 DOI: 10.33892/aph.2021.91.300-301

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Some Interactions of the Novel Photoswitchable Compound Phototrexate

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Keywords: *Anticancer, antirheumatic, photoswitchable, scavenger, thermodynamics*

1. Introduction

Methotrexate (MTX) is a first line drug in the therapy of some sorts of cancer (e.g. leukaemia), rheumatoid arthritis (RA) and psoriasis. However, its drug safety profile is relatively poor, it can cause several adverse effects. Alternative strategies to overcome low therapeutic indices, nonspecific targeting, and the off-target toxicity of chemotherapeutics have emerged over the last years. Phototrexate (PHX) is a photoswitchable azobenzene analogue of MTX that has been synthesized for the first time in 2018. PHX contains a diazene stereogenic unit and its pharmacological activity is higher in its *cis* state than in the more thermodynamically stable *trans* state. It can be effectively isomerized from *trans* to *cis* with UVA light and back-isomerized from *cis* to *trans* with blue or white light (*Figure 1*).

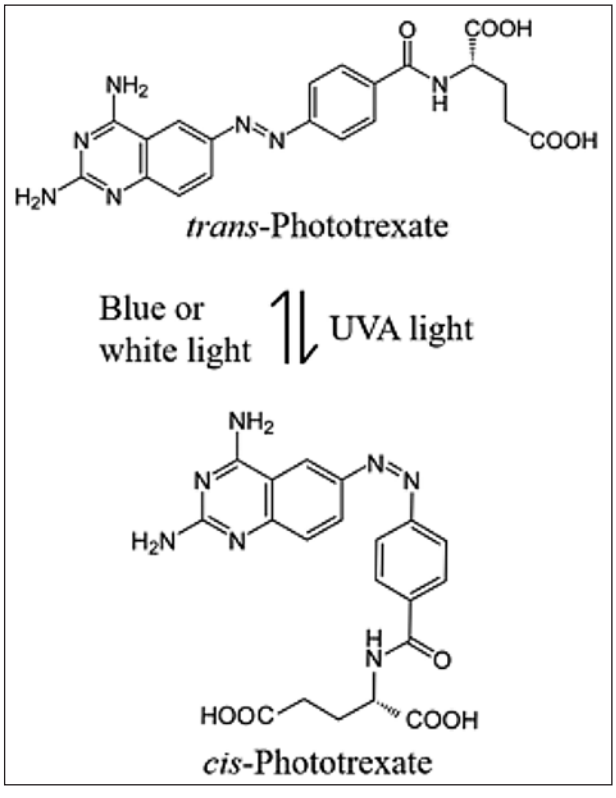

Figure 1 *The reversible isomerization of Phototrexate (PHX).*

This transition is reversible and can be repeated several times. The antineoplastic effect (and the adverse effects caused by the cytotoxic activity) appears only in light-exposed regions and decreases in dark regions. In the last years, MTX is being studied because of its assumed cardioprotective effect caused by its radical scavenging properties. The aim of this study was to compare the scavenging properties of MTX with that of *trans*-PHX and *cis*-PHX. The thermodynamics of the host-guest complex formation of the isomers of PHX with two cavitand derivatives (TAC and TDC) were also studied.

2. Materials and methods

The superoxide scavenging effect of MTX, *trans*-PHX and *cis*-PHX was investigated by EPR spectroscopy (superoxide radicals were generated by a hypoxanthine/xanthine oxidase system). Direct antioxidant properties of the compounds were determined by ABTS scavenging assay. The thermodynamic properties of the isomers of PHX were studied by fluorimetric measurements and by molecular modeling.

3. Results

Isomerization of Trans-PHX

To reach the complete isomerization of *trans*-PHX,

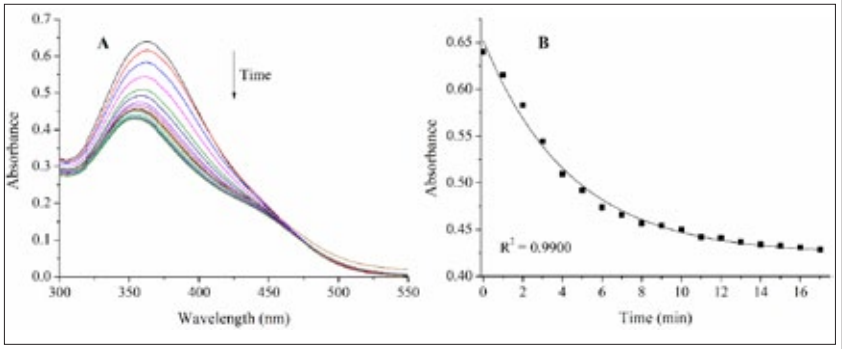

Figure 2 *UV-vis spectra of trans-PHX solution exposed to 366 nm light (**A**) and the intensity of the peak at 360 nm plotted against time (**B**). The first spectrum was recorded before the beginning of the UV-light exposure, then measurements were taken every minute.*

UV-light exposure was used (λ=366 nm). The process was followed by spectrophotometric measurements (*Figure 2*).

Investigation of the scavenging properties

EPR spectra of samples containing different concentrations of MTX, trans-PHX and cis-PHX were recorded. The average amplitudes of the EPR-peaks were investigated in all cases. The rate of the production of reactive oxygen species (ROS) was applied to describe the scavenging effect of MTX and PHX.

All the compounds decreased the ROS production rate. This decrease was more pronounced in the presence of MTX than in the presence of *cis*-PHX, but *trans*-PHX reduced ROS production the most.

Trolox equivalent antioxidant capacity (TEAC) values were determined by using ABTS scavenging assay. There was no significant difference between the TEAC values of *trans*-PHX and *cis*-PHX, but both isomers of PHX showed a higher antioxidant capacity than MTX.

Investigation of host-guest complexes

Thermodynamic properties of 1:1 complexes of the isomers of PHX and two cavitand derivatives (TAC and TDC) were determined applying fluorimetric measurements.

Stability constants were calculated by the Benesi-Hildebrand method, then enthalpy change, entropy change, and Gibbs free energy values were determined using the van 't Hoff equation.

Opposite temperature dependence of the stability of PHX-TAC and PHX-TDC complexes was found. These results imply different complex formation mechanisms at different temperature range.

Molecular modelling studies suggest formation of complexes with different conformations as background of the experimental results.

4. Conclusions

Based on the investigation of the scavenging properties it can be concluded that *trans*-PHX may have more pronounced anti-inflammatory and tissue-protective effects than MTX, despite the lack of its antineoplastic effect.

The host–guest complex formation of PHX was described for the first time. PHX is a promising new molecule whose cytotoxic effect can be regulated with light. Complex formation can be another method that could make the physiological effect of this molecule more adjustable.

5. Acknowledgements

This work was supported by the 2019-2.1.11-TÉT-2019-00042 and the OTKA K-137793 projects.

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