

NANOCAPSULES OF METHOTREXATE CROSS BOTH THE BLOOD BRAIN BARRIER OF INTACT C57BL/6 MICE, IN VIVO, AND TUMOR CELL MEMBRANE, IN VITRO.

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Glioblastoma multiforme (GM) is a severe disease and the therapeutic ineffectiveness of available treatments is enormous, especially by causing adverse reactions. Methotrexate (MTX) is a cytotoxic drug used to treat GM, however, its use is limited by poor bioavailability and adverse effects. Nanotechnology has been employed to enhance efficacy of drugs to treat cancer, and therefore, our hypothesis is that nanocapsules (LNC) may enhance the therapeutic efficacy of MTX and decrease the toxic effects, as they are driven to the tumor site.

The initial assays were carried out to investigate the ability of MTX LNC to cross the BBB after intravenous (i.v.) injection, without affecting the BBB permeability. Therefore, female C57Bl/6 mice were injected with rhodamine-labeled MTX LNC (n = 4) and its distribution in the brain was quantified by intravital microscopy at different interval times. In addition, the BBB integrity was evaluated by Evans blue extravasation in brain tissue 24 hours following treatments (MTX LNC and MTX in solution, and respective vehicle solutions, n = 4). Subsequent assays were performed to investigate the mechanism of uptake of rhodamine-labeled MTX LNC in tumor culture cells (GL261) using the following blockers of endocytosis: cytochalasin B (10 μ M) and sucrose (0.5 M).

MTX LNC was mostly observed inside the blood vessels 10 minutes after i.v. injection (0.6477 ± 0.01865 , $P < 0.05$) when compared to the time of 30 (0.9729 ± 0.1516), 60 (0.9060 ± 0.1161), or 120 minutes (0.9540 ± 0.01666). However, great dispersion of MTX LNC into the cerebral parenchyma tissue was noticed after 30 minutes following i.v. injection. In addition, no treatment changed significantly the BBB integrity. In vitro studies, demonstrated that rhodamine-labeled MTX LNC cross tumor cell membrane and cytochalasin B or sucrose do not reduce that entrance.

For now, we can conclude that methotrexate LNC cross both the BBB without disrupting its permeability and cross the tumor cell membrane, being a promising treatment for brain cancer.

Fapesp, CNPq.