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Distribution of a highly lipophilic drug cannabidiol into different lymph nodes following oral administration in lipidic vehicle

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ABSTRACT

Efficient delivery of highly lipophilic drugs or prodrugs to the mesenteric lymph nodes (MLN) can be achieved following oral administration with lipids. However, it remains unclear which specific MLN can be targeted and to what extent. Moreover, the efficiency of drug delivery to the retroperitoneal lymph nodes (RPLN) has not been assessed. The aim of this study was to assess the distribution of a highly lipophilic model drug cannabidiol (CBD), known to undergo intestinal lymphatic transport following administration with lipids, into specific MLN and RPLN in rats at various time-points post dosing. *In vivo* studies showed that at 2 h following administration, significantly higher concentrations of CBD were present in the region second from the apex of the MLN chain. From 3 h following administration, concentrations in all MLN were similar. CBD was also found at substantial levels in RPLN. This study demonstrates that drug concentrations in specific MLN are different, at least at the peak of the absorption process. Moreover, in addition to the MLN, the RPLN may also be targeted by oral route of administration, which may have further implications for treatment of a range of diseases.

1. Introduction

Mesenteric (MLN) and retroperitoneal (RPLN) lymph nodes play an important role in the pathology of a number of diseases including inflammatory and autoimmune diseases, lymphomas, cancer metastasis and infections such as human immunodeficiency virus (HIV) [1–4]. Targeted drug delivery of highly lipophilic compounds to the MLN can be achieved through incorporation into dietary lipid absorption pathways following oral delivery [1–4]. Importantly, the design of different activated ester lipophilic prodrugs was recently shown to significantly enhance active drug concentrations achieved in the MLN for compounds that otherwise could not be delivered to the intestinal lymphatic system. These include the antiretroviral drug lopinavir and cancer chemotherapeutics bexarotene and retinoic acid [2,4]. Furthermore, a triglyceride mimetic prodrug of the immunomodulatory agent mycophenolic acid resulted in higher drug concentrations in the MLN and enhanced pharmacodynamic activity [3].

The primary focus of these previous studies has been the enhancement of drug concentrations in the MLN collectively. However, the MLN are comprised of a chain of multiple individual lymph nodes which lie parallel to the intestine (Fig. 1). Studies have indicated that the intestinal lymph nodes drain distinct segments of the gastrointestinal tract [5]. However, potential segregation of lymphatic drainage from different parts of the GI tract into these nodes has been largely overlooked in previous studies. Since lipid absorption varies along the small intestine [5], it can be hypothesised that the actual concentrations of orally administered drugs in the individual lymph nodes draining the intestine will also vary. However, the distribution of drugs into individual lymph nodes and how this compares to pharmacological thresholds remains unknown. Moreover, how the drug distribution into these nodes changes over time also remains unclear. Since intestinal lymph nodes have been shown to have distinct immunological functions [5], knowledge of which lymph nodes can be targeted has particular importance in inflammatory disorders where specific regions of the bowel are implicated, such as ulcerative colitis. In addition, since primary tumours in the bowel are common and metastases into MLN are well described, knowledge of which MLN and RPLN nodes could potentially be targeted by chemotherapeutic drugs could also be

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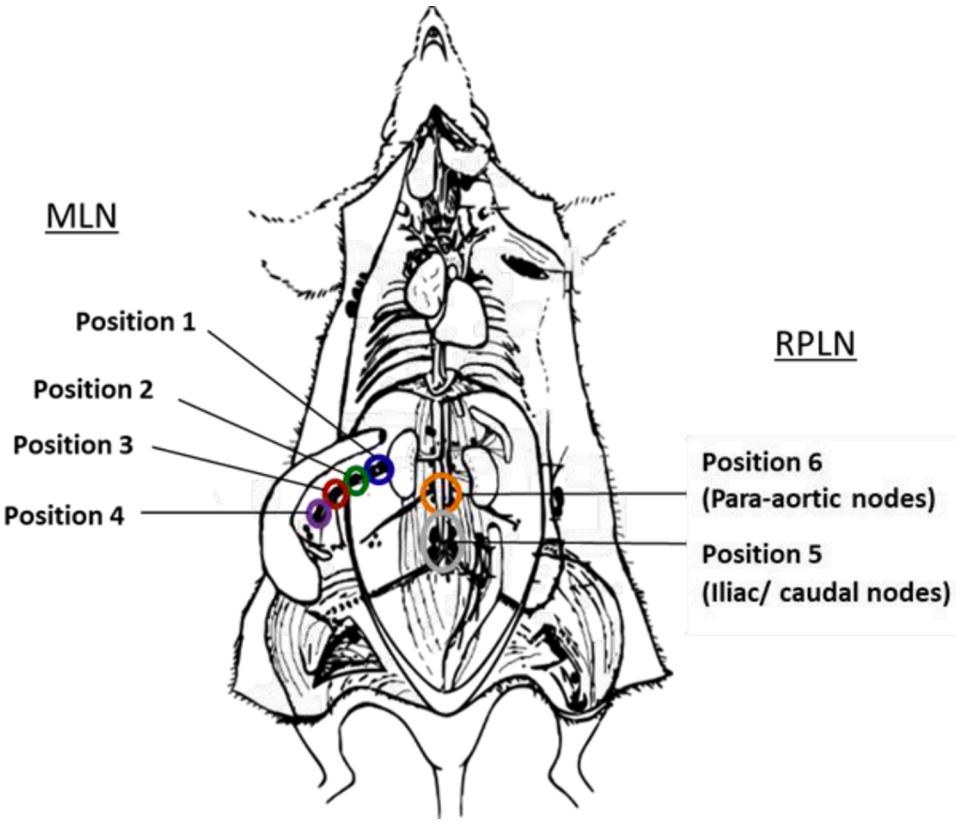

Fig. 1. Schematics showing the anatomical localisation of the MLN (position 1–4) and RPLN (position 5&6) collected as in this work. Modified from [6].

relevant in treatment of cancer associated with the abdominal and pelvic regions. More specifically, targeting of immune oncology agents to tumour-draining lymph nodes has high clinical potential, and the anatomy of lymph drainage becomes critical for efficient targeting of these agents to relevant lymph nodes.

Despite the fact that human mesentery contains comparatively more lymph nodes (100–150), the overall organisation of the gastrointestinal tract and associated lymph nodes is comparable in rats and humans [7]. Following collection into the superior MLN, lymph draining from the duodenum, small bowel and ascending and transverse colon then enters the superior mesenteric duct [6]. Lymph from the superior MLN is then directed through the pre-aortic nodes (part of RPLN) which lie in front of the aorta before entry into the cisterna chyli and thoracic duct [6]. It can therefore be hypothesised that the RPLN may also be targeted by lipophilic compounds undergoing intestinal lymphatic transport, although perhaps less efficiently than MLN due to a dilution factor. Effective targeting of the RPLN could be important, not only for drug delivery for immunological disorders, but also in cancer, where RPLN metastasis has a poor prognosis [8]. Despite this, RPLN have to date been largely overlooked in terms of lymphatic delivery research and little is known about how efficiently they can be targeted via the oral route.

Cannabidiol is a highly lipophilic compound shown to undergo substantial intestinal lymphatic transport following oral administration with long-chain triglycerides, reaching concentrations above immunomodulatory thresholds in the MLN [1]. By acting on the cells within these nodes, CBD therefore has high clinical potential as an immunomodulatory agent in inflammatory and autoimmune diseases. In addition, CBD has also received interest as an anticancer agent [9]. However, it has been unclear in previous work which specific lymph nodes can be targeted by CBD.

The aim of this study was to determine how the distribution of CBD compares throughout the individual MLN and RPLN nodes following oral administration in a lipid-based formulation. The impact of time on CBD distribution has also been investigated. The data from this study will therefore provide more specific information on the potential of lymph node targeting via the oral route for treating disorders in which the lymphatic system plays an important role.

2. Materials and methods

2.1. Materials

Plant-derived CBD was purchased from THC Pharm GmbH (Frankfurt, Germany). Sesame oil was purchased from Sigma Aldrich (Gillingham, UK). All other solvents and reagents were of HPLC grade and were purchased from Fisher Scientific (Loughborough, UK).

2.2. Animals

All experiments and procedures were approved by the UK Home Office in accordance with the Animals [Scientific Procedures] Act 1986. Experiments were performed using male Sprague-Dawley rats (Charles River Laboratories) weighing 300–349 g. The rats were housed in the University of Nottingham Bio Support Unit, and kept in a temperature-controlled and 12 h light–dark cycle environment.

2.3. Bio-distribution studies

Following 5 days of acclimatization, animals were fasted overnight with free access to water. CBD solution in sesame oil was administered via oral gavage (12 mg/ml, 12 mg/kg) as previously described [7]. Maximum CBD concentrations in MLN following administration in sesame oil has been previously shown to be at 2 h following administration (plasma t_{max}-1) [1]. Since it was hypothesised that CBD would enter RPLN after MLN, it was sensible to assume that CBD would reach peak concentrations in RPLN at time points at or later than 2 h post administration. Based on this, following administration of CBD in sesame oil, tissues were collected 2, 3, 5 and 8 h following administration (n = 4 per time point). Animals were euthanized by means of carbon dioxide gas and individual lymph nodes were dissected and separated from the surrounding tissue. The anatomical location of the nodes dissected and analysed are depicted in Fig. 1. RPLN collected at position 5 & 6 were the iliac/caudal lymph nodes and para-aortic nodes, respectively.

2.4. Sample preparation

Individual lymph node samples were added to 1.5 ml green RINO bead lysis tubes (WebScientific, Crewe, UK) in 175 μl water and homogenised using Bullet Bender Gold 24 Tissue Homogeniser (Next Advance, USA) at 4 °C. Dichlorodiphenyltrichloroethane (DDT) at a final concentration of 5 μg/ml was used as an internal standard. Following homogenisation of lymph nodes samples, 100 μl suspension was prepared for protein precipitation and liquid – liquid extraction. Samples were vortex-mixed with 600 μl of cold acetonitrile for 1 min. Water (450 μl) was then added, followed by 3 ml n-Hexane. Samples were vortexed for a further 5 min and centrifuged at 4000 g at 10 °C for 10 min. The upper organic layer was removed and evaporated to dryness under nitrogen at 35 °C (Techne DRI-Block type DB-3D). The residue was reconstituted in 100 μl mobile phase. Calibration curves were prepared in blank MLN tissue at concentrations of 0, 0.025, 0.05, 0.1, 0.5, 1, 5 and 10 μg/ml CBD. The final concentrations in tissue samples were calculated according to the weight of the tissue and expressed as μg/g. The lower limit of quantification (LLOQ) for CBD using this method is 10 ng/ml.

2.5. Chromatography conditions

A Waters Alliance 2695 separations module equipped with a Waters 996 photodiode array ultraviolet (UV) detector was used for sample analysis. Separation was achieved using an ACE C18-PFP 150 mm × 4.6 mm, 3 m particle size column (Hichrom Ltd., Reading, UK), protected by an ACE C18-PFP 3 m guard cartridge. The mobile phase was an isocratic mixture of acetonitrile and water in a ratio of 62:38 (v/v) at a flow rate of 1 ml/min. Samples were stored at 4 °C during analysis and the column was maintained at 55 °C. Injection volume was 40 μl and CBD was detected at a wavelength of 220 nm. Data collecting and processing was carried out by means of the EmpowerTM 2 software (Waters).

2.6. Data analysis

All data are presented as mean ± standard error of the mean (SEM). One way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test was used to assess significance of differences between individual lymph nodes at different time points. A p value < 0.05 was considered statistically significant. All figures and statistical tests were generated in GraphPad Prism (version 7). Potential outliers were excluded using a Grubb's test where alpha = 0.05 was deemed signifcant. Half-lives were calculated using Phoenix WinNonlin 6.3 software (Pharsight, Mountain View, CA, USA).

3. Results and discussion

3.1. Bio-distribution of CBD into individual MLN following oral administration in sesame oil vehicle

For this work, sesame oil was used as a vehicle as it was previously shown to be highly efficient for delivery of CBD to MLN [1]. The concentrations of CBD in individual MLN and RPLN at 2, 3, 5 and 8 h following oral administration are shown in Fig. 2. At 2 h post administration, concentrations of CBD were significantly higher in the lymph nodes positioned second from the apex of the chain (position 2), when compared to lymph nodes at the bottom of the MLN chain (position 4). Based on the positioning of lymphatic vessels that drain lymph from the gut tissues into the MLN, we hypothesise that the upper nodes in the chain drain lymph from the duodenum and upper jejunum whereas the lower nodes drain the ileum, caecum and partially the colon [6] (Fig. 1). Data from this work describing the distribution of a lipophilic drug are therefore in agreement with previous studies that suggested the majority of dietary lipid absorption occurs in the duodenum and jejunum [5]. The calculated half-lives at position 1, 2 and 3 were 1.75, 2.27 and 3.08 h respectively.

At 3 h post administration, concentrations in MLN at position 1, 2 and 3 appeared to be higher compared to position 4, however this was not statically significant. At 5 and 8 h post administration concentrations in MLN at all positions were similar. The average concentration of CBD across all MLN was 8.3 ± 1.8 μg/g, 7.8 ± 2.2 μg/g, 3.6 ± 0.4 μg/g and 2.2 ± 0.4 μg/g for 2, 3, 5 and 8 h post administration respectively. Concentrations of CBD across all MLN in this work are therefore comparable to concentrations previously reported in MLN following oral administration in sesame oil formulation at the same dose (12 mg/kg) [1].

Using the same data as presented in Fig. 2, the concentrations in each lymph node over time were also compared (Fig. 3). Semi-log plots of these data are also shown in Supplementary Figure 1. For all lymph nodes, concentrations of CBD were not statically different at 2, 3, 5 and 8 h following administration. Data from this work therefore suggest that exposure to CBD is widespread across lymph nodes positioned throughout the mesenteric chain. In addition, based on our understanding of lymph drainage, the fact that CBD is present at substantial levels in the lymph nodes positioned lower in the chain may also indicate that some absorption occurs in the lower GI tract. Confirmation of drug absorption from the lower GI tract would however require future work.

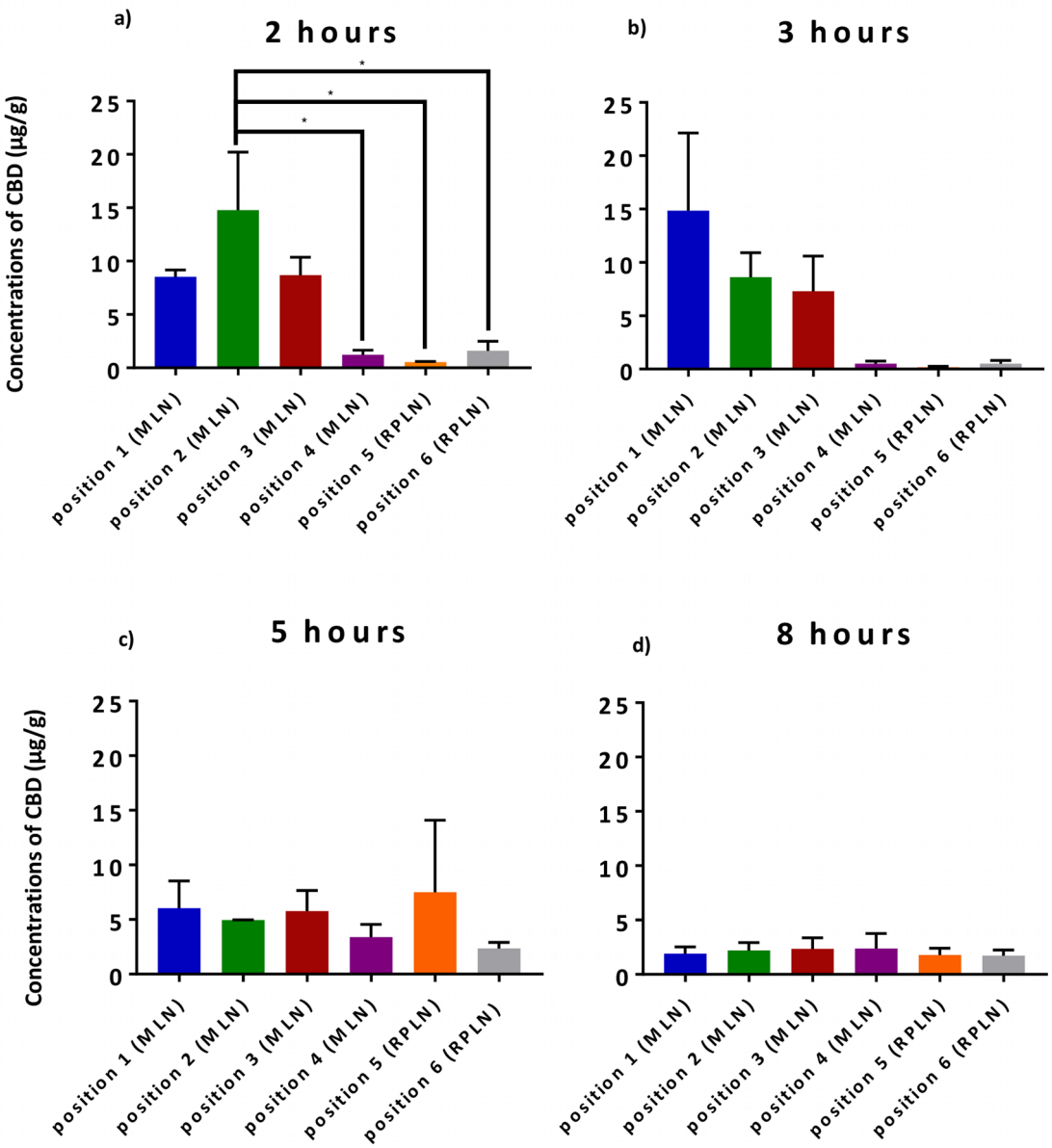

Fig. 2. Distribution of CBD into MLN and RPLN at various time points following oral administration in sesame oil. The bar colours in this figure correspond to the positions of lymph nodes collected as described in Fig. 1. All data are presented as mean ± SEM, n = 3. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons test. *P < 0.05. No outliers were identified (Grubb's test, alpha = 0.05).

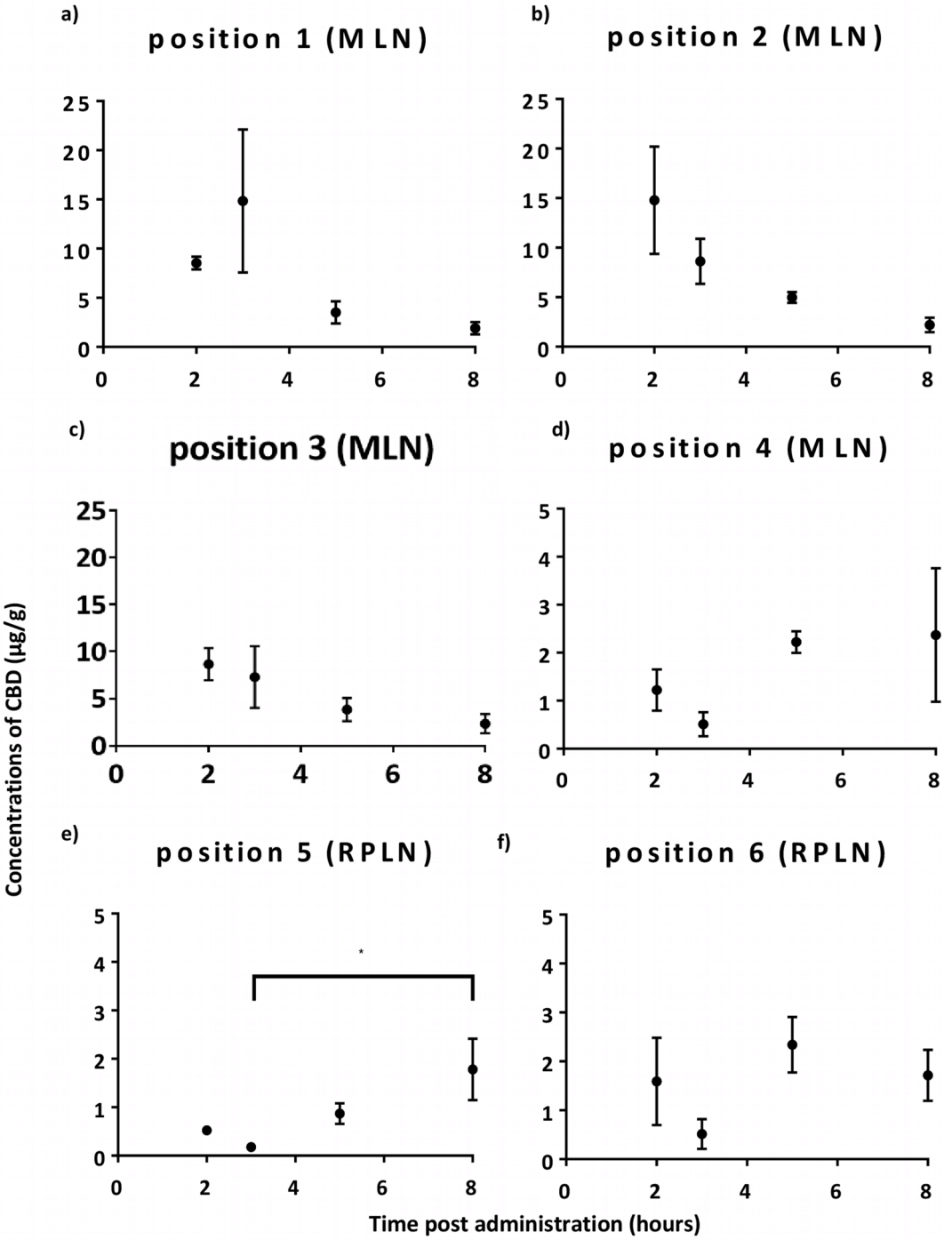

Fig. 3. Distribution of CBD at various time points post oral administration in sesame oil in MLN and RPLN at various positions (a-f). The data in this figure is the same as the data in Fig. 2, but presented differently. All data are presented as mean ± SEM, n = 3. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons test. *P < 0.05. No outliers were identified (Grubb's test, alpha = 0.05).

3.2. Bio-distribution of CBD into individual RPLN following oral administration in sesame oil vehicle

At 2 h post administration, MLN at position 2 contained significantly higher concentrations of CBD compared to both the iliac and *para*-aortic lymph nodes at position 5 and 6, respectively. Concentrations at position 5 and 6 were similar to those previously shown in plasma [1]. Relatively low concentrations in the RPLN 2 h post administration are consistent with previous reports which showed no uptake of dye into the iliac or caudal nodes following injection into the small intestine and colon shortly after administration [10].

At 3 h post administration, concentrations in RPLN had tendency to be lower than in MLN (but not significantly different). However, at both 5 and 8 h post administration, concentrations of CBD were comparable in the MLN and RPLN at all positions. In addition to widespread drug delivery to MLN at multiple positions, this data demonstrates that the RPLN can therefore also be targeted through oral administration in lipidic vehicles. Importantly, at 5 and 8 h post administration, concentrations of CBD in the RPLN were more than 20 times higher than concentrations previously reported in plasma at the same time points following oral administration of the same dose and formulation [1]. Based on this, the accumulation of CBD in RPLN is not likely to be a

result of redistribution from plasma following entry into systemic circulation. When comparing the differences in concentrations of CBD in the iliac / caudal nodes at position 6, at each time point, concentrations were not significantly different. However, at position 5 concentrations were significantly higher 8 h post administration compared to 3 h (Fig. 3). Increasing drug concentrations over time may also indicate a delayed uptake as a result of lymph being directed from the MLN into the RPLN.

It should be noted that the concentrations observed in the RPLN at 3, 5 and 8 h post administration were above the immunomodulatory threshold as previously determined [1]. CBD is likely therefore to exert pharmacological effects on the cells of RPLN, in addition to the MLN. It can also be hypothesised that through the mechanism of association with chylomicrons and lymphatic transport, other highly lipophilic compounds may be distributed in a similar way. This has relevance for metastatic cancers, in particular testicular and colon cancers, where RPLN are involved and avoidance of invasive lymph node dissection would be beneficial.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2022.03.014.

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