Budapesti Műszaki és Gazdaságtudományi Egyetem

Ph.D. thesis

Accumulation and QSAR study of photosensitizers

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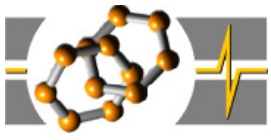

Chemical Research Center, Hungarian Academy of Sciences
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2004.

INTRODUCTION

Photodynamic therapy (PDT) is preferentially used for tumor cell killing using porphine-based photosensitizers and related molecules. It is based on the action of a selectively accumulated photosensitizer molecule and light in the presence of oxygen in a given tissue. PDT is also used to treat non-carcinogenic ailments, such as psoriasis, atherosclerosis, vascular restenosis, rheumatoid arthritis and age-related macular degeneration. It is used for blood and blood product sterilization, but its insecticide and bactericide effects have also been reported.

The bottleneck of PDT application in the clinics is the impurity of the used sensitizers, as well as their low effectivity. So, development of new porphine-based sensitizers is one of the main goals of synthetic chemistry. Several laboratories, also in Hungary, are engaged in the synthesis of new photosensitizers, but the biological activity testing remains a problem.

The aim of this study was to help the development of new effective photosensitizers by testing and analyzing the activity of porphine-based compounds as sensitizing molecules under the same laboratory conditions.

AIMS

In this study, we decided to adopt and develop an analytical method for sensitizer measurement and to select the appropriate quantitative structure-activity relationship method for assessment of activity data.

These are:

1. adaptation and improvement of a system not commercially available, which is suitable for determination of photosensitizer accumulation in cells and animal tissues based on *in situ* fluorescent measurements using fiber optics;

2. development of a new method based on fiber optic fluorescence measurement, which makes possible the determination of the tissue concentrations of a large number of porphine-based sensitizers using a technique much simpler and less time-consuming than chemical extraction, with a minimal use of experimental animals;
3. selection of the most appropriate model building methods for prediction of photodynamic activity, selective accumulation and anti-HIV-1 activity of the compounds based on literature data. Comparison of several chemometric methods for selection of linear and non-linear descriptors, as well as for model building based on their predictive ability using a small number of congeneric and non-congeneric sensitizer molecules.

These methods will serve as a base for testing a bigger number (35-40) of molecules in our own laboratory in the frame of an international cooperation. QSAR analyses will follow the experimental measurements.

METHODS

We have set up our own system to measure sensitizer concentrations *in situ* using parts (fiber optics, reflection probe, AVS S2000 detector and SPECTRAWIN 4.2 software) provided by the Dutch AVANTES company.

Animal experiments were performed according to the guidelines of the Chemical Research Center, Hungarian Academy of Sciences protocols and were approved by the local Animal Ethics Committee. Male NMRI mice weighing 20±1 g were used (Toxicoop Ltd., Budapest).

Geometrical optimization and conformation analysis of the 3-dimensional structures of porphyrins was performed based on ChemPlus^{TM} 2.0 program (part of the HyperChem 4 program, HyperCube Inc., Canada, 1997**)** using MM^{+} molecular mechanics method. Data were organized

into a database using IsisBase 2.3 program (Molecular Design Ltd., USA, 1996). Statistica™ program package (Statsoft, USA, 1999) was used to construct MLR and PLS models, while ANN models were built using the 3DNET 1.0 program (CompElit Ltd., Hungary, 1998).

RESULTS

We have developed and optimized methods indispensable for testing and analyzing the biological activity of photosensitizing molecules:

1. An equipment suitable for *in situ* fluorescent detection of porphine-based and related compounds has been devised. Using appropriate parts (filters, lenses and fiber optics), the quality of spectra taken in different animal organs by the developed reflection probe approached those taken in chemical solvents. This way, the fluorescence intensity of *in situ* accumulated sensitizer proportional to its concentration can be measured quickly and reliably.
2. We have developed a new method for calibration of the correlation between the *in situ* measured fluorescence intensity and the amount of accumulated sensitizer in the given organs. This makes determination of the accumulated sensitizer in the tissues easier and faster than using general extraction methods. Tissue homogenates of different concentrations were prepared from organs of untreated animals and known amounts of sensitizers were added to construct calibration curves. Then, they were used to determine the amounts of sensitizers in the tissue homogenates of pretreated animals. Using samples prepared from liver and tumor, we have shown that homogenates containing 50% of tissue are just as suitable to predict the sensitizer content of the intact organs as by the more complicated linear extrapolation made based on measurements of homogenates of different concentrations.
3. Calibration curves were constructed based on *in situ* measured fluorescence intensities in the organs of pretreated animals and the sensitizer concentrations determined in the tissue samples.

Using these calibration curves, the amounts of the accumulated sensitizer can be estimated immediately based on the measured fluorescence intensities. The method was used to calibrate the sensitizer content of liver, skin, tumor and blood plasma samples. The differences between the photophysical properties of tetrapyrrole-based molecules belonging to the same family are negligible due to the substituent effect. Therefore, the *in situ* prepared calibration curve for one compound is expected to be valid for other members of the family as well. The optimal excitation time of the sensitizer necessary for PDT activity measurements can be determined within a short period of time, and the accumulation of different sensitizers can be compared based on the determined concentrations using a minimal number of animals.

4. The descriptive and predictive abilities of models built using quantitative structure-activity relationship studies based on the three-dimensional structures, tumor accumulation data and PDT activities of a homologous series of pyropheophorbide derivatives were compared. We have shown that the accumulation of the compounds can be described and predicted by models built using both multiple linear regression (MLR) and artificial neural networks (ANN). The most important descriptor for predicting sensitizer accumulation turned out to be log*P*, which was sufficient on its own for prediction using ANN.
5. According to our calculations, linear methods were not suitable to build good models for the description and prediction of PDT activities, while ANN gave very good results for prediction of tumor growth retardation. In this case, log*P* was not enough to predict the activity. Models containing the Wiener index and WHIM descriptors characterizing molecular size of the molecules (AMASS, AVDW, AEN) in addition to log*P*, resulted in much more reliable models.
6. We have built models using three different methods (MLR, and partial least squares (PLS) and ANN) to predict the non-photodynamic anti-HIV1 activities of a larger and more heterogeneous group of porphine-based photosensitizers. We have determined that non-linear methods (MLR

and PLS) can describe well the aforementioned activities, but are not suitable for prediction (based on leave-one-out, leave-n-out and external validation results). Internal and external validations showed that only ANN was able to render useful models.

7. The most important descriptor for predicting anti-HIV-1 activities in all ANN models was the freedom of chemical bond rotation (DF). It was not enough though to predict the activities. At least one more descriptor was necessary for this purpose: the double bond equivalent (DBE) or the electrostatic total acidity of hydrogen bonds (ESTA). Internal validation results could be further improved by addition of a third descriptor: HOMO or KMASS.
8. We have shown that new antiviral compounds can be designed based on molecular characteristics influencing the anti-HIV-1 activities. New compounds with low and high antiviral activities were designed and tested based on our models. The expected results were obtained: the models predicted high activities for compounds designed to have high activities and lower activities for compounds designed to have lower activities. The method was also used to predict the activities of missing members of a series. We have confirmed literature data on that, in case of carboxylated tetraphenylporphyrins, at least three carboxyl groups are necessary to achieve good activity.
9. Based on the analyzed data, it has been established that MLR and PLS are not, while ANN is a suitable method for conducting quantitative structure-activity relationship studies of smaller and larger groups of homologous and non-homologous tetrapyrrole molecules in case of biological activities depending on structural parameters in a linear and non-linear ways.

CONCLUSION

1. An equipment suitable for *in situ* fluorescent detection of porphine-based and related compounds has been devised. Unlike the others, we apply halogen lamp instead of laser.
2. Fluorescence intensity, which is proportional to the concentration of the accumulated sensitizer, can be measured reliably by the used reflection probe.
3. The sensitizer content of liver, skin, tumor and blood plasma can be estimated quickly using calibration curves resulted by the tissue homogenization method. This method could be used not only for measurements of sensitizer levels in a specific organ, like the simple fluorescence intensity measurement, but is suitable for comparison of its concentrations between different organs.
4. Photosensitizer accumulation and clearance in murine organs can be monitored using a limited number of animals.
5. Accumulation and photodynamic activity could be predicted using ANN on a small number of congeneric pyropheophorbide molecules.
6. Non-photodynamic anti-HIV-1 activities of tetrapyrrole molecules could only be predicted using models build by ANN.
7. The leave-one-out cross-validation procedure, for itself, is not enough to give reliable representation of predictive ability of the models. Applying leave-n-out cross-validation and external validation together results in a much more reliable method.
8. Possibility of designing new antiviral molecules based on their molecular properties has been exemplified.

UTILIZATION POSSIBILITY

1. The applied transportable fluorometer can be used in human treatment for *in vivo* monitoring of sensitizer levels in tumors and surrounding tissues.
2. The anti-HIV-1 activity predicting models built by ANN can be used either to select the promising antiviral agents or the inefficient ones from molecules from among a porphyrin data base.
3. Using the adopted and newly developed methods, a quantitative structure-activity relationship analysis can be applied, which could be widely used to predict PDT activity of porphyrin derivatives.

9. PUBLICATIONS

Publications related to the thesis

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2. Vanyúr R., Héberger K., Kövesdi I., Jakus J.:
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