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Summary of the doctoral thesis "CONTRIBUTIONS TO THE STUDY OF MONOSODIUM GLUTAMATE'S METABOLISM AND TO THE IDENTIFICATION OF OTHER POTENTIAL BIOLOGICAL EFFECTS OF GLUTAMIC ACID AND OTHER STRUCTURAL DERIVATIVES THROUGH *IN SILICO* AND *IN VITRO METHODS*"

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Background

Glutamic acid (GLA) is a non-essential amino acid with a key role in multiple metabolic pathways. It has an advantageous chemical structure, easy to optimize for obtaining structural derivatives with a new biological effect. Monosodium glutamate (MSG) is used as a food additive. Still, the possible toxicity with long-term use raises questions about its safety. Glutamine (GLN) is the amide of GLA with an essential role in cell development, including cancer cells; developing new anticancer agents targets GLN metabolism. GLA derivatives can be optimized to obtain compounds with anticancer or antibacterial effects or that influence bacterial biofilm formation.

Study no. 1. The evaluation of the potential toxicity of monosodium glutamate sub-chronic consumption in two-year-old Wistar rats

The aim of this study was the comparative evaluation of positive and negative metabolic aspects of MSG sub-chronic consumption in two-year-old rats. The impact of the acceptable daily intake (ADI) and no-observed-adverse-effect level (NOAEL) of MSG was monitored. The evaluated serum biochemical and metabolic parameters include aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol (TC), triglycerides (TG), total bilirubin (TB), direct bilirubin (DB), creatinine (CR) and urea (UR). The targeted histological samples are liver, kidney and stomach tissue. The working protocol was applied to two-year-old Wistar rats who were administered several doses of MSG. Statistically significant differences were observed for ALT, ALP and CR. Modifications of possible biological importance are ALT, ALP, DB, TB and UR values, lower at high doses of MSG; a decrease of AST, TC, TG, UR, DB and TB from the MSG non-consuming group to group 1 (MSG dose equivalent to the ADI dose in humans), or groups 2 (1500 mg/kg body weight/day MSG) and 3 (3000 mg/kg body weight/day MSG – close dose to the NOAEL). The histological changes observed at the hepatic and renal levels cannot be correlated with the sub-chronic consumption of MSG. The stomach has not undergone histopathological changes. The positive metabolic aspects of AGL are better highlighted than the negative ones in ageing rats. The hormesis effect, with the protective effects of low doses of reactive oxygen species and the biochemical implications of GLA, support the hypothesis of a potential defence mechanism triggered by sub-chronic MSG consumption in ageing rats.

Study no. 2. *In silico* evaluation of some new glutamic acid-fluoroquinolone hypothetical hybrids as potential human topoisomerase II inhibitors with anticancer effect

The hypothesis of this study is the interaction of some new theoretical potential hybrids obtained from molecules of common fluoroquinolones (FQ) and GLA with the human type II topoisomerase (the target enzyme of FQ). The hybrids can act on some cancer cell lines, similar to other human topoisomerase II inhibitors with anticancer effects (for example, etoposide). The *in silico* evaluation of 27 GLA-FQ theoretically formulated hybrids suggested a possible inhibitory activity of type II topoisomerase, and the most sensitive cancer cell lines could be adult acute myeloid leukaemia (OCI-AML2), bladder carcinoma (UMUC3) and promyelocytic leukaemia (HL-60). The hybrid OF-3-EDA-GLA presents the best results of molecular docking to the human topoisomerase II beta-DNA complex using the binding site of etoposide. MF-7-GLA proved to be the ideal candidate to become a lead compound because of its high similarity of the physico-chemical properties to etoposide, the favourable Tanimoto coefficient, the topoisomerase II inhibitory capacity and the promising results of the interaction with the target enzyme (human topoisomerase II beta-DNA complex).

Study no. 3. The interaction study of some glutamic acid structural derivatives with the human glutaminase through *in silico* methods

This study aimed to theoretically design a series of compounds derived from GLA structure with potential anticancer activity. One hundred twenty-three theoretical derivatives of GLA were obtained and subjected to a selection algorithm based on *in silico* methods, eliminating the derivatives with unsuitable properties. The nine selected derivatives were included in two molecular docking studies to the human glutaminase, using the binding site of a non-competitive allosteric inhibitor of glutaminase and of a glutaminase inhibitor that „mimics" GLN structure. The glutaminase inhibitory capacity and the Tanimoto coefficient concerning GLN were determined *in silico*. Correlating the results of the interaction with the human glutaminase with those of glutaminase inhibitory capacity and Tanimoto coefficient, derivative 1 (2-amino-5-ethoxy-5-oxopentanoic acid) and derivative 3 (N,N-dimethylglutamic acid) could be candidates to become a lead compound.

Study no. 4. The evaluation of glutamic acid and some structural analogues' effects on the bacterial activity and the ability of bacteria to form biofilm

The aim of this study was the *in vitro* evaluation of the antibacterial activity by the determination of minimum inhibitory concentration, minimum bactericidal concentration and the influence on bacterial biofilm formation of GLA and some derivatives (GLN, MSG and the diethyl ester of GLA (DEGLA)) on Gram-positive and Gram-negative reference bacterial strains. The most promising results were obtained for compound DEGLA, which had antibacterial activity on all six tested bacterial strains; this compound had bacterial inhibitory activity at concentrations 12,75 mg/ml and 25,5 mg/ml and bactericidal activity at 51 mg/ml and 25,5 mg/ml, depending on the tested bacterial strain. The results indicate a biofilm formation stimulating tendency or a lack of influence of the four tested compounds, depending on the bacterial strain or compound.