ANTITUMOUR EFFECT OF PLANT PEPTIDE EXTRACT PE-PM: PRELIMINARY *IN VIVO* TESTINGS IN MOUSE MODELS OF BREAST CANCER

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Breast cancer (BC) is mostly common among female malignancies. Multistage mechanisms of aetiology and pathogenesis of this disease comprise numerous molecular interactions. Thus, combined therapies are expected more effective against BC. Complementary phytotherapy after radical mastectomy is currently under clinical trial. Moreover, plants are well known resources of new chemotherapeutics. For instance, many currently used drugs were obtained from plants, at least initially (taxol, oncovin *etc.*). Recently discovered anti-cancer properties of plant cyclotides challenged us to search for new potent plant peptides against BC. Therefore, we created data base and analyzed information (including in Russian) about plants with anti-cancer properties used in folk medicine. We isolated a peptide extract – plant mixture (PE-PM) from the mixture of the plants: Chelidonium majus L., Inula helenium L., Equisetum arvense L., and Inonotus obliquus. The extraction was performed according to the method described earlier; peptide fractions were characterized by Ion exchange -HPLC, Matrix-Assisted Laser Desorption / Ionization spectrometry (MALDI) and amino acid analysis. No data were available concerning the influence of PE-PM on mammary adenocarcinoma (MAC) growth *in vitro.*

The aim of this study was to reveal local and/or systemic anti-cancer effect of PE-PM *in vivo* using original panel of mouse models that reproduced a number of morphological forms of mammary cancer in human and veterinary patients (especially MAC in cat). Four tests called “point” experiments (5-6 mice of the same sex and similar age *per* group) were performed using transplanted and spontaneous mouse models and local application of the peptide. The mouse models differed in (1) time of drug application in relation to a tumour growth stage (in start model – next day after tumour transplantation or spontaneous tumour detection, and in therapeutic model – application to advanced tumour burden); (2) site of tumour transplantation (sub cutaneous, s.c. or intra peritoneal, i.p. models); (3) an extent of transplanted tumour lethality for the untreated control group (lethal and sublethal models); (4) rate of palpable tumour appearance before their visible manifestation (lag^+ models with at least two week lasted latent palpable period and lag^- models with the absence of a palpable period); and (5) tumour growth rate after visible tumour manifestation (standard and fast growth). PE-PM (10 mg in 1 ml of physiologic solution) was applied locally (s.c. around a tumour burden in s.c. model and i.p. in i.p. model). Tumours in control mice were injected with physiologic solution alone at the same time and manner. Tumour growth delay and improved survival of treated mice in comparison to the parameters in the control mice showed local and systemic anti-cancer effects, respectively.

A local effect of a single PE-PM injection was firstly probed in a lethal transplanted s.c. start model of slowly appearing lag^+ MAC of the CBRB females. Earlier this model was shown to reproduce invasive lobular adenocarcinoma in human and veterinary patients. We propose a start mouse model to replicate the situation after radical mastectomy in patient: massive tumour burden removed but residual tumour cells may persist leading to local and/or systemic recurrence later. Need of an additional local treatment is evident as a distinct proportion of BC patients with local relapses require secondary operations. A long lasting palpable tumour growth period in lag^+ MAC allows revealing even a weak local effect of the testing drug on tumour growth. Moreover, local application of the drug provides detecting both direct and indirect (via immune system) anti-cancer effects. Here, on day 0 CBRB females were s.c. injected to right axillary fat pad with 10^6 tumour cells obtained from spontaneous slowly growing syngeneic MAC. On day 1 mice were locally treated with 0.1ml PE-PM solution *per* mouse. The local PE-PM effect was monitored for four weeks *post* transplantation (*pt*) by delay in palpable tumour appearance and growth rate in treated mice comparing with those parameters in control mice. As a result, only 67% of treated mice demonstrated tumour palpable nodules at the second week *pt* versus 100% of females in the control group. Significant tumour growth delay was observed on the forth week *pt* as treated animals demonstrated smaller nodules (25% on average) than ones in control mice. Finally, we showed that even a single local PE-PM application to slowly appearing lag^+ palpable CBRB MAC resulted in prolong local anti-cancer effect.

As a next step, sublethal i.p. transplanted slowly growing BLRB MAC was used as a model to test anti-cancer effect of a single i.p. PE-PM application. This *in vivo* model is a prototype of in *vitro* test as both drug and tumour cells are interacting at the same location during a long period of time. Moreover, presence of immune components (lymphocytes, macrophages, neutrophils, and mast cells) in peritoneal cavity of tumour bearing host may facilitate indirect drug effect manifestation/s. Furthermore, highly expressed direct and/or indirect anti-cancer effect may cause tumour grafting and growth prevention in treated animals as solid i.p. MAC growth is normally observed in only about half of untreated animals in sublethal model. Here we obtained TC from slowly

growing spontaneous BLRB MAC, transplanted them i.p. to syngenic male recipients using dosage of 10^6 TC *per* mouse. Next day experimental mice received single i.p. injection of PE-PM solution (0.1 ml *per* mouse). Anti-cancer peptide activity was estimated locally by delay of palpable i.p. solid MAC appearance (until the 13th week *pt)* in treated mice and reduced proportion of treated recipients with tumour manifestation (20% versus 57% in control, 16th week *pt*). This implies prevention of tumour grafting and growth in 57-20=37% of treated animals. As a result, survival of treated recipients was improved significantly as they all were alive at the 16th week *pt* when only 72% control recipients survived. Therefore, single local PE-PM treatment caused long term local and systemic antitumour effect in transplanted sublethal i.p slowly growing BLRB mouse model.

As PE-PM exhibited significant anti-cancer effect in slowly appearing and growing transplanted models we further tested it's activity in fast tumour growing model using lethal transplantable s.c. mammary carcinoma in BALB/cJCitMoise (B/c) females and multiple PE-PM local injections. This model characterized by aggressive growth with visible metastatic foci in the lungs. Here syngeneic B/c females received 10^6 TC *per* mouse at day 0. Local PE-PM treatments were provided at days 1, 3, 5, 7, 9 *pt* in a dosage of 0, 1 ml PE-PM solution *per* mouse *per* injection. Multiple treatments resulted in significant s.c. tumour growth delay (starting from the second week *pt*) and survival improvement. All control animals died at the day 44 *pt* whereas 80% of treated recipients survived. Gross morphology examination *post mortem* showed in PE-PM treated animals less visible metastatic foci in the lungs and three fold increased spleen weight comparing with these parameters in control animals. As a result, multiple PE-PM local treatments exhibited significant long term local and systemic anticancer effect in fast growing s.c. BLRB model, probably due to the metastasis spread postponement and immune component involved in the mechanism(s) of action.

As anticancer activity of PE-PM was clearly revealed in both slowly and fast growing transplanted s.c. and i.p. mouse models of human BC we further tested whether PE-PM affect spontaneous MAC growth in females of the BLRB-Rb(8.17)1Iem strain (BLRB, about 90% MAC and/or about 5-10% T-lymphoma/leucosis). It is known to distinguish in this model: (1) lag^- and lag^+ tumours before visible tumour manifestation, latter exhibit distinct palpable latent period during at least during two weeks (morphology of well differentiated MAC, similarly to lobular carcinoma of the human and veterinary patients) and (2) fast and slowly growing tumours after visible tumour manifestation (as a rule, latter are morphologically characterized by the absence of cystic tumour structures, which are common in fast growing mouse MAC, similarly to MAC in the cat). Average tumour growth rate dynamic of 10-20 spontaneous tumours represents "normal" tumour growth rate curve. Tumour growth above this curve is considered to be fast. Information about tumour morphology, growth rate, and survival of some hundreds females with lag^- and lag^+ (about ⅓ of all MAC) tumours is accumulated in our data base. This information was used as historic control data to analyse tumour growth rate and survival of treated mice. According to earlier described approach, five females aging 12 ± 2 months with spontaneous MAC of various tumour growth steps without associated lymphoma/leucosis were selected to test PE-PM activity. At the therapy start two females exhibited advanced lag^- tumours growing fast during the third week after visible tumour manifestation (lag^- therapeutic model, tumour diameter 11.9 ± 0.5mm) and three females had palpable tumours at the first detection (lag^+ start model, tumour diameter 2.0 ± 0.2mm). Those five females were locally treated weekly, seven PE-PM applications. Peptide dosages were dependent on tumour size: 0.1 ml of PE-PM solution was locally applied to tumours up to 5mm of diameter, above this size, 0.1 ml of peptide solution *per* each 5mm of tumour diameter was additionally applied each time. Fast initial tumour growth was detected in therapeutic model before PE-PM application. Therefore, tumour growth rate in treated females was compared with average growth dynamic of fast growing historical controls. Multiple PE-PM injections turned initially fast tumour growth down significantly (tumour diameter of 6, 9, 12, 13, 14, 16, 18, 20 mm at weeks 1, 2, 3, 4, 5, 6, 7, 8, respectively) comparing with tumour growth in historical control (tumour diameter 6, 9, 12, 15, 18, 21 mm at weeks 1, 2, 3, 4, 5, 6, respectively). Tumour growth delay in treated females resulted in two fold prolonged survival comparing with survival (6.0±0.2 weeks) of untreated control females bearing similar lag^- fast growing MAC. In start lag^+ model therapy was applied to palpable tumours at the time of the first detection; so, information of initial tumour growth speed was not available. Palpable latent period of tumour growth in PE-PM treated females seemed to be not prolonged. Average tumour growth dynamics of treated tumours after visible tumour manifestation and their average survival time were compared with "normal" tumour growth curve and average survival of historic controls. No differences were detected in nor tumour growth rates and survival. Thus, multiple local PE-PM applications appeared to be less effective in lag^+ spontaneous start model in comparison to promising results in lag^- advanced tumours with fast tumour growth rate.

Data obtained and conclusion made here remind in a way of our wealth experience with local application of immunotherapy by interleukin-2 to treat spontaneous mammary cancer, where therapy benefit was strongly dependent on initial tumour growth rate and time of application in both transplanted and spontaneous mouse models. Together with previously pub-

lished data this may indicate a presence of indirect immune component in a mechanism of PE-PM action. Immunotherapeutic approaches are well known to be tightly dependent on a step of tumour-host interactions and, therefore, to be beneficial for recipients only within distinct period of time. Consequently, immunotherapy would not be worthwhile to apply before or later, as it is appeared here with PE-PM application. In conclusion, both local and systemic effect of multiple PE-PM local applications to advanced mammary tumours by mounting dosages with tumour diameter increasing was detected in fast growing lag$^-$ spontaneous BLRB model (morphologically resembling cystic papillary and/or medullary carcinoma of the human and veterinary patients).

"Point" experiment approach proposed here during initial steps of anti-cancer PE-PM efficacy testing procedure *in vivo* revealed mono-directed effect, i.e. tumour growth delay and survival prolongation in all mouse models (CBRB, BLRB and BALB/cJCitMoise). And the amount of used animals is sufficiently decreased.

Finally, our data permit to recommend PE-PM for extensive anti-cancer drug testing and to hope that this kind of therapy would be of benefit for local application after radical mastectomy.

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METABOLOIC DISORDERS IN ELDERLY DIABETES PATIENTS

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The purpose of the work is studying biochemical factors of blood against metabolic syndrome in diabetes patients – elderly men and women.

The object of the investigation was the blood of 20 diabetes patients (the average age of the men was 61, 7±1, 5 years; women - 63, 1±1, 3 years), the disease duration was from 7 to 34 years. As the control the blood of 20 practically healthy donors matching in sex and age was used.

The quantity of leucocytes, the concentration of glucose, total protein, cholesterol (CL), triacylglycerols (TG), low density lipoproteins (LDLP), high density lipoproteins (HDLP), urea, creatinine, also amylase, alanine aminotransferase (ALT), aspartate aminotransferase (AST) were determined in the blood by the unified methods applied in clinical laboratory diagnostics. The concentration of Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+} was determined by the method of flame atomic absorption spectroscopy (Quantum-2A, Russia). The WBC differential was developed; the intoxication leukocytic index (ILI) and allergization index (AI) were calculated.

In diabetes men-patients the glucose concentration in blood made 11, 6±1, 3 mmol/l^{-1}, women-patients - 9, 4±0, 7 mmol/l^{-1}, that is authentically higher than in the control group donors. In the diabetes men-patients' blood the concentration of CL (by 72, 7%), TG (by 43, 1%), LDLP (by 55, 4%) and K^+ (by 19, 4%) is authentically higher and the concentration of HDLP (by 14, 2%), Na^+ (by 6, 5%), Cl^- (by 5, 5%), Mg^{2+} (by 20, 6%) and Ca^{2+} (by 57, 6%) is lower. In the diabetes women-patients the content of CL (by на 70, 1%), TG (by 44, 4%), LDLP (by 63, 0%) and K^+ (by 8, 7%) is also authentically higher, and that of HDLP (by 17, 4%), Na^+ (by 5, 9%), Cl^- (by 4, 2%), Mg^{2+} (by 17, 6%) and Ca^{2+} (by 68, 9%) is lower.

As a tendency one can consider the amount decrease of amylase, ALT and the increase of creatinine, AST by 16, 0% and 6, 3%; 5, 4 and 15, 7; 6, 3 and 13, 1; 14, 9% and 54, 5% accordingly in men and women, in the blood of diabetes patients.

In the examined healthy people and diabetes men- and women-patients the average ILI values correspond to a light form of endointoxication, the AI values in the diabetes patients are authentically higher and reflect the presence of an allergic process in them.

Thus, hyperglycemia, provoking serious metabolic disorders, retains against the insulinic therapy in elderly men and women suffering from diabetes; a light form of endointoxication and the presence of allergic process have been detected.

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