Andrea Hunn, 2000 | Malters, LU

The aim of this project was to use laboratory experiments to find out whether selected natural remedies, namely wogonin, curcumin, epigallocatechin gallate (EGCG) and mistletoe extract, have a toxic effect on cancer cells in the cell model and thus kill them. Another part of the work was to verify the impedance flow cytometry (IFC) measurement system used as functioning and suitable for such investigations. An IFC, which has never been used for such a toxicity study before, determines the vitality of the cells based on the membrane differences that occur during cell death. The measurements were carried out on different cell lines in order to determine differences in effectiveness. Blood cancer cells, cervical cancer cells and embryonic kidney cells that do not originate from a tumour and are therefore less degenerate were used. In addition, different concentrations of the active substance and different incubation times were tested to improve the significance of the effects. Thus, the dependence of the effect on the cell type, the natural remedy, the concentration and the incubation time was determined.

Question

This project deals with the question whether the addition of natural remedies can be used to observe cell death. The aim was to measure this cell death with the IFC, which had never before been used for such a toxicity study on human cells. In addition, the influence of natural remedy concentration and incubation time on cell death was investigated. The question arose whether the natural remedies only kill blood cancer cells or also other types of cancer, but not minor degenerated human cells.

Methodology

The experiments can be divided into three steps: (I) Validation of the measurement system, (II) measurement series 1 and (III) measurement series 2. First, it had to be ensured that reliable results could be obtained with the selected method of impedance flow cytometry. The IFC measures the differences in current permeability between a dead and a living cell. Then the experiments were carried out with the blood cancer cells. These results, related to concentration and incubation time, were used for measurement series 2, in which cervical cancer cells and slightly degenerated kidney cells were examined.

Results

The results show that the IFC method is suitable for such toxicity studies, because its measured data deviate from those of a conventional method by only about ten percent on average. This led to the establishment of a system that enables the rapid initial analysis of cells in a short time (up to 1000 cells per second). In addition, the results show that all natural remedies used, with the exception of mistletoe extract, have a toxic effect on blood cancer cells from a concentration of 10 µM and reduce vitality by up to 50 percent compared to untreated cells. In addition, the killing effect on more degenerated cells, i.e. blood cancer and

other cancer cells, is stronger. It was also clearly shown that a higher concentration of the active ingredient causes more severe death, but a longer incubation period does not. On the contrary: the cells begin to recover and their percentage vitality increases sharply after 24 hours.

Discussion

The cell types used naturally grow together and must be separated from each other for the measurements. This can damage the cell membrane. This in turn leads to a falsification of the measurement results, as it is precisely the damage to the cell membrane that serves as an indicator of whether a cell is classified as living or dead. A further point of criticism is that the killing effect of the natural remedies in the first

24 hours is significantly stronger than in the hours after. The assumption that the active substance has been used up or broken down and that the still vital cells can multiply again afterwards is obvious.

Conclusion

The investigations underline the suitability of the IFC as a cell analysis system. In addition, it is interesting to see that the selected natural remedies are effective cancer cell killers in the cell model within the cell lines used. These initial results suggest that it might be worthwhile to extend this study to other cell lines and, if this tendency is confirmed, their effects should be investigated in a living organism.

Appreciation by the expert

Prof. Beat Suter

Mrs. Hunn found answers to 2 different questions. Can an impedance-flow cytometer, which was developed for other purposes, also measure the vitality of cell culture cells? Her YES answer to this question allowed her to test some natural remedies for their activity, killing cancer cells but not less degenerate cells. Among the substances tested, there were some that did not stop any cell line from growing under the chosen conditions. Others, however, were toxic for both or only one cancer cell line. Mrs. Hunn now proposes justified, further tests.

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