Subcutaneous slow-release metformin formulation grants high tissue drug levels and induce antitumoral effects. An in vivo feasibility pilot study.

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Metformin (MET) is the first line treatment for type-2 diabetes, being nowadays administered to millions of patients. However, a potential, although still controversial, antiproliferative activity for this drug was also reported. Starting from epidemiological studies showing a decreased cancer incidence in individuals with type-2 diabetes taking MET when compared with patients receiving sulfonylureas, few clinical studies confirmed this observation, and more are ongoing. Mechanistically, preclinical studies identified multiple mechanisms affected by MET in cancer cells. According to the current vision, MET anti-tumor effect is based on a dual action, affecting systemic insulinemia and directly impairing cancer cell proliferation, with relevant higher efficacy towards cancer stem cells. A major problem encountered in translating these studies in a clinical setting is represented by the high concentrations (within the mM range) required to observe antiproliferative effects in tumor cells that are at least 10-fold higher than the plasma concentration attained with typical dosing in diabetics. Therefore, very large doses should be administered by oral route to obtain effective plasma levels, with consequent risk of adverse effects. Moreover, MET is highly hydrophilic and possess a quite unfavorable pharmacokinetic profile, having low and variable oral bioavailability (F=55±16%) due to an absorption mechanism based on easily saturable transporters, which does not guarantee a linear relationship between the administered dose and the plasma levels. Altogether, these observations suggest that a local delivery system could be useful to concentrate the drug at the tumor sites. Local administration may be achieved through systemic delivery of nanodispersed systems (liposomes, nanospheres), which, however, have the drawbacks of potential dispersion, low drug loading or sequestration by the reticuloendothelial system. On the other hand, a viscous system could localize and decrease the release rate of the drug close to the lesion, favouring the absorption by the neoplastic cells. Importantly tumor cells overexpress ionic transporters able internalize MET with high efficiency. Therefore, with the aim to potentiate the efficacy of MET in cancer treatment, here we describe the development of a series of sterile MET-loaded formulations based on poloxamers P407 and P124, injectable at room temperature and gellifying at body temperature, granting a sustained slow release of MET. In vivo testing of this new MET preparation by single subcutaneous administration in mice, showed sustained levels of the drug (measured by HPLC) in the plasma even after 2 days from the administration, while, as expected, administration of soluble preparation of MET resulted in a plasma level lower by 40% after 6 hours from the injection and below the detection limit after 24 hours. Antitumoral effects were shown in vivo in NOD-SCID mice xenotransplanted with human breast mammary carcinoma cells. MET gel (administered every other day for 3 weeks) to mice caused a reduction of the tumor growth by about 3 folds as compared with untreated controls or mice treated with empty gel. Histochemical analysis of the explanted tumors showed that MET-treated tumors showed lower proliferating cells and

neovascularization and larger necrotic areas than the controls. Biochemical analysis by Western blot, demonstrated that cells from MET-treated tumors showed increased activated caspase 3, and reduced phosphorylated ERK1/2. Finally, to demonstrate the specificity of the effects of metformin we measured by HPLC its content in plasma, liver and tumors of the treated mice, demonstrating drug concentration 6 times higher in the tumor than in plasma and 3 times higher than in liver.

In conclusion, we report for the first time the possibility to use a subcutaneous slow-release MET formulation that, administered every 48 hours, grants high tissue levels of drug able to induce antitumoral effects.