EuroSciCon

International Conference on
Biotechnology, Biomarkers & Systems Biology

March 04-05, 2019
Amsterdam, Netherlands

Wassil Nowicky et al., Biochem Mol biol J 2019, Volume:5
DOI: 10.21767/2471-8084-C1-023

MIXTURE OF ALKALOIDS AFFECTS MMP9 PROTEIN EXPRESSION IN AN INFLAMMATORY *IN VITRO* MODEL

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Background: Matrix metalloproteinas-es (MMPs) are a large family of ubiqui-tously expressed zinc-dependent enzymes with proteolysis activities. They are ex-pressed in physiological situations and pathological conditions involv-ing inflammatory processes, Epithelial to Mesenchymal Transition (EMT), neuronal injury and cancer. There is also evidence that MMPs regulate inflammation in tumor microenvironment, which plays an im-portant role in cancer progression. Looking at both inflammatory and neuronal damag-es, MMP, specially MMP-2 and MMP9 are involved in both processes and their modulation seems to be regulated by two major actors as tumor necrosis factor alpha (TNF-alpha) and interleukin 6 (IL-6). The BV-2 cells (microglial cells of mouse) were been used as *in vitro* model fto simulat both inflammatory and neuronal injury pathologies. In these models, MMP9 seems to be involved in cellular migration throughout inflammatory activation in de-pending manner. Leonurine, an alkaloid derived from Herbal Leonuri, seems to af-fect the induced inflammatory expression in BV-2 cells, while, the effects of alka-loids against MMP9 seem not to be demonstrated in BV-2 cells. Nevertheless, Ukrain (UK) a mixture of alkaloids had demonstrated to regulate the MMP9 ex-pression. Aim of this study was to investi-gate the role of alkaloids against MMP9 in BV-2 cells.

Materials & Methods: The immortalized murine BV2 cell line (ATCC Cell Line Collection, Milan, Italy) was cultured in RPMI 1640 medium with phenol red (nvi-trogen) supplemented with 10% fetal bo-vine serum (FBS), 1% penicillin-streptomycin (Invitrogen), and 1% gluta-mine (Invitrogen). Cultures were grown at 37 °C in 5% CO2 until 50% confluence. BV-2 cell culture was used to investigate the MMP9 expression by ELISA test and for Immunofluorescence (IF) assay. BV2 mouse microglial cells were seeded in 12-well plates, in order to obtain three differ-ent experiments for UK concentration 5 µM. The inflammatory stimulation was induced by lipopolysaccharide (LPS). We used BV-2 treated with UK alone, as con-trols. In all experiments the cells were treated for 24 hrs. ELISA tests: the medi-ums were harvested for ELISA analyses of MMP9. 2) IF Analyses. BV2 mouse mi-croglial cells were seeded in 8-well Cham-ber Slides (CS) (Lab-Tek1 Chamber Slide™ system, Nalge Nunc International, Naperville, IL, US), putting in 5000 cells/well in a 650 µ L final volume. CS was prepared in order to obtain three dif-ferent experiments in triplicate. After treatments, cells were fixed directly on the slides by Carnoy's solution for 10 min and the chamber slide wells were removed by mechanical support following manufactur-er's instructions. The IF for MMP9 protein detection was performed using a monoclo-nal primary antibody anti-MMP9, followed by Fluorescent secondary antibody. The nuclei of cells were counterstained using a DAPI solution.

Results: We performed the calibration curve of MMP9 and we tested the presence of MMP protein in BV-2, before the treatments. The MMP9 protein expres-sion was present inside BV-2 before the chemical treatment. The MMP9 expression was down regulated in both cultures LPS+UK and UK with respect to their controls. In particular, we showed that MMP9 concentration gone down during UK treatment (p=0.0001). Indeed, looking at IF profiles, the levels of MMP9 decreased drastically with respect to those observed in their respective controls.

Conclusions: There is increasing evidence that mixture of alkaloids can affects MMPs protein expression not only in cancer, but in other *in vitro* models. Additional precise information on the MMP interaction with other protein might open novel therapeutic treatments for inflammatory diseases and cancer blocking overexpressed actions of MMPs

Biography

Wassil Nowicky, Director of "Nowicky Pharma" and President of the Ukrainian Anti-Cancer Institute (Vienna, Austria). Has finished his study at the Radiotechnical Faculty of the Technical University of Lviv (Ukraine) with the end of 1955 with graduation to "Diplomingeniueur" in 1960 which title was nostrificated in Austria in 1975. He is the Inventor of the anticancer preparation on basis of celandine alkaloids "NSC-631570". He is an Author of over 300 scientific articles dedicated to cancer research. He is a real Member of the New York Academy of Sciences, Member of the European Union for Applied Immunology and of the American Association for scientific progress, Honorary Doctor of the Janka Kupala University in Hrodno, Doctor "honoris causa" of the Open international university on complex medicine in Colombo, Honorary Member of the Austrian Society of a name od Albert Schweizer. He has received the award for merits of National guild of pharmacists of America. the award of Austrian Society of sanitary, hygiene and public health services and others.

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