Nanoparticle radiosensitization experiments at IFIN-HH, Romania

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Abstract

• Translational cancer radiotherapy aims to deliver a high dose at the tumor site, to inhibit the development of cancer cells, while, in the meantime, trying to protect the healthy cells. In order to reach this goal and to minimize the adverse systemic effects, targeted nanoparticle therapies have been proposed as a solution.

• We have employed different types of radiation therapy in combination with iron oxide nanoparticles (IONP) to be used as radiosensitizers.

• Due to the secondary reactive species production following the interaction with radiation, intermediate Z IONP determined the alteration of the tumor cells metabolic function, DNA damage, protein expression, division or even induction of cell death.

• We have proved that the sensitizing response is influenced by the nanoparticles targeting ability and intracellular localization, as well as radiation type and properties, like energy, dose and flow.

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Introduction

• **Nanotechnology** → potential improvement of cancer treatment efficiency ↔ ability to target the tumor area;

• Cancer therapy using **radiation** → high dose at the tumor site → inhibit the growth of tumor cells → protect the surrounding healthy cells;

• **Radiosensitizers based on high Z elements** → absorb incident radiation → deliver secondary radiation → enhances the production of reactive oxygen species, interaction and intercalation into the DNA.

• The **biological effects** of nanoparticles radiosensitization imply the alteration of cellular signaling pathways involved in:
  • oxidative stress;
  • cell cycle disruption;
  • DNA repair inhibition;

• **Shortcomings** to clinical translation
  • non-specific targeting;
  • inability of internalization and interaction with the cell compartments;
  • inability of nanoparticles to be intracellularly retained.
Treatment with radiation followed by NPs

Cell survival of MG-63 previously exposed to 0 Gy, 1 Gy X-Rays, 500 µg/mL, 100 µg/mL Fe₃O₄@DOX or combined treatment, during 48h; data is shown as mean ±SEM; *P<0,05 **P<0,01 and ***P<0,001.

Quantity (pg) of internalized Fe₃O₄ in MG-63 osteosarcoma cells exposed to different concentrations of Fe₃O₄@DOX during 24, respectively 48h; a group of cells as previously exposed to 1 Gy; data is shown as mean ±SEM; *P<0,05 **P<0,01 and ***P<0,001.

Cell cycle distribution for MG-63 cells exposed to radiation treatment (0, 1 Gy) and NPs (0, 500 µg/mL Fe₃O₄@DOX) at different time intervals; *P<0,05.

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Treatment with radiation followed by NPs

- The potential cytotoxic effects of radiation treatment followed by exposure to Fe₃O₄@DOX in MG-63 human osteosarcoma cells. MG-63 cells at confluence were irradiated with 1 Gy (150 KeV), allowed to attach during 4h and then exposed to different concentrations of Fe₃O₄@DOX in the range of 0-500 µg/mL.

- Combined treatment $\Rightarrow$ increase of the cytotoxic effects compared to NP alone.
- Temporary cell cycle arrest in G₂/M $\Leftarrow$ cells previously exposed to 1Gy X-Rays $\Leftarrow$ enhanced internalization of Fe₃O₄@DOX.
- 1Gy + 500 µg/mL NP $\Rightarrow$ early G₂/M at 12h from irradiation, maximum at 24h after treatment.

[Int. J. Molec. Sci., 2020, 21(19), 7220;]
Treatment with NPs followed by radiation

Clonogenic survival rate of HeLa cells exposed to 100 µg/mL \( \text{Fe}_3\text{O}_4@\text{PEG} \) 6K during 16h and followed by 50kV X-Ray exposure. Data is shown as mean ± STDEV (n=3);

Clonogenic survival rate of HeLa cells exposed to 0, 100, respectively 200 µg/mL \( \text{Fe}_3\text{O}_4@\text{PEG} \) 6K/DOX during 16h and followed by 150 kV X-Ray at different doses; data is shown as mean ± STDEV (n=3);

Clonogenic survival rate of HeLa cells exposed to 100 µg/mL \( \text{Fe}_3\text{O}_4@\text{PEG} \) 6K during 16h and followed by 6MV X-Ray exposure. Data is shown as mean ± STDEV (n=3);


dose (Gy)

Acknowledgement: Prof. M. R. Veldwijk, Univ. Heidelberg

[Sci. Rep., 2020, 10, 10530]
Treatment with NPs followed by radiation

Internalization of Fe₃O₄@PEG 6K/DOX in SW1353 cells incubated with 200 µg/mL Fe₃O₄@PEG 6K/DOX during 16h, at 24h from NP removal;

Cell survival of SW1353 cells exposed to 200 µg/mL Fe₃O₄@PEG 6K/DOX during 16h and followed by 155 MeV proton exposure. Data is shown as mean ±STDEV (n=3); *P<0.05 **P<0.01 and ***P<0.001

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In order to evaluate the radiosensitization potential of Fe$_3$O$_4$@PEG 6K(\text{/DOX}) nanoparticles, human cervical adenocarcinoma HeLa and human chondrosarcoma SW1353 were incubated with NP during 16h and then irradiated. X-Rays with different energies were used: high energy (6MV), as well as medium (150 kV) and low energies (50 kV), respectively protons (155 MeV).

- 100 µg/mL DOX- free Fe$_3$O$_4$@PEG 6K + 6MV X-Ray → no statistically significant effect compared to radiation alone in HeLa.
- 100 µg/mL DOX- free Fe$_3$O$_4$@PEG 6K + 50 kV X-Ray → radiosensitizing effect DMF$_{SF\text{0,1}}$ = 1.13 ±0.05 in HeLa.
- 100 µg/mL Fe$_3$O$_4$@PEG 6K/DOX + 6 MV X-Ray → radiosensitizing effect DMF$_{SF\text{0,1}}$ = 1.3 ±0.1 in HeLa.
- 100 µg/mL Fe$_3$O$_4$@PEG 6K/DOX + 150 kV X-Ray → radiosensitizing effect DMF$_{SF\text{0,1}}$ = 1.29±0.04 in HeLa.
- 200 µg/mL Fe$_3$O$_4$@PEG 6K/DOX + 150 kV X-Ray → radiosensitizing effect DMF$_{SF\text{0,1}}$ = 1.55±0.12 in HeLa.
- 200 µg/mL Fe$_3$O$_4$@PEG 6K/DOX + 155 MeV protons → increased cytotoxicity in SW1353.

Acknowledgement: Prof. M.R. Veldwijk, Univ. Heidelberg Dr. A. Rzhanina; Dr. G. Mytsin, JINR Dubna [Sci. Rep., 2020, 10, 10530]
Conclusions

- The implication of magnetite nano-systems in the dual treatment of cancer involving radiotherapy and chemotherapy was evaluated through the monitorization of cytotoxic effects by applying the ionizing radiation treatment before or following the incubation of cells with nanoparticles.

- In the first case, was investigated the correlation between the increase of Fe₃O₄@DOX NP internalization following the irradiation of the cells with the cell cycle progression, as well as the characterization of the cytotoxicity. A higher significant percent of magnetite nanoparticles was measured in cells that were irradiated previous to NP exposure, phenomena associated with a more rapid entrance of the cells in the G₂/M phase of the cell cycle.

- The incubation of the tumor cells with Fe₃O₄@PEG 6K (/_DOX) nanoparticles followed by irradiation at different energies and doses proved a radio-sensitization effect dependent on the concentration of the nanoparticles, concentration of DOX, but also the energy and dose of ionizing radiation. The obtained results proved that DOX free Fe₃O₄@PEG 6K determined a decrease of cell survival in HeLa cells following the radiation with low energy X-Rays (50 kV), but not following radiation with high energy (6MV). Through DOX encapsulation, a radio-sensitizing effect was obtained on HeLa cells at both high (6MV) and medium (150 kV) energies. Irradiation with 155 MeV protons showed a significantly increased cytotoxicity against chondrosarcoma cells for dual treatment.

- Results proved the efficiency of functionalized magnetite nanoparticles in the controlled delivery of anti-tumor substances and the chemical and/or radiological sensitization of human tumor cells. These observations confirm the potential use of the resulted nano-systems as potential candidates in the chemo- and radio-therapy mediated by nanoparticles.