ABSTRACT

Charles University
Faculty of Pharmacy in Hradec Králové
Department of Biochemical Sciences

Candidate: Bc. Natálie Klusová
Supervisor: prof. PharmDr. Martin Beránek, Ph.D.
Consultant: RNDr. Milada Šírová, Ph.D.
Title of diploma thesis: Anti-tumor and immunomodulatory effect of polymer conjugates based on HPMA carrying gemcitabine

The diploma thesis is focused on the anti-tumor and immunomodulatory effect of polymer conjugates based on HPMA carrying gemcitabine (Gem). We investigated the effects of four polymer conjugates (P-Gem1–P-Gem4), which had similar molecular weight and carried approximately the same amount of the drug. The only difference were the used spacers for Gem linkage. We used the following spacers: β-Alanin (P-Gem1); glycyl-phenylalanyl-leucyl-glycyl (P-Gem2); aminocaproic acid (P-Gem3); valeric acid (P-Gem4).

Based on the assessed IC_{50} values for tumor cell lines 4T1, LL2, Panc02, MiaPaca2 and Panc1 the samples were divided into two groups: a) samples with quick rate of Gem release (P-Gem1, P-Gem2); b) samples with slow rate of Gem release (P-Gem3, P-Gem4). The *in vivo* stability of the conjugate affects systemic toxicity and anti-tumor activity, which was proven by the experiment performed on BALB/c mice bearing murine mammary carcinoma cells (4T1). We enrolled significant anti-tumor activity of the samples P-Gem3 and P-Gem4 in all observed parameters (tumor size, complete blood count, metastatic assay) after single intravenous administration of the samples. Experiments performed on B/6 mice carrying LL2/Panc02 tumors didn't show similar effect. Nevertheless, after series of experiments, it was found out that the MTD values for B/6 mice are approximately 50 % higher than for BALB/c mice. Considerable anti-tumor effect and prolonged survival was observed in immunodeficient mice $Rag2^{-/-}$ bearing human pancreatic carcinoma cells (MiaPaca2) treated with sample P-Gem4. Flow cytometry analysis of the number of myeloid suppresor cells (MDSC) in the spleens of BALB/c mice bearing 4T1 tumors showed significant decrease in the number of MDSC after single dose administration of P-Gem1. The degree of suppresion was dependent

on the dose. The stable samples P-Gem3 and P-Gem4 were evaluated as more therapeutically convenient and less systemically toxic.

Key words: anti-tumor effect; immunomodulatory effect; gemcitabine; polymer conjugates; HPMA; MTD; MDSC; pancreatic carcinoma, mammary carcinoma.