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Biotechnology, Biomarkers & Systems Biology 2020: Mixture of alkaloids affects MMP9 protein expression in an inflammatory in vitro model - Wassil Nowicky - Austria University of Pisa, Italy

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Extended Abstract

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

In nature there are many natural compounds. From among many classes of naturally occurring organic compounds such as carbohydrates, lipids, proteins, amino acids, anthocyanins, flavonoids, and steroids, the one that seems to be quite special is alkaloids. What makes them special? They derived from amino acids and can be synthetized as secondary metabolites by plants and some animals. These compounds play an important role in living organisms. Alkaloids occurred to be extremely important for human beings for ages, besides they are secondary metabolites, what could suggest that they are useless. Alkaloids showed strong biological effects on animal and human organisms in very small doses. Alkaloids are present not only in human daily life in food and drinks but also as stimulant drugs. They showed anti-inflammatory, anticancer, analgesics, local anesthetic and pain relief, neuropharmacologic, antimicrobial, antifungal, and many other activities. Alkaloids are useful as diet ingredients, supplements, and pharmaceuticals, in medicine and in other applications in human life. Alkaloids are also important compounds in organic synthesis for searching new semisynthetic and synthetic compounds with possibly better biological activity than parent compounds.

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Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atom or atoms (amino or amido in some cases) in their structures. These nitrogen atoms cause alkalinity of these compounds. These nitrogen atoms are usually situated in some ring (cyclic) system. For example, indole alkaloids are those that contain nitrogen atom in indole ring system. Generally based on structures, alkaloids can be divided into classes like indoles, quinolines, isoquinolines, pyrrolidines, pyridines, pyrrolizidines, tropanes, and terpenoids and steroids. Other classification system is connected with a family of plant species that they occur. One of the examples is the opium alkaloids that occur in the opium poppy (Papaver somniferum) [1]. These two different classification systems cause confusion between their biological distribution and the chemical types of alkaloids, because there is not unmistakable correlation.

Alkaloids (whose name originally comes from “alkali-like”) can react with acids and then form salts, just like inorganic alkalis. These nitrogen atoms can behave like a base in acid-base reactions. In general alkaloids, which are treated as amines, the same as amines in their names, have suffix -ine. Alkaloids in pure form are usually colorless, odorless crystalline solids, but sometimes they can be yellowish liquids. Quite often, they have bitter taste. Now more than 3000 of alkaloids are known in over different 4000 plant species. These compounds are produced generally by many plant species, mainly by flowering plants and also by some animals. Plants produce and store many organic compounds like amino acids, proteins, carbohydrates, fats, and alkaloids, which are usually treated as secondary metabolites. They are stored in each part of the plant—leaves, stem, root, and fruits of plants—but in different amounts. It was suggested that they are plants’ waste product, but now evidence suggests that they play some important biological function in plants.

Some groups of structurally related alkaloids are present in plants from few to even 30. These alkaloids belong to the same class but have some differences in their structure and one of them usually occurs in majority. Some plant families are very rich in alkaloids. For example, in plants like opium poppy (Papaver somniferum) and the ergot fungus (Claviceps), there are about 30 different alkaloid types. In plants, their function is still mostly unknown. Alkaloids because of their bitter taste are natural compound to deter herbivorous organisms. In some plants they are used as natural pesticides. It was suggested that alkaloids in plants have a function to protect them from destructive activity of some insect species. Alkaloids are also present in some animal species like frogs (poison dart frogs (Phyllobates)), New World beaver (Castor canadensis), and lizards, and they are produced by fungi species and ergot.

Besides having the same general name—alkaloids—they have an extreme variety of chemical structures. Some of these compounds seem to have people known for ages because of their wide range of activity on human organisms and also other animals. For thousand years, extracts from plants containing alkaloids had medicinal use as drugs, and they owe their powerful effects thanks to the presence of alkaloids. Morphine was the first alkaloid which was isolated about 1804 from opium poppy in crystalline form. Alkaloids are an interesting group of compounds with a wide range of activities, undesirable and desirable, on animal and human organisms. Alkaloids have diverse physiological effects: antibacterial, antimitotic, anti-inflammatory, analgesic, local anesthetic, hypnotic, psychotropic, and antitumor activity and many others. Nowadays, alkaloids usually from plants rather than from animals are still of great interest to organic chemists, biologists, biochemists, pharmacologists, and pharmacists. Well-known alkaloids include morphine, strychnine, quinine, atropine, caffeine, ephedrine, and nicotine

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