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Penetration of PLGA Nanoparticles into the Intracranial Rat C6Glioma: INFLUENCE OF SURFACTANT COATING

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Introduction

Our previous results have shown that PLGA nanoparticles (PLGA NPs) coated with poloxamer 188 (P188) enable the delivery of drugs across the blood–brain barrier (BBB) after intravenous injection. Doxorubicin loaded PLGA NPs (Dox-PLGA) coated with P188 produced a considerable anti-tumour effect against the intracranial glioblastoma in rats [1]. The objective of the present study was to evaluate the internalization of the P188-coated PLGA NP in the intracranial C6 glioma in rats.

Experimental Methods

For visualization using scanning laser confocal microscopy (SLCM) (Nikon A1 MP) and the intravital fluorescence imaging system Ivis®Spectrum CT (Perkin-Elmer) the NP were labeled with DiI (DiI-PLGA NP).

Preparation of drug-loaded PLGA nanoparticles.
The DiI-PLGA NP were prepared by an emulsion-solvent evaporation technique. The DiI:PLGA ratio was 1:750. The solution of PLGA and DiI in dichloromethane was added to 1% aqueous solution of PVA (9-10 kDa) and passed through a high-pressure homogenizer (Panda Plus2000) at 1000 bar. The organic solvent was evaporated under vacuum followed by addition of 2.5% mannitol and lyophilization. Free DiI was removed from the nanosuspension by gel filtration chromatography using a Sephadex G-25 column.

Nanoparticle characterization.
The average particle size and zeta-potential were measured using a Zetasizer Nano ZS (Malvern, GB) and were found to be 42,8 ± 2,2 nm and -11,4 ± 0,4 mV respectively.

Nanoparticle administration.
The freeze-dried NP were resuspended either in P188 or in water for injections, incubated for 30 min and administered i.v. into rats with intracranial C6 glioma on day 15 after tumour inoculation. The presence of mass lesion was verified by previous MRI. Two hours after administration of the NP, the rats were perfused transcardially with 4% p-formaldehyde solution, organs were recovered, and the fluorescence intensity was assessed using an Ivis® Spectrum CT system.

Histological analysis.
Brains were removed and fixed with 4% paraform solution for at least 24h, afterwards 50 micron-thick sections were prepared using vibrotome. To assess NP localization in brain sections immunohistochemical staining with antibodies against GFAP (astroglial marker), beta-III Tubulin (neuronal marker), was performed. Goat anti-mouse Alexa Fluor 633 and Goat anti-rabbit Alexa Fluor 488 (Invitrogen) were used as the second antibodies.

References

[1] Gelperina S et al. Drug delivery to the brain using surfactant-coatedpoly (lactide-co-glycolide) nanoparticles: influence of the formulation parameters. Eur J Pharm Biopharm. 2010; 74(2): 157-163.

Acknowledgements

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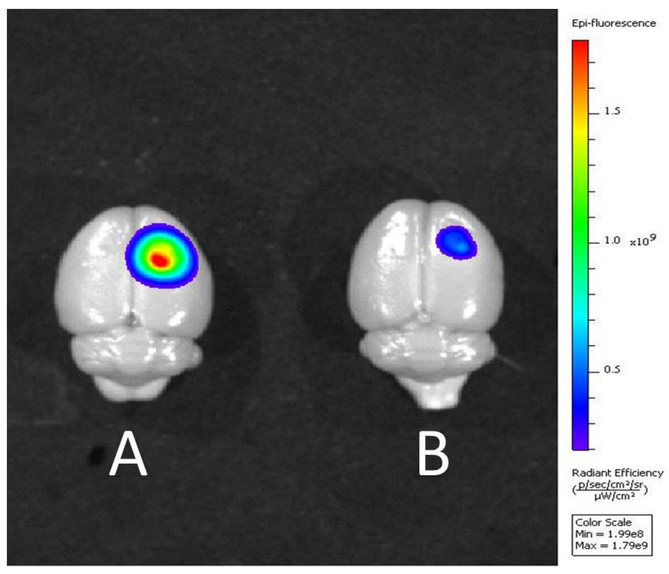
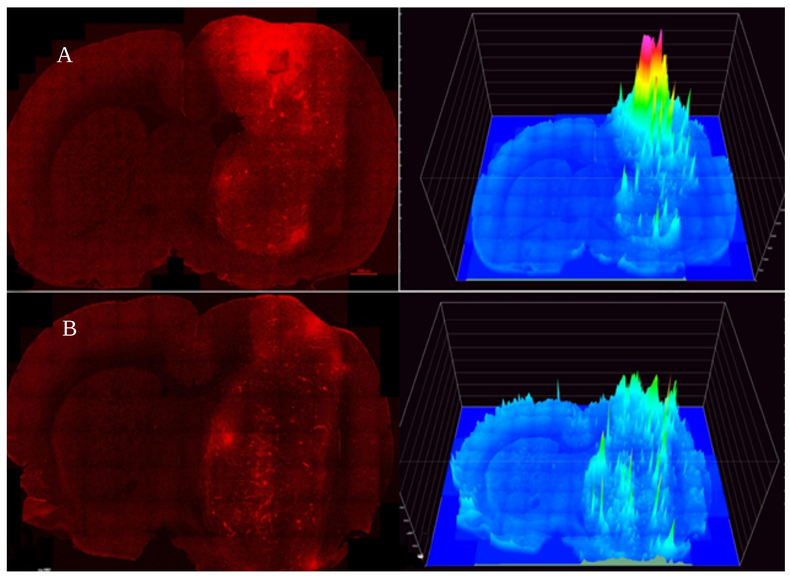
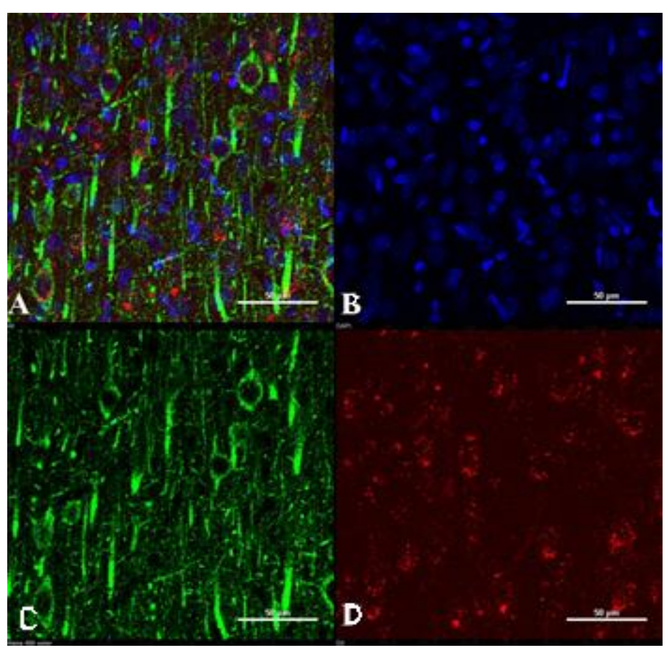
Results



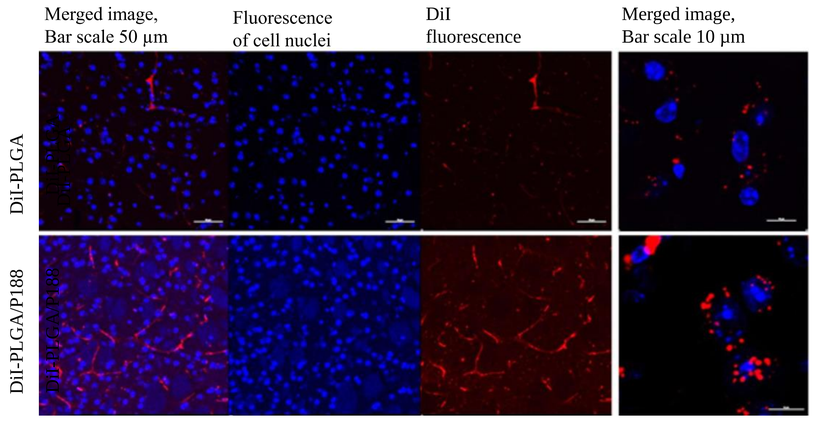
Brain fluorescence (Ivis® Spectrum CT) in rats with glioma C6 after transcardial perfusion.
A. DiI-PLGA/P188 NP; B. Uncoated DiI-PLGA NP.



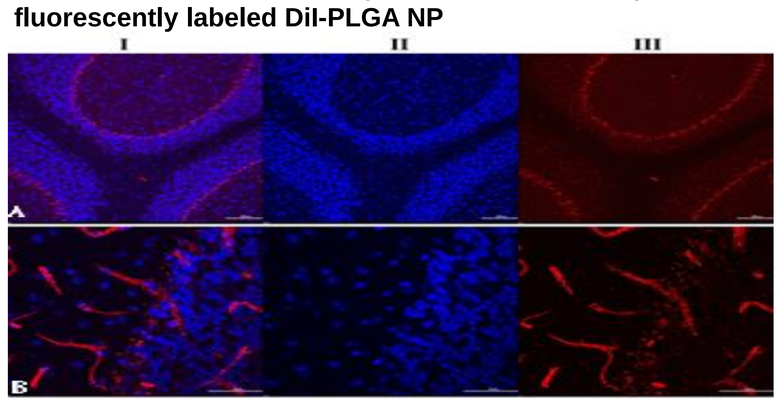
Quantitative fluorescence analysis (SLCM) on rat brain sections with C6 glioma 2 h after i.v. administration of DiI-PLGA NPs. A-B. Panoramic images - DiI-PLGA/P188 NP (A) and uncoated NP (B). Bar=1000 µm. C-D. 3-D fluorescence intensity histograms of the same sections.



Accumulation of DiI-PLGA/P188 NP in some populations of neurons of contralateral hemispere. A. Merged image. B. Fluorescence of cell nuclei (Hoechst staining). C.Beta-III-tubulin positive neurons of the cerebral cortex. D. DiI fluorescence . Bar scale 50 µm. SLCM.



Rat brain sections 2 h post intravenous injection of fluorescently labeled DiI-PLGA NP



Accumulation of DiI-PLGA/P188 NP in Purkinje cells, 2 hours after i.v. administration. A. Overview of the cerebellar cortex. Bar scale 100 µm. B. Magnified fragment of cerebellar cortex, bar scale – 50 µm. I Merged image. II.Fluorescence of cell nuclei (Hoechst staining). III.DiI fluorescence. SLCM.

The fluorescence intensity of the hemisphere with the implanted glioma was 4-fold higher for the P188-coated NP (DiI-PLGA/P188 NP), as compared to the uncoated NP (45.1×10^6 vs 9.5×10^6 photons/sec/cm^2, respectively according to intravital fluorescence imaging data.
The quantitative fluorescence analysis of the tumor sections using SLCM showed a significantly higher accumulation of the DiI-PLGA/P188 NP, as compared to the uncoated DiI-PLGA NP. Mean fluorescence intensity values in the tumor were 1698.9±536.6 and 558.9±181.0 CU for the P188-coated and uncoated NP, respectively. The intensity values in the contralateral hemispheres for the same preparations were 293.4 ± 32.3 and 203.2 ± 22.9 CU, respectively. Thus, according to the SLCM data, the penetration of the DiI-PLGA/P188 NP into the tumor was 3 times more effective than that of the uncoated NP. The analysis of the magnified fluorescence images showed considerable accumulation of the DiI-PLGA/P188 NP both in the tumor interstitial fluid and inside the C6 glioma cells. At the same time, DiI-PLGA-NPs were mainly localized in epithelial cells of cerebral microvessels of the contralateral hemisphere. Relatively intense DiI fluorescence was also observed in Purkinje cells of cerebellar cortex.

Conclusion

Together with the data obtained previously, the results of the present study demonstrate that coating of the PLGA NP with poloxamer 188 considerably enhances NP delivery to the brain tumor.