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Good oral absorption prediction on non-nucleoside benzothiadiazine dioxide human cytomegalovirus inhibitors using combined chromatographic and neuronal network techniques

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Abstract—The current drugs available against human cytomegalovirus (HCMV) suffer from a number of shortcomings such as toxic side effect, poor bioavailability and/or risk for emergence of drug-resistance virus strains. Due to these limitations, the development of new drugs against HCMV is of great interest. Taking into account the therapeutic potential of benzothiadiazines dioxides (BTD) derivatives, it is most important to know their oral bioavailability because all the current clinical drugs are poorly absorbed. In this work, the utility of CODES neural networks and biopartitioning micellar chromatography (BMC) in predicting pharmacokinetic properties has been used to estimate the oral absorption of BTD derivatives and their efficacy has been verified. The results indicate higher values for BTD derivatives than the currently licensed anti-viral agents.

1. Introduction

The limitations of current therapies for human cytomegalovirus (HCMV) coupled with the continued impact of HCMV disease on the immunocompromised host are the driving force for the development of new drugs against HCMV.[1] The currently available anti-HCMV drugs (ganciclovir, foscarnet, cidofovir, fomivirsen and valganciclovir) suffer from a number of shortcomings such as toxic side effect, poor bioavailability and/or risk for emergence of drug-resistance virus strains.[2] This has prompted the search for new anti-HCMV agents that are more specific in their anti-HCMV action and will not show the shortcomings of the older compounds.[3] This search has yielded a wealth of novel compounds, most of which are, or behave as, non-nucleoside, that is, CMV423, pyrrolopyrimidines, dihydroisoquinolines, indolo-carbazoles, β-L-ribofuranosyl benzimidazoles and benzothiadiazines dioxides (BTD) derivatives. All of these compounds act on molecular targets that are different from that of the older compounds, targeted at the viral DNA polymerase.[4,5]

In our search for new anti-viral agents, we discovered the BTD-modified acyclonucleosides with marked activity against HCMV and varicella-zoster virus.[6] The optimization of their anti-viral activity is already described,[7,8] and the structural requirement for potency and toxicity has also been defined.[9,10] BTD are potent anti-HCMV drugs with a mechanism of action completely different from the current clinical drugs. They act in the first stages of the viral replicative cycle and are effective when viral resistance appears. Taking into account the therapeutic potential of BTD derivatives, it is most important to know their oral bioavailability because the great majority of the current clinical drugs are poorly absorbed. Only valganciclovir, the valine ester of ganciclovir, has been recently approved to overcome the poor oral absortion of the current clinical drugs, acting as oral pro-drug of ganciclovir.[11]

We report here the oral absorption prediction of BTD using two different methods. The first one is the in silico CODES/neural network model while the second is the experimental biopartitioning micellar chromatography (BMC). These methods are quite effective in predicting drug-like properties and will allow the selection of drug candidates to be introduced in the early stages of drug discovery programs.

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2. Results and discussion

The early ADME considerations in preclinical development would help to avoid costly late stage preclinical and clinical failures.[12] A number of in silico, in vitro and in vivo techniques are available to screen compounds for ADME characteristic.[13] Among them, the well known 'rule of five', neural networks or artificial membrane permeability have been successfully used for the prediction of properties and drug-likeness.[14–16] Good absorption is one of the most important criteria in selecting new drug candidates for development, because the oral route generally is the preferred one in clinical management for reasons of ease and compliance by the patient.[17]

Recently a new neural network model able to predict oral absorption of structurally diverse drugs in use clinically has been described in our group using CODES/neural networks.[18] CODES is an efficient and easy way to encode the molecules and has been used in the QSAR field[19] and in the determination of pharmaco kinetic properties of very different structural compounds.[20] CODES generates descriptors, which contain all the information that underlines the chemical structure of the molecule. This model showed a good agreement in experimental versus calculated results with $r = 0.95$, which led us to use it as a predictive tool.

In this work we have applied the methodology previously reported[18] to predict the oral absorption of some BTDs derivatives (compounds **1**–**3** in Table 1). As reference standard, we have chosen two drugs currently used in clinical HMCV therapy, acyclovir and ganciclovir. For these five molecules CODES descriptors were generated and the reduction of dimension was performed. These last codes were used as input data in our previous training neural network. The predicted values, expressed as the percent of drug absorbed after oral administration, are reported in Table 1. From these data we can conclude that BTD derivatives have a much better oral absorption profile than the standard reference.

On the other hand, BMC have shown to emulate in vitro the biological barriers and mimic the drug partitioning process in biological systems based on the measure of their retention data (K_{BMC}). The K_{BMC} of compounds in BMC depends on their interactions with modified reversed stationary phase and micelles presents in the mobile phase. These interactions are governed by hydrophobic, electronic and steric properties of compounds, in the same way as they occur in natural biomembranes, showing the usefulness of BMC as an in vitro technique for predicting human drug absorption by passive diffusion.[21]

Table 1. Data of predicted percent absorbed using CODES oral absorption model and BMC methodology

Compound	% Absorption using CODES	% Absorption using BMC
Acyclovir[a]	41	56
Ganciclovir[a]	3	<10
BTD 1	67	90–95
BTD 2	99	90–95
BTD 3	94	90–95

[a] Blood level percentage after oral administration of acyclovir (15%) and Ganciclovir (3%).[23]

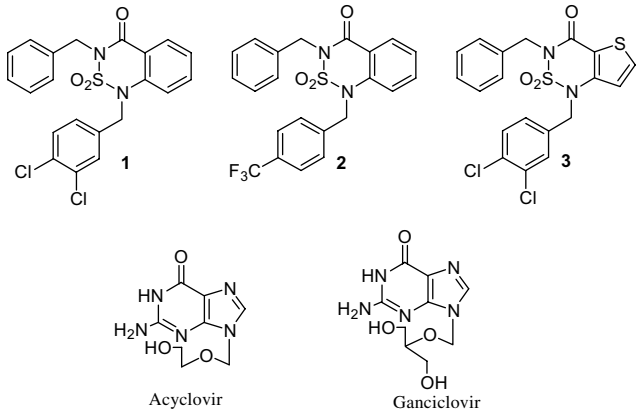

Figure 1. BTD derivatives and standard compounds used in the study.

This chromatographic system uses aqueous solution of Brij 35 as micellar mobile phase, at pH 6.5 and 7.4, and the column (Kromasil octadecyl-silane C_{18}) was thermostated at 36.5 °C. Statistical analysis of the calculated K_{BMC} data was evaluated using linear regression techniques. These values showed that the relationship between % oral drug absorption and K_{BMC} data was statistically significant at 95% confidence level.[22]

This methodology was employed here for the prediction of the oral absorption of BTD derivatives **1**–**3** as well as the reference standard compound (Fig. 1).

As a result, the BTD oral absorption determined by this methodology was comprised between 90% and 95%, while the same data for acyclovir and ganciclovir was much lower (Table 1).

3. Conclusions

Anti-viral agents currently licensed for the treatment of HCMV infection include ganciclovir, foscarnet, cido fovir, fomivirsen and valganciclovir. However, toxicity associated with these drugs, poor oral bioavailability and high relapse rates have made their use less than optimal. In this work, the utility of CODES neural networks and BMC in predicting pharmacokinetic properties has been used to estimate the oral absorption of BTD derivatives and their efficacy has been verified. The results indicate higher values for BTD derivatives than the currently licensed anti-viral agents. A good correlation between predicted dose absorbed by using in silico or experimental methods was observed. Because a successful drug is really a combination of biological activity and drug-like properties, BTD derivatives appear as promising compounds for the treatment of HCMV infection.

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