC absorption and processing.

Overall, this is the first evidence suggesting that PSCs are absorbed by intestinal lymphatic transport. Further research could focus on a number of open questions: Is this absorption pathway generalizable across herbivorous taxa and various classes of PSCs? How does lymphatic absorption influence systemic exposure to PSCs, and scale to influence foraging behaviors of herbivores? If specialist herbivores have decreased intestinal lymphatic absorption to avoid PSC exposure, what are the molecular mechanisms, and are there any tradeoffs (for example lipids and fat-soluble vitamins are also absorbed via lymphatic absorption)? Inclusion of lymphatic absorption will increase our understanding of plant-herbivore interactions and their consequences for ecology.

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