Passive and specific targeting of lymph nodes: influence of the administration route

Marion Pitorre, Guillaume Bastiat, Elodie Marie Dit Chatel, Jean-Pierre Benoit

Passive and specific targeting of lymph nodes: influence of the administration route

Marion Pitorre,[a,b] **Guillaume Bastiat,***[a,b] **Elodie Marie dit Chatel**[a,b] **and Jean-Pierre Benoit**[a,b]

[a] *LUNAM Université – Micro et Nanomédecines Biomimétiques, F-49933 Angers, France.*
[b] *Inserm – U1066 IBS-CHU, 4 rue Larrey, F-49933 Angers Cédex 9, France.*
Fax: +33 2 44 68 85 46; Tel: +33 2 44 68 85 31;
E-mail: marion.pitorre@etud.univ-angers.fr ; guillaume.bastiat@univ-angers.fr

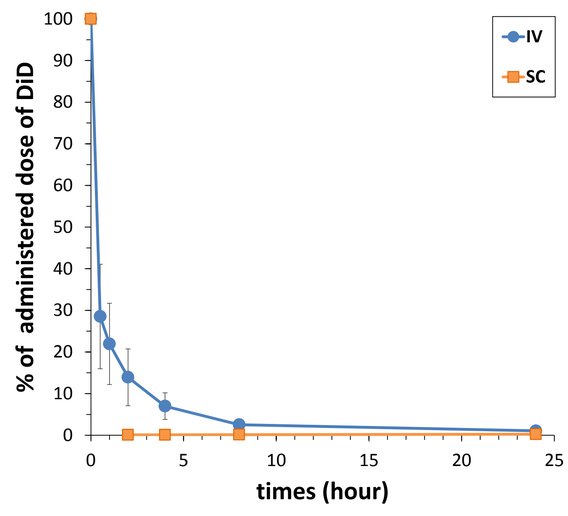
Introduction

Patients with cancer, diagnosed in advanced stage with the presence of metastases in lymph nodes, present a dismal prognosis. Therefore, the targeting of lymph nodes and/or metastases need to be improved in the design of future chemotherapy. Using a lymphogenous metastatic model, the tumor cells (human Ma44-3 cells) implanted in lung were spontaneously disseminated to mediastinum. Using lauroyl modified gemcitabine-loaded lipid nanocapsules (LNCs) to treat the tumor, an accumulation of nanocarriers in mediastinum and associated lymph nodes was observed, after subcutaneous (*SC*) and/or intravenous (*IV*) administration.[1] In this study, a correlation between *SC* sites for LNC administration and specific lymph node accumulation was achieved.

Materials & methods

LNCs were prepared as previously reported with a size of 30 nm (Z-Average) and with the encapsulation of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine 4-chlorobenzenesulfonate (DiD) for fluorescence labeling.[1,2] LNC suspensions were *IV* (tail vein) and *SC* (behind the neck, left and right flanks, above the tail) administered to Sprague Dawley rats (9-week females). Blood samples and specific organs (liver, spleen, left and right cervical, axillary, inguinal lymph nodes) were recovered *vs.* time (until 28 days) to establish pharmacokinetic and biodistribution profiles using fluorescence techniques (spectroscopy and imaging). Fluorescence–labeling of the nanocarriers was performed with the encapsulation of DiD inside LNCs to follow the *in vivo* LNC future. This labeling presents a great stability.[3]

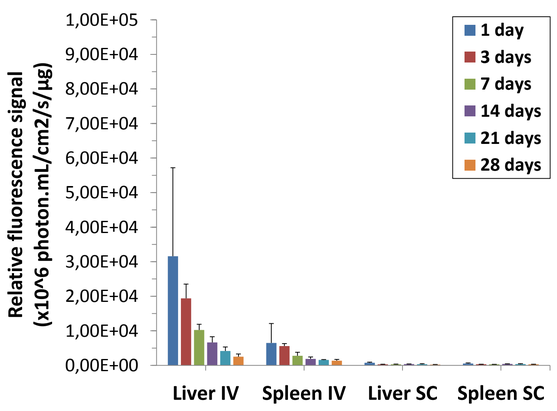
Pharmacokinetics



After *SC* administrations, no LNC was observed in systemic circulation whatever the injection site while a typical pharmacokinetic profile was obtained after *IV* administration.

fig. 1 Pharmacokinetic profiles (% of administered dose of DiD *vs*. time) of DiD-LNC in healthy rats, after (●) intravenous (IV) injection (tail vein) and (■) subcutaneous (SC) injection (on the left flank). (n=4, mean ± SD).

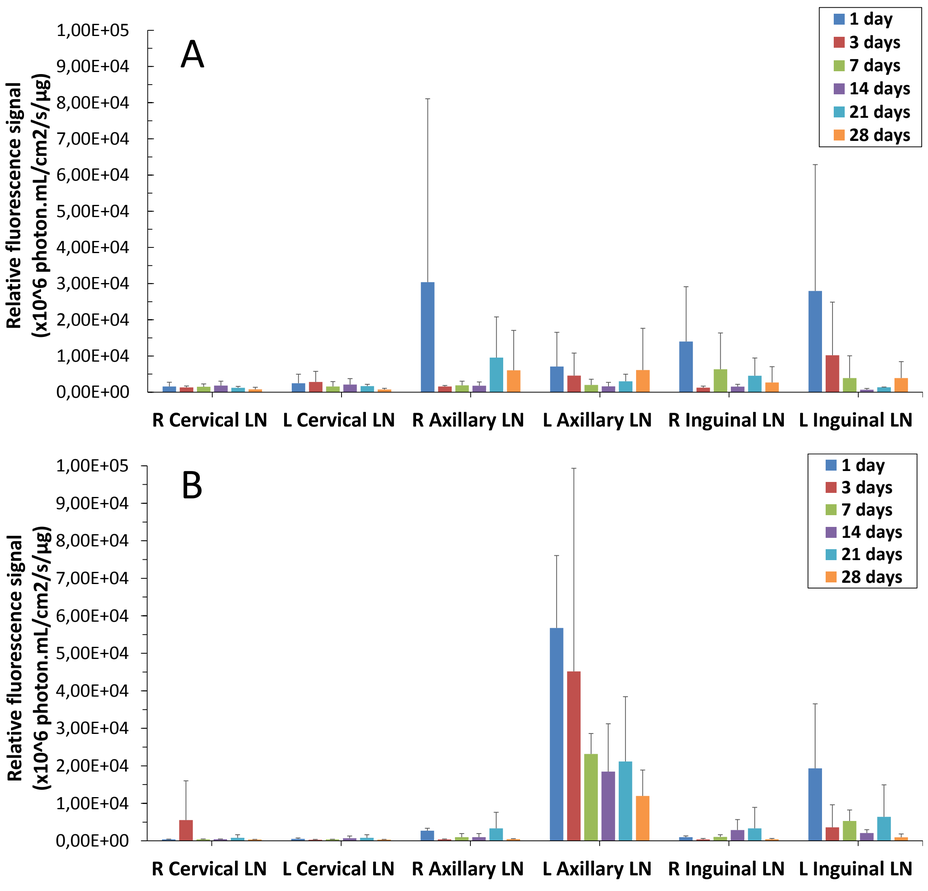
In vivo biodistribution in liver and spleen



LNCs accumulated in liver and spleen after *IV* administration while no LNC was observed in these organs after *SC* administrations. These results confirmed the absence of LNC in systemic circulation after *SC* injection, observed in the pharmacokinetics part.

fig. 2 *In vivo* biodistribution of DiD-LNC suspension in healthy rats, after intravenous injection (tail vein) and subcutaneous injection (on the left flank). Semi-quantification of the fluorescence signals (relative to DiD injected dose) of the removed liver and spleen using fluorescence images (10 ms integration time) at various times *post* LNC administrations (1, 3, 7, 14, 21 and 28 days) (n=4, mean ± SD).

In vivo biodistribution in lymph nodes



The *IV* administered LNCs are spread in all lymph nodes.
The SC administration in left flank showed a specific distribution of LNCs only in the left axillary and the inguinal lymph nodes.

Fig. 3 *In vivo* biodistribution of DiD-LNC suspension in healthy rats, after intravenous injection (tail vein) (A) and subcutaneous injection (on the left flank) (B). Semi-quantification of the fluorescence signals (relative to DiD injected dose of the removed left (L) and right (R) cervical, axillary and inguinal lymph nodes (LN) using fluorescence images (10 ms integration time) at various times *post* LNC administrations (1, 3, 7, 14, 21 and 28 days) (n=4, mean ± SD).

In vivo biodistribution overview

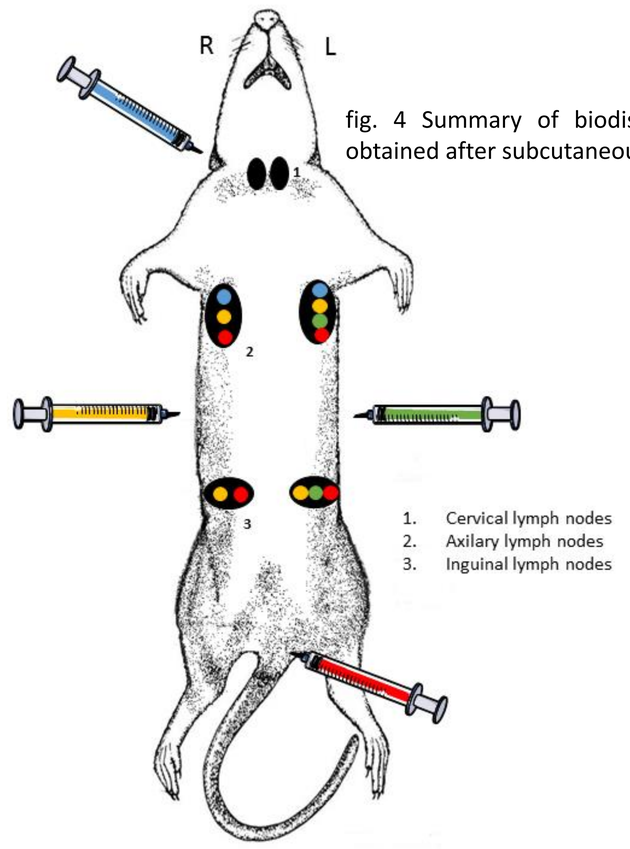


fig. 4 Summary of biodistribution results obtained after subcutaneous injections

After IV administration, a passive targeting was observed in all the studied lymph nodes (left and right cervical, axillary, inguinal ones).
On the other hand, after *SC* administration, a specific targeting of lymph nodes was observed, depending on the injection site.
Whatever the *SC* injection site, no LNC accumulated in the cervical lymph nodes (left and right).
After *SC* administration above the tail, LNCs accumulated in all the other lymph nodes (left and right) while after *SC* administration behind the neck, only the axillary (left and right) lymph nodes (and not the inguinal ones) were targeted.
After *SC* administration in the left flank, only the left lymph nodes (axillary and inguinal ones) were targeted while after *SC* administration in the right flank, LNCs accumulated in the right lymph nodes but also the left ones.

Conclusion

Passive but specific targeting of lymph nodes was observed, depending on the administration site. With an appropriate *SC* administration, LNCs can accumulate in specific lymph nodes, while *IV* administration led to an accumulation of LNCs in overall lymph nodes. A personalized therapeutic scheme for patients could be envisaged when specific lymph node targeting is needed.

References

1, N. Wauthoz, G. Bastiat and E. Moysan, *submitted to J. Control. Release*.
2, E. Moysan, Y. González-Fernández, N. Lautram, *Soft Matter*, 2014, **10(11)**, 1767.
3, G. Bastiat, C.O. Pritz and F. Fouchet, *J. Control. Release*, 2013, **170(3)**, 334.

Acknowledgment

This work was financially supported by EuroNanoMed2 ERA-NET within the research program NICHE.