Iron oxide nanoparticles for theranostics applications

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Introduction

In the field of radiation therapy, high-Z nanoparticles (NPs) have been studied for their ability to increase tumor cell death upon irradiation. The mechanism(s) responsible for the radiosensitization effect remains poorly understood and mainly focused on the physical phenom. Recent studies suggest the role of some biochemical mechanisms on the observed radiosensitizing effect. A correlation has been made between the inhibition of the detoxification enzyme in GNP-treated cells and the magnitude of the radiosensitizing effect. Given these elements, we were interested to study if such inhibition behavior could be demonstrated for other kinds of NPs. Because of their biocompatibility and superparamagnetic properties, iron oxide nanoparticles (IONPs) were selected.

Methods

Preliminary in vitro test

- Cell viability was determined by MTT assay. A549 cells treated with an increasing concentration of IONPs
- Iron content in A549 was determined by Perl's Prussian Blue colorimetric assay at 6h; 24h; 48h for A549 cell line.

Evaluation of inhibited enzyme

The activity of the detoxification enzyme for A459 cell line incubated with and without 50 µg of Fe/mL of IONPs for 24h was evaluated.

Effects of IONPs on irradiation

X-rays irradiation was administrated on cells pre-incubated with and without IONPs Radiosensitizing effect of IONPs, was determined by :

- Amplification factor (AF)
- α/β (parameter extract to the fitted curve)

Relaxometric properties

- Preliminary relaxometric properties determined by MR phantom image
- Biodistribution of IONPs was evaluated in a mouse model by intravenous (IV).

Results

In vitro test

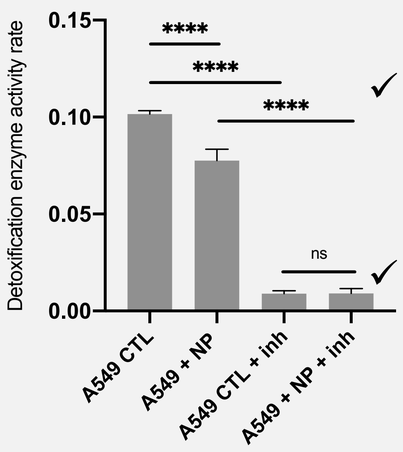
✓ No major cytotoxicity was observed at 10 to 200 µg of Fe/mL of 7 nm IONPs PEG_{5000} (MTT test)

✓ Cellular iron content quantification (Table 1)

Incubation time (hours)	Internalization (pg of Fe/cell)
6h	0.8 ± 0,1
24h	1.6 ± 0,4
48h	0.25 ± 0,03

Table 1. Cellular iron content quantified by Perl's Prussian blue stain method.

Evaluation of the activity of the detoxification enzyme for cells incubated with IONPs:



✓ Inhibition of 26% of detoxification enzyme in the presence of IONPs

✓ Significant decrease

Figure 1. Detoxification enzyme activity rate

X-ray irradiation

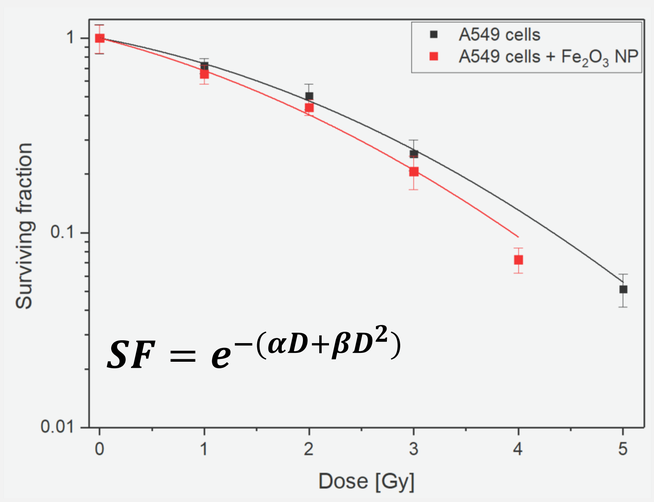


Figure 2. Survival fraction determined by clonogenic assay

Radiosensitizing properties were demonstrated by :

1. The decrease of the survival fraction curve in the presence of IONPs (**Fig. 2**)
2. AF_{2Gy} of 12% highlight the increase in cell death at a given dose (2 Gy) in the presence of IONPs
3. α/β from 3.2 to 4.6 Gy respectively without and whit IONPs.

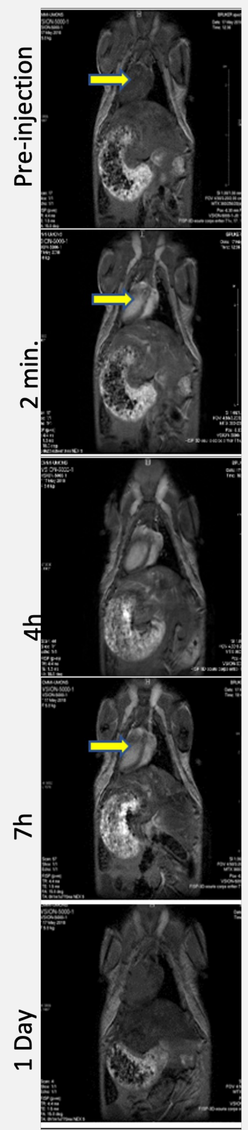
This increase in the ratio reflects a predominance of the impact of "not repairable" damage to the cells after X-ray irradiation.

Biodistribution

Relaxometric properties determined by :

✓ Relaxometric parameter (r_1; r_2)

✓ MR phantom image



MRI probe evidenced by : The monitoring of signal of the cardiac left ventricle.

➔ Persistent signal after 7h highlighting the long-time circulation.

Elimination pathways evaluation by T_2-weighted images :

1. Urinary system: strong darkening observable more than 300 min post-injection.
2. Liver: T_2 decrease until reaching is minimum at 1 day post-injection, recovery signal obtained 12 weeks later.

Discussion

Iron oxide nanoparticles are well known in MRI applications but less in irradiation fields. This study aims to demonstrate the possible use of IONPs as a theranostic agent. First, the evaluation of the activity of the detoxification enzyme of cells incubated with IONPs was determined and an inhibition 26% was shown after 24h of preincubation with IONPs. To attest that such inhibition can give rise to a radiosensitizing effect, A549 cells were irradiated with X-ray. IONPs showed a radiosensitizing effect at 2 Gy to induce a 12% increase in cell death in IONPs treated cells compared to untreated cells. Superparamagnetic properties of PEGylated iron oxide nanoparticles were evaluated by recording relaxometric parameters and phantom MR images. *In vivo* Magnetic resonance imaging (MRI) experiments demonstrated circulation times exceeding 7 hours by observing the signal of the cardiac left ventricle.

Conclusion

Even though these nanoparticles are not defined as high-Z nano-objects, radiosensitizing properties were demonstrated by the decrease in the survival fraction and an AF_{2Gy} of 12%. Radiosensitisation seems to be related to the inhibition of detoxification enzyme in presence of IONPs. The evaluation of the biodistribution was then studied in mouse model. These elements make them good candidates as theranostic agents.

References

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