Project description

Title: *In vitro* and *in vivo* evaluation of the pharmacokinetic and pharmacodynamic profile of triterpenic compounds nanoformulations with antitumoral effect

Finding the treatment for cancer has been, is and will represent a real interest for the researchers due to the fact that it is an incompletely elucidated field, taking into consideration the complexity of the mechanisms involved in the development and the progression of this disorder and the lack of a curative treatment. The study of some natural compounds as alternative therapy for cancer brought a new breath in this direction. Betulin (Bet) and its oxidized form, betulinic acid (BA) are two natural compounds that present a variety of pharmacological effects, including: anti-inflammatory effects, anti-HIV, immunomodulatory, hepato-protective, antiangiogenic and, the most important, antitumoral activity [1].

BA and Bet are members of pentacyclic triterpenes family; they possess a lupan skeleton characterized by the presence of 4 cycles of 6 C atoms and 1 cycle of 5 C atoms, feature that is responsible for the strong lipophilic character of these compounds. A great interest was drawn by these two compounds starting with 1995, when Pisha *et al.* showed that BA was a potent anti-melanoma agent during a screening program of natural compounds developed by The National Institute of Cancer, USA [2]. Both compounds can be obtained from multiple natural sources, but it was discovered that the highest content of Bet was isolated from the bark of the birch tree, this compound representing an important source of BA by a simple chemical reaction, an oxidation process [1, 3].

Betulin (lup-20(29)-ene-3β,28-diol), also known as betulinol exerts antitumoral effects *in vitro* against a panel of tumor cells, but the cytotoxic effect of BA is much higher as compared to betulin [3, 4]. Mullauer *et al.* demonstrated that betulin induces apoptosis in a similar way with BA, via mitochondrial pathway, and the combination with cholesterol led to an increased antitumor effect [5]. In a recent study developed on patients with chronic hepatitis C, which received as treatment an extract of birch tree bark (content: betulin 75% and BA 3%) it was observed a decrease of the unpleasant symptoms associated to this disease and a reduction of hepatic enzymes values [6]. In another study it was showed that after administration of betulin to mice with seizures it was observed an anticonvulsant effect of this compound by binding to GABA receptors [7].

Betulin formulated as a nanoemulsion induced an antiangiogenic effect on embryonated egg and in addition, after topical application improved the physiological skin parameters in a mouse model of skin carcinoma [8].

Betulinic acid (3β, hydroxy-lup-20(29)-en-28-oic acid) was described as a potent antitumoral agent both *in vitro* in a broad panel of tumor cell lines (neuroblastoma, human and mouse melanoma, breast cancer, ovarian cancer, prostate cancer, hepatocellular carcinoma, lung and colon cancer, leukemia) [1, 9, 10], and *in vivo* acting as an inhibitor of tumor initiation at nude mice with induced human melanoma and at mice with skin cancer obtained by UV exposure [11]. BA treatment proved to be effective in a colon cancer mouse model obtained by inoculation of RKO cells, the mechanism of action being suppression of tumor development [12]. Moreover, BA inhibited the bone metastases associated to breast cancer induced by injection of metastatic breast cancer cells – MDA-MB-231 [13]. In a recent study developed on human melanoma cells –A375, it was shown that BA interferes with epithelial-to-mesenchymal transition, a key process in metastasis and tumor progression, leading to an anti-metastatic effect of this compound [14].

Albeit the number of studies that refer to BA and Bet pharmacological effects is quite impressive, these compounds are still in the preclinical phase of research due to their very low solubility in aqueous solutions, the feature that limits their administration *in vivo* (DMSO – dimethyl sulfoxide is toxic in high doses *in vivo*).

The subject of this project consists of the *in vitro* and *in vivo* evaluation of pharmacodynamic and pharmacokinetic profile of a novel nanoformulation of BA and Bet. The preparation of these compounds as nanoformulations was chosen in order to correct their solubility problem. There will be tested different types of nanoformulations and the nanoformulation chosen for the *in vivo* administration must meet several requirements such as: to be physico-chemically stable, to be biocompatible, to exert a potent antitumoral effect on tumor cells and a lack of toxicity on normal cells. The *in vitro* study will be conducted on normal (human keratinocytes – HaCat cells) and tumor cell lines (A375 – human melanoma, B164A5 – murine melanoma, A549 – lung carcinoma, Hep G2 – hepatocellular carcinoma, MCF-7 – non-invasive breast cancer and MDA-MB-231 – invasive breast cancer), and the *in vivo* part will be realized on embryonated eggs and healthy mice and mice with induced human melanoma and breast cancer. We chose the human melanoma and breast cancer animal models due to the fact that melanoma is ranked in top 3 of

most common types of cancer among males and breast cancer occupies the first place among females [15].

The number of the articles that describe the quantification of BA and Bet in biological liquids and organs after *in vivo* administration of these compounds at healthy mice and mice with induced carcinogenesis is very small [16, 17], what represents an element of novelty and originality of this project.

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