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Investigation Report on Project Kre-Celazine®:

Antiproliferative effects of creatine mixed/bonded to fats – non-stabilized or buffered in a panel of human tumor cell lines.

Experimental design

The antiproliferative effects of a buffered formulation of creatine & fats was investigated in a comparative fashion vs. non-stabilized creatine & fats in a panel of tumor cell lines, representative to some important kinds of human cancer. The panel included the acute promyelocyte leukemia HL-60, the chronic myeloid leukemia LAMA-84, the Hodgkin-lymphoma HD-MY-Z and the multiple myeloma-derived cell lines OPM-2, U-266 and RPMI-23366. All cells where obtained from the German Collection of Microorganisms and Cell Cultures (Brounschweig, Germany) and were routinely maintained under standard conditions – RPMI-1640 medium, supplemented with 10% fetal calf serum and L-glutamine, in a 5% CO_2 humidified atmosphere (at 37ϲC).

Experimental protocol

For the cytotoxicity assessment exponentially growing cells were plated in 96-well flat-bottomed microplates and after 24 h were treated with the tested fats. The tested compounds were dissolved in DMSO and serially diluted in RPMI-1640 to the desired level. For each concentration at least 8 wells were used. After 72 h exposure the cellular viability was monitored by the standard MTT-dye reduction assay.
The tested fats – both conventional and processed, exerted strong inhibition of the proliferation of malignant cells, in a concentration-dependent manner. This allowed the calculation of the corresponding IC_{50} values i.e. concentration causing half-maximal inhibition of cell viability, as merit of the antiproliferative potency of tested compounds.

Results

The acute promyelocyte leukemia HL-60 demonstrated significant sensitivity to both conventional and stabilized creatine & fats, although the latter proved to be significantly more active as evident by the point-to pint comparison of survival fractions for every concentration.
Significant antiproliferative effects were established in LAMA-84 cells as well. As in the preceding cell line the stabilized, buffered creatine & fats proved to be superior antiproliferative agents as evidenced by the MTT-data

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The evaluation of the antiproliferative effects of tested compounds against the Hodgkin lymphoma derived cell line HD-MY-Z revealed that they exerted strong inhibitory activity, again superior for the processed creatine & fats. The results for the three multiple myeloma-derived cell lines also demonstrated the superiority of the processed creatine & fats, vs. the non-stabilized ones.

Conclusions:
The presented data indicate that throughout the panel of malignant cells the processed creatine & fats proved to exert superior antiproliferative effects vs. the non-buffered ones, as evidenced by point-to –point comparison of survival fractions after treatment with equivalent concentrations as well as by juxtaposition of the calculated IC_{50} values. It is well appreciated, that the antiproliferative potency of a given compound in vitro is governed by its stability under the experimental conditions. Thus, considering the equivalent controlled conditions of the experiment the observed greater activity of processed creatine & fats could be ascribed solely to the superior stability afforded by the processing manipulations. These types of cancer cells are directly and indirectly related to inflammation.

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Sofia August 25th 2007

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