INDOMETHACIN/INDOMETHACIN ESTER NANOCAPSULES, BUT NOT INDOMETHACIN IN SOLUTION, REDUCE BRAIN TUMOR OF MICE.

RODRIGUES S.F.[1]; FIEL L.[2]; SHIMADA A.L.[1]; LOIOLA R.A.[1]; PEREIRA N.[1]; GUTERRES S.S.[2]; POHLMANN A.[2]; FARSKY S.F.[1]

[1]Department of Clinical and Toxicological Analysis, FCF/USP, Sao Paulo, SP, Brazil. [2]Department of Organic Chemistry, Institute of Chemistry, UFRGS, Porto Alegre, RS, Brazil.

Once indomethacin/indomethacin ester nanocapsules (LNC) were demonstrated to reduce tumor cell proliferation, in vitro, we aimed to investigate the antitumoral effect of LNC in vivo and the mechanisms of action and of entrance of them into tumor cells. For that, ten days after tumor cell implant (in female C57Bl/6 mice), LNC, indomethacin in solution or vehicle control solution were intravenously given to mice on alternate days for 20 days and tumor volume was measured after brain excision. In another series of experiments, twenty eight days after the tumor cell implant, 2% Evans blue (EB) was intravenously injected into untreated mice. Twenty four hours later, EB extravasation was quantified using a spectrophotometer. Uptake of rhodamine-labeled LNC was visualized, *in vitro*, using a fluorescence microscope and pharmacological blockers (cytochalasin D, ouabain, and sucrose) were employed to study the mechanism of internalization. Finally, GL261 proliferation curve and apoptosis rate were measured in presence of LNC or indomethacin in solution. Tumor volume was dramatically reduced after LNC treatment (16±5%), but not significantly altered after indomethacin treatment in solution (37±12%), compared to control group (66±3% of hemisphere, n=3). EB amount was three times higher in the ipsilateral hemisphere than in the contralateral hemisphere (5.6±0.8 vs 1.5±0.9, n=3). LNC entered GL261 cells 30 minutes after incubation and none of the blockers reduced the LNC entrance (n=3). LCN reduced GL261 proliferation in a larger extension than indomethacin in solution (625±125 vs 5750±250, n=3) and did not increase apoptosis (n=3). This way, we demonstrated that LNC reach the tumor site probably as consequence of the BBB disruption, reduce the tumor volume and tumor cell proliferation much more than the compound in solution and enter into the tumor cells by a mechanism independent of phagocytosis or clathrin-mediated endocytosis.

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