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Natural alkaloids noncovalently bound to C_{60} fullerene are potent anticancer phytotherapeutic agents

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Background. Conventional anticancer drugs have a number of known limitations but the development of new ones takes a long time, requires significant funding, and has a low success rate. Repurposing of pharmacologically active plant alkaloids Berberine (Ber) and Piperlongumine (Pl) and finding nanoplatforms for their delivery to cancer cells seems promising to be addressed. The ability of the C_{60} fullerene (C_{60}) carbon nanostructure to be functionalized with therapeutic agents makes it a potential drug carrier. The aim of this study was to prove a non-covalent functionalization of C_{60} with Ber or PL and to check the effectiveness of these nanocomplexes against Lewis lung carcinoma (LLC) both *in vitro* and *in vivo*. **Methods.** The stable C_{60}-Ber and C_{60}-Pl nanocomplexes were obtained and charachterized by UV-Vis spectroscopy, AFM and DLS measurements as described in [1, 2]. MTT test, migration and invasion assays, Western-blot analysis, qRT-PCR, a syngeneic mouse model for spontaneous metastasis, and histological analysis were used [1–3]. **Results.** It was shown that IC_{50} for Ber and PL in a free form for LLC cells were 43 and 15μM while after complexation with C_{60} decreased by 21 and 2 times respectively. Treatment with complexes was followed by a more significant inhibition of migratory and invasive potentials at a low 10 μM alkaloids concentration compared to the effect of alkaloids alone; apoptosis induction due to increased Bax/Bcl-XL ratio; EMT suppression through the Twist1 down-regulation and E-cadherin up-regulation; repression of CSCs-like features due to almost complete CD44 down-regulation. No therapeutic effect of Ber or Pl alone was found in a tumor-bearing animals after treatment at a dose of 7.5 mg/kg, while treatment with nanocomplexes at equivalent dose resulted in a 50% inhibition of tumor growth, substantial reduction of distant lung metastases number and induction of immunomodulatory favourable outcome due to immunosurveillance. **Conclusions.** The proposed nanocomplexes of alkaloids with C_{60} represent promising drugs for the treatment of metastatic lung cancer. **Funding.** The study was granted by the National Research Fund of Ukraine (project N 2020.02/0060).

Keywords: C_{60}, alkaloids, nanocomplexes, lung carcinogenesis, tumor growth, EMT, metastasis.

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