Radiosensitizing Magnetic Nanoparticles as a Targeted Theragnostic Agent for Cancer Therapy

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Introduction

Since the early 2000s, high-Z nanoparticles (NPs) have been studied as radiosensitizing agents. Recent research indicates that certain metabolic processes are crucial to the observed radiosensitizing effect (1). Specifically, the magnitude of the radiosensitizing effect has been correlated to the residual activity of a detoxifying enzyme (thioredoxin reductase (TrxR)) in NP-treated cells. Regarding these considerations, our laboratory has previously shown that similar enzymatic behaviour is observed in cells exposed to iron oxide nanoparticles (IONPs), which appear as a promising theragnostic nanoplatform due to their biocompatibility and magnetic properties (2). To further improve our previously described nanoplatform, we evaluated *in vitro* the biological impact of a vectorisation strategy using the H1299.3 peptide (3).

Methods

IONPs synthesis

Magnetic core were produced by co-precipitation in organic medium and subsequently grafted with PEG (5 kDa).
Further functionalisations have been conducted by clip photochemistry and EDC.HCl coupling reactions

Radiosensitizing potential measurement

In vitro tests have been conducted on A549 cell line exposed or not to IONPs at 50 µg Fe/ml for a defined duration.
Residual TrxR activity has been assessed via a commercial kit whereas radiosensitizing effect of IONPs was determined by amplification factor at 2 Gray (AF_{2Gy}) calculations

Confocal Microscopy

IONPs were visualized in confocal microscopy using fluorescent anti-PEG antibody.

Biodistribution studies

Biodistribution was evaluated in a tumour bearing mouse model after intravenous injection (200 µmoles of IONPs/ kg of body mass).

Results

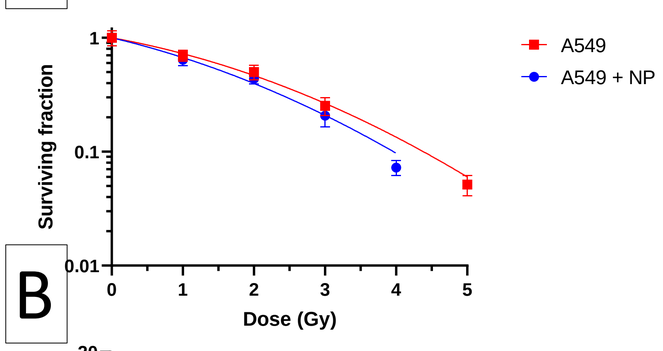
X-ray irradiation

Radiosensitizing properties were demonstrated by :

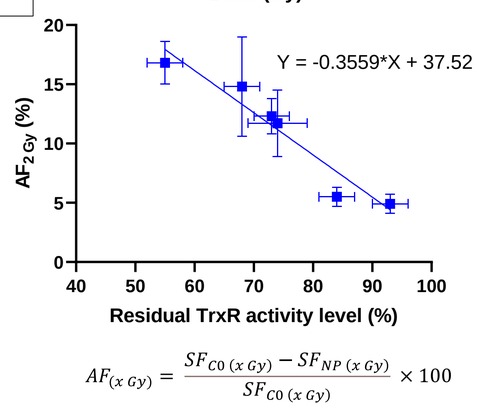
- ✓ The decrease of the survival fraction curve in the presence of IONPs (Fig. A)
- ✓ AF_{2Gy} superior to 0 % for every experimental condition

We also evidenced a strong correlation between residual TrxR activity and AF_{2Gy} (Fig. B)

A



B



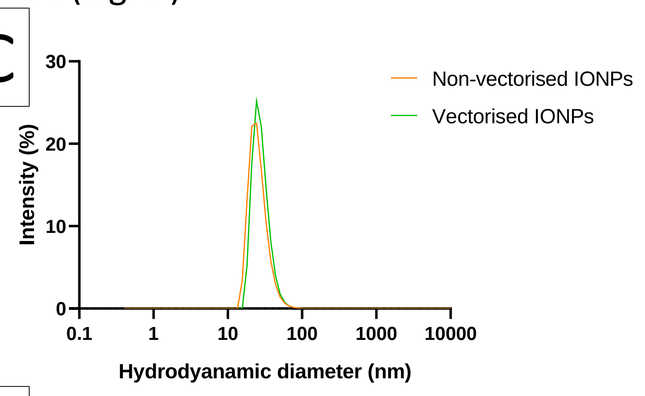
$$AF_{(x\,Gy)} = \frac{SF_{C0\,(x\,Gy)} - SF_{NP\,(x\,Gy)}}{SF_{C0\,(x\,Gy)}} \times 100$$

Internalisation assay

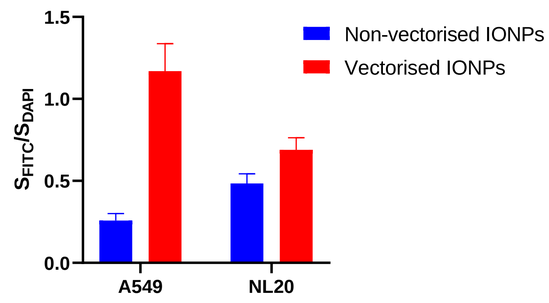
Functionalisations of the PEG coating have been conducted with clip photochemistry and subsequent peptide grafting through peptide bond formation.

- ✓ No destabilisation of the IONPs visible in dynamic light scattering (Fig. C)
- ✓ Internalisation assessed by confocal microscopy after 24h of exposition to the IONPs (Fig. D)

C



D

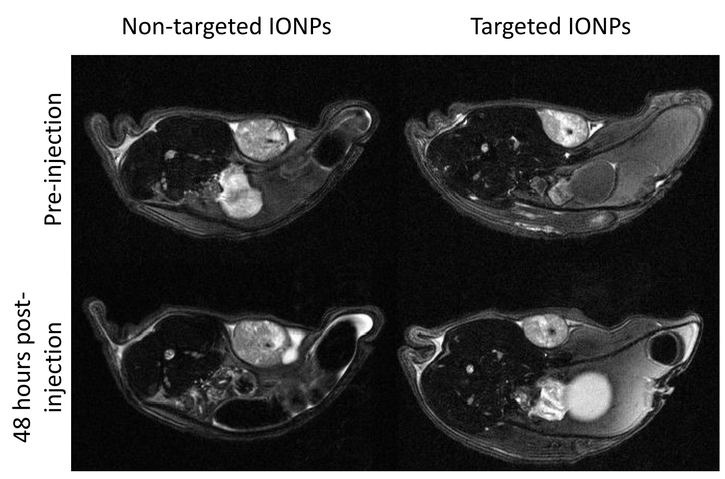


Biodistribution study

The monitoring of signal on T_2 Rapid Acquisition Rapid Echo (RARE) following intravenous injection of IONPs evidenced (Fig. E):

- ✓ Induction of a slight contrast in the tumour (white arrow).
- ✓ IONPs do not accumulate specifically in the tumour, as darkening can also be seen in other organs (mainly the liver).
- ✓ No difference in terms of internalisation between vectorised and non-vectorised particles can be determined on the analysis of MRI scans.

E



Discussion and conclusion

This study aimed to demonstrate the possible use of IONPs as a theragnostic agent. First, their radiosensitizing potential has been evidenced *in vitro* through calculations of the amplification factor at 2 Gy. We developed a stable and peptide-vectorised formulation of IONPs whose internalisation levels were studied by confocal microscopy. This grafting process appears to increase the internalisation of IONPs in cancer cells to a greater extent than in healthy cells. Besides, the potential as contrast agent of PEGylated iron oxide nanoparticles was evaluated by recording the evolution of MRI signal intensity within the tumour following intravenous injection. *In vivo* magnetic resonance imaging experiments demonstrated an accumulation of IONPs in the tumour zone, potentially by EPR effect. However, we were not able to detect a significant difference between the two formulations of IONPs in terms of biodistribution.

Although it will be necessary to assess in future studies whether the radiosensitizing potential and vectorisation capacity of the peptide observed *in vitro* are reflected *in vivo*, the current results make IONPs good candidates as theragnostic agents.

References

[1] S. Penninckx, A.-C. Heuskin, C. Michiels, S. Lucas, Cancers. 12 (2020).
[2] I. Ternad et al., Nanomaterials. 13, 201 (2023).
[3] B. J. Umlauf, J. S. Mercedes, C.-Y. Chung, K. C. Brown, Bioconjug. Chem. 25, 1829–1837 (2014).