CHEMICAL ENGINEERING TRANSACTIONS
VOL. 32, 2013
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at: www.aidic.it/cet
Chief Editors: Sauro Pierucci, Jiří J. Klemeš

ISBN 978-88-95608-23-5; **ISSN** 1974-9791
DOI: 10.3303/CET1332132

Combination of classical and molecular modeling approaches to investigate the effect of antipsychotic drugs on cell proliferation kinetics

Enrico Pieroni[a], Amit Kumar[a], Massimo Pisu[a], Vito Genna[a], Alessandro Concas[a], Giacomo Cao*[,a,b]

[a]CRS4, Parco Scientifico e Tecnologico, Edificio 1, 09010 Pula, Cagliari, Italy
[b]Dipartimento di Ingegneria Meccanica, Chimica e dei Materiali, Piazza d'Armi, 09123 Cagliari, Italy
giacomo.cao@dimcm.unica.it

A novel approach to investigate the effect of drugs on cell proliferation is proposed and addressed in a case study concerning Human Umbilical Vein Endothelial Cells (HUVECs) *in vitro* cultivation in the presence of an antipsychotic drug, i.e. Clozapine. A mathematical model based on mass structured population balance (PB) has been proposed to simulate experimental data concerning cell count and its size distribution, as well as the nutrient (glucose) concentration, as a function of cultivation time. Molecular modeling studies have been in parallel performed to investigate the drug interaction with serotonin receptor (5-HT_{2A}R) and the dopamine one (D_2R). In particular, by combining classical and molecular modeling approaches, it is shown that it is possible to quantitatively estimate one of the most relevant receptor parameter (i.e. the allosteric constant related to the equilibrium between active and inactive receptor state). Results show also that Clozapine plays a role as an inverse agonist with serotonin and dopamine receptors providing a contribution to the cell growth inhibition mechanism. The obtained results are in good agreement with the known pharmaceutical actions of the drug and show the viability of the proposed methodology.

1. Introduction

The investigation of inhibitory/toxicity effect of drugs on cell proliferation is undoubtedly useful in drug testing and development. *In vitro* expansion of a cell population is in general an essential step for the systematic optimization of culture conditions. At the same time, a targeted investigation on *in vitro* mammalian cell expansion, may contribute to evaluate the effect of drugs in both the cell metabolism and the mitotic process. The evaluation of the intrinsic kinetics of cell proliferation may be performed using static cultivation system (i.e. Petri dishes). The interpretation and rationalization of the corresponding experiments, which provide a clear contribution to the understanding of the complex biological mechanisms involved in stem cell expansion/differentiation, may be achieved by means of suitable mathematical models based on PB (Mancuso et al, 2010). A deeper analysis on inhibition mechanisms of drugs in human cell expansion may be performed by taking advantage of molecular dynamics (MD) approach (van Gunsteren et al., 1990). In this way, the investigation of the interaction between ligands and their respective receptors can be carried out at molecular scale. Typically, the first step in molecular approach consists in modeling drugs structure and related force field, required when performing MD simulations, by means of suitable database and software tools (van Gunsteren et al., 1990). Structure of receptor, if is not solved by crystallographic techniques, as for the case of 5-HT_{2A}R, may be modeled by means of homology modeling technique (van Gunsteren et al., 1990). Next, after the identification of potential binding sites for the investigated drug, docking analysis may be performed by using suitable software tools. Finally, molecular analysis of the dynamic interaction between ligands and receptors can be carried out by taking advantage of MD techniques. The investigation of the affinity between drug and

Please cite this article as: Pieroni E., Kumar A., Pisu M., Genna V., Concas A., Cao G., 2013, Combination of classical and molecular modeling approaches to investigate the effect of antipsychotic drugs on cell proliferation kinetics, Chemical Engineering Transactions, 32, 787-792 DOI: 10.3303/CET1332132

the putative receptor, evaluated by docking and MD, provides significant insights to interpret cell expansion inhibition mechanism.
In this work our attention has been focused on the combination of classical and molecular modeling approaches in order to elucidate the inhibitory mechanism of an atypical antipsychotic drug (i.e. Clozapine) on HUVECs cell growth and proliferation. To the best of our knowledge the combination of these two distinct modeling approaches constitutes a first attempt characterized by a high degree of novelty in the field of cell proliferation kinetics of pharmacological interest.

2. Classical and molecular modeling

The mathematical model has been developed following a classical chemical engineering approach on the basis of a recent published paper (Mancuso et al., 2010). In particular, a mass structured PB to simulate cell growth and proliferation, has been considered by assuming uniform spatially distribution of spherical cells (Pisu et al., 2003). In the present work we report only model equations properly modified, with respect to the above mentioned paper, with the aim of taking into account glucose as limiting nutrient instead of O_2. The latter one, as verified experimentally, is not kinetically limiting since its concentration in the culture medium remains constant during cell cultivation at a value close to its saturation level (i.e. 7 mg/L). Thus, considering the glucose concentration in the culture medium, C_{GLU}, the following mass structured population balance can be written:

$$\frac{\partial \psi(m,t)}{\partial t} = -\frac{\partial [v\,\psi(m,t)]}{\partial m} + 2\int_{m}^{\infty} \psi(m',t)\ \chi_M\ (m',C_{GLU})\, p(m,m')\ dm' - \psi(m,t)\,\chi_M(m,C_{GLU}) \tag{1}$$

along with initial and boundary conditions

$$\psi(m,t) = \psi_0(m) \quad for \quad t = 0 \quad and \quad m > 0 \tag{2}$$

$$\psi(m,t) = 0 \quad for \quad t > 0 \quad and \quad m = 0 \tag{3}$$

The cell division rate, χ_m, appearing in Eq(1), is a function of the time rate of change of cell mass, v_m, defined as follows:

$$v(m,C_{GLU}) = \left(\frac{3}{d_c}\right)^{2/3} (4\pi)^{1/3} m^{2/3} \frac{\mu'\ C_{GLU}}{(C_m + C_{GLU})\left(1 + \dfrac{C_I}{K_I}\right)} \Phi(t) \tag{4}$$

In Eq (4) v_m is expressed in the Michaelis-Menten form where the metabolic term is proportional to cell surface. The complex cellular processes, when a specific drug inhibits cell growth and its division, are "lumped" by means of a simple non-competitive inhibition typical of biological enzymatic systems through a properly modified Michaelis-Menten equation. Thus, when a specific drug is added to the culture medium, model equation accounts for the drug effect through the introduction of a specific inhibition constant, K_I. Eq (4) also describes the so-called "contact inhibition" which occurs when the monolayer cultivation on Petri dish is close to reach the confluence, where the rate of proliferation starts to decrease. This effect is accounted for through a specific function, $\Phi(t)$, defined in detail elsewhere (Mancuso et al., 2010). Finally, glucose consumption is simulated through the following material balance:

$$\frac{\partial C_{GLU}}{\partial t} = -\left(\frac{3}{d_c}\right)^{2/3} (4\pi)^{1/3} Y \frac{\mu'\ C_{GLU}}{(C_m + C_{GLU})\left(1 + \dfrac{C_I}{K_I}\right)} \int_0^{\infty} m^{2/3}\ \psi(m,t)\ dm \tag{5}$$

with

$$C_{GLU} = C_{GLU}^0 \quad for \quad t = 0 \tag{6}$$

The rate of glucose consumption is related to the cell mass growth by a specific yield, Y, which represents the mass of nutrient consumed per unit of cell mass produced. The numerical solution of partial differential Eq (1) along with the initial and boundary conditions Eq (2) and Eq (3) is performed by means of the well-known method of lines which allows one to obtain a system of ordinary differential equations in time. The

latter one, together with the differential Eq (5), is then integrated by means of standard numerical libraries (Gear method, IMSL) as an initial value problem.
As discussed in the introduction, we also perform investigation at the molecular scale level by considering proper docking and MD calculations taking advantage of public domain software tools such as AutoDock and NAMD. Specifically, Clozapine targets, i.e. Serotonine (5-HT_{2A}) and Dopamine receptors (D_2), are modeled by homology (since their structure is not available) and refined by MD simulations. Subsequently, the binding of drug with the receptors is extensively analyzed by long MD runs and in parallel by flexible dockings to double-check the results. It is well known that molecular simulations can provide free energy variation, ΔG, of the binding process between ligand drug and receptor, that can be converted into the affinity constant for the drug to the receptor, K_{aff}, through the Gibbs-Helmholtz equation:

$$\Delta G = -RT \ln(K_{aff}) \tag{7}$$

$$K_{aff} = \frac{[RD]}{([R][D])} \tag{8}$$

where K_{aff} is the equilibrium constant of the reaction R+D=RD, which is typically adopted to quantitatively describe the drug (*D*) /receptor (*R*) interaction leading to the formation of the drug/receptor complex (*RD*).
Let us now analyze how classical and molecular approaches can be combined to provide data integration and working hypothesis for functional mechanisms of drug receptor interaction at both molecular and cellular level, in the attempt to interpret cell growth inhibition. To this aim we consider the so called “two-state model”, widely used in pharmacology (Giraldo et al., 2007) and reported in Appendix. Such a model is particularly useful in describing the action of a drug on the basal activity of cell and consequently on its proliferative capacity. Assuming two possible states of receptor (i.e. active or inactive) the equilibria reported in Eq (A1) hold while the related equilibrium constant are represented in Eq (A2). The main result of the “two-state model” is the capability to predict an inhibitory action induced by the drug (acting as an inverse agonist) on the biological basal activity through the definition of the so-called drug efficacy index *IE*. The latter one may be evaluated as shown in Eq (A3) from the knowledge of the activity *E*, as well as the constitutive E_C, and minimal E_∞ activities, defined in Eq (A4). Next, to link classical and molecular modeling approach, we propose to define a biological inhibitory effect on cell growth, *BIE*, that can be applied to classical PB model and finally compared to inhibition index *IE*. Since the inhibition effect due to the drug reduces cell growth rate and consequently the mitotic process such biological inhibitory effect can be defined as follows:

$$BIE = \frac{\nu(C_I = 0) - \nu(C_I \neq 0)}{\nu(C_I = 0)} = \left(1 + \frac{K_I}{C_I}\right)^{-1} \tag{9}$$

where ν is the time rate of mass change of the classical PB model calculated in absence ($C_I = 0$) or in the presence of drug ($C_I \neq 0$), respectively. Finally, by comparing the biological cell-scale inhibition index *BIE* reported in Eq (9) with the inhibitory effect of the drug *IE* reported in Eq (A3), we can obtain a useful biological interpretation of the inhibition constant K_I:

$$K_I = EC50 \tag{10}$$

which, by considering Eq (A5), can be expressed as

$$K_I = \frac{1}{K_{aff}} \tag{11}$$

where the link between classical and molecular modeling approach may be clearly seen.
On the other hand, since Clozapine displays a strong inverse agonism behavior the parameter *EC50*, given by Eq (A5), may be expressed as in Eq (A6). Moreover, if MD standard simulations are related only to the receptor in the inactive state, it is apparent that the affinity constant, K_{aff}^I, obtainable through MD calculations, corresponds to the inverse of the constant K_{in} Eq (A6). It is then possible to identify a new relationship between the affinity constant K_{aff}^I and the parameter *EC50* as reported in the same Eq(A6).

Therefore, by taking into account Eq (10) and Eq (A6), one can evaluate the allosteric constant, K_{ai} related to the equilibrium between active and inactive receptor state as follows:

$$K_{ai} = \frac{1}{K_I K_{aff}^I - 1} \tag{12}$$

Eq(11), in the general case, and Eq (12), when it is possible to assume strong inverse agonism behavior for the investigated drug and for MD calculations referring only to the inactive state of the receptor, may represent simple and valuable tool tools to combine classical and molecular modeling approaches in the field of cell proliferation kinetics of pharmacological interest.

3. Results and discussion

Results of the mathematical model developed by a classical approach are compared with experimental data in terms of cells size distribution, total cell number and glucose concentration as a function of the cultivation time. In particular, in the present work, we take advantage of experimental data published in a previous paper (Mancuso et al., 2010) concerning cell count and its size distribution. Data on glucose concentration as a function of time, considered in this paper, refer to the same work although they were not yet published. By direct comparison between model results and experimental data in terms of total cell count and glucose concentration as a function of cultivation time for trials in absence of drug (CTRL) two model parameters (i.e. maximum rate of cell growth, μ', and inhibition contact parameter, α_P) have been estimated by means of a non-linear fitting procedure. All model parameters are reported in Table 1. The agreement between model results (solid line) and experimental data (symbols) appears rather good both in terms of total cell count (Figure 1a) and glucose concentration (Figure 1b). The relative error obtained from the fitting procedure is very low (i.e. 4 %), thus confirming the model reliability. From Figure 1a it may be also observed that the model well interprets the exponential growth which occurs during the first days of the cultivation (up to 2-3 days) and properly simulates the effect of the contact inhibition which arises after to about 3 days of cell expansion. The confluence is not fully reached after six days of culture and therefore the expansion curve doesn't display the typical plateau. The predictive capability of the model is also demonstrated in Figure 2 where the comparison between model results and experimental data is shown in terms of cell distribution (percentage) as a function of cell diameter. For the sake of brevity, the comparison between experimental data and model prediction is shown only after 2 days of cultivation. Similar results have been obtained for other cultivation times investigated. Next, the inhibition constant K_I of the investigated drug, has been estimated by a fitting procedure where model results are compared with experimental data in terms of total cell count as a function of cultivation time for trials performed by adding the drug. Model parameters used in this simulation and the tuned value of K_I are reported in Table 1. In particular, the inhibition constant resulted to be K_I = 38.5 μM with a relative error of the fitting procedure of about 10 %. The comparison between model results (dashed line) and experimental data when drug is added in the culture medium is shown in Figure 1a in terms of total cell count and in Figure 1b in terms of glucose concentration as a function of time. As expected, drug affects the proliferative capacity of cells (Figure 1a) thus reducing the consumption of glucose (Figure 1b).

Analysis of the inhibitory effect of Clozapine on cell expansion has been also performed at molecular level. First, the starting structure of the 5-HT_{2A} receptor was identified as discussed in the modeling section. The corresponding result is shown in Figure 3. From the model inspection and the corresponding docking analysis three potential binding sites for Clozapine have been identified (Figure 3). Going from the extra-cellular environment to the intracellular space, the first binding site is localized in the extra-cellular domain, the second one is in a bit deeper trans-membrane region, and corresponds to the putative binding site for the 5-HT endogenous ligand, and the third one results close to the intracellular loop at the interface with the cytoplasm. Results of binding free energies shows that the most probable binding between ligand and receptor may occur in the first putative site (ΔG_1 = -7.63 kcal/mol, ΔG_2 = -3.0 kcal/mol, ΔG_3 = -6.25 kcal/mol). On the other hand, the value of K_{aff}^{-1} = 39.2 μM estimated for the intracellular binding site is very close to the one of K_I = 38.5 μM calculated by the classical approach as it could have been expected by taking advantage of Eq (11) which represents one of the results obtained when combining classical and molecular ones, as proposed in this paper. This is a quite intriguing issue. The drug cannot of course enter the cell from the intracellular region, but one can speculate that a saturation effect can occur at increasing drug concentration. At small drug concentration, the receptor can change configuration upon Clozapine binding to the extracellular site, thus allowing the drug, at higher concentration, to pass the energy barrier

and move through the intra-barrel area, and finally reach the intracellular site where it can exert a loop restraint function and then a G-protein decoupling action. Further studies are required to elucidate this aspect and the possibility of a drug transport across the serotonin receptor. Concerning the dopamine one, drug dock to an unique putative binding site with extremely high affinities (ΔG = -7.8 kcal/mol). Results obtained by means of docking analyses, in terms of free energies, have been fully confirmed by means of MD simulations. Let us finally take into account the case when molecular analysis is considered to capture only receptor and complex drug-receptor in inactive state. By taking advantage of equation (12) we can then estimate the allosteric constant K_{ai} related to the equilibrium between active and inactive receptor state from the main relevant parameters of classical and molecular modeling. Result show that the values of K_{ai} are similar for both receptors (i.e. K_{ai} = 0.12 for serotonin receptor and K_{ai} = 0.09 for the dopamine one). These results appears to be reasonable, thus explaining the constitutive activity of the two receptors through a dominance of the active form (~10 fold) with respect to the inactive state.

Finally, for the correct interpretation of our attempt to link the molecular to the cellular proliferation inhibition, it should be noted that a simplified model of drug interaction with a single site has been considered, while *in vitro* inhibitory mechanisms will be the combination of several, largely unknown, molecular mechanisms including interaction with different sites of other enzymes, receptors and proteins not considered in this work. With an improved computational and modelling effort, these additional contributions could be integrated, thus helping to better understand the cell growth inhibition and receptor molecular mechanisms.

Table 1: Parameter values of PB based model used in simulations

Parameter	Meaning	Value	Unit	Reference
N	Number of cells	90,000	Cells	Mancuso et al. (2010)
ϕ_a	Petri area	920	mm^2	Mancuso et al. (2010)
V	Volume	1,500	mm^3	Mancuso et al. (2010)
C_{GLU}	Glucose concentration	4.44	mM	Mancuso et al. (2010)
C_I	Inhibitor concentration	40	μM	Mancuso et al. (2010)
d_C	Cell mass density	1.14 10^6	ng/mm^3	Mancuso et al. (2010)
μ	Average mass of dividing cells	4.0	ng	Mancuso et al. (2010)
σ	Standard deviation of *f(m)*	1.57	ng	Mancuso et al. (2010)
q	Parameter of β function	40	-	Mancuso et al. (2010)
C_m	Michaelis Menten constant	6	mM	Busik et al. (2002)
$Y\mu'$	Model parameter (Eq(5))	6.44 10^{-7}	$mmol/(mm^2h)$	Busik et al. (2002)

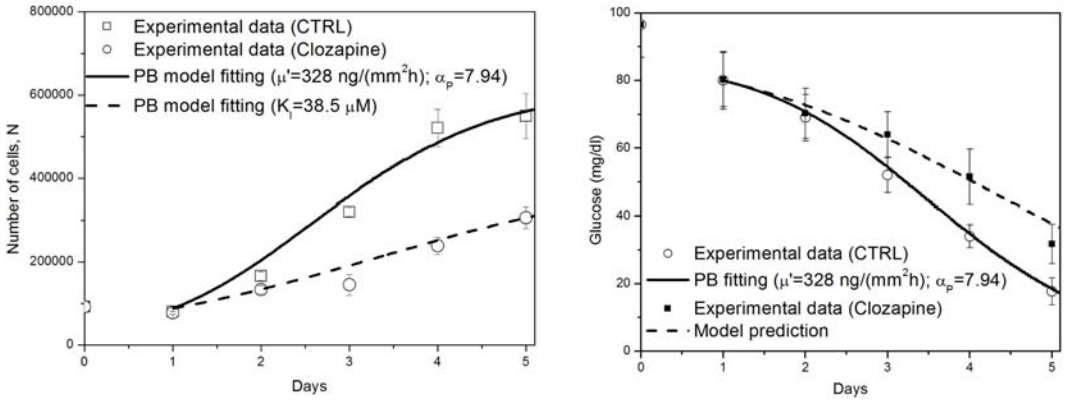

Figure 1: Comparison between model results and experimental data in terms of total cell number (a) and glucose concentration (b) as a function of cultivation time

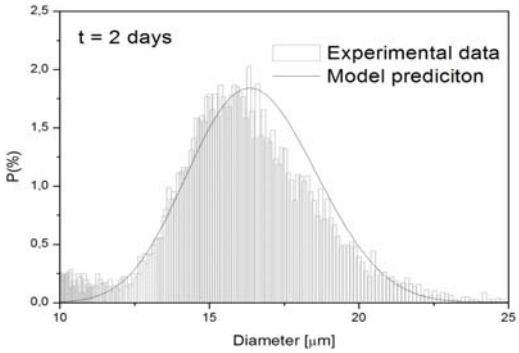

Figure 2: Comparison between model predictions and experimental data in terms of cell distribution (percentage) as a function of cell diameter

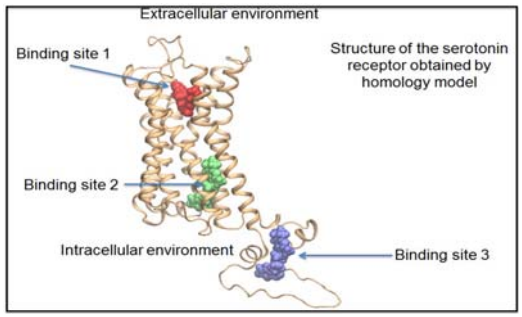

Figure 3: Serotonin receptor (5-HT$_{2A}$R) obtained by homology model and the three putative binding sites identified by docking analysis

References

Busik J.V., Olson L.K., Grant M.B., Henry D.N., 2002. Glucose-induced activation of glucose uptake in cells from the inner and outer blood-retinal barrier, Invest Ophthalmol Vis. Sci., 43, 2356-2363.

Giraldo J., Serra J., Roche D., Rovira X., 2007. Assessing receptor affinity for inverse agonists: Schild and Cheng-Prusoff methods revisited, Curr. Drug. Targtes, 8, 197-202.

van Gunsteren W.E., Berendsen H.J.C., 1990, Computer Simulation of Molecular Dynamics, Angrw. Chem. Int. Ed. Engl., 29, 992-1023.

Mancuso L., Scanu M., Pisu M., Concas A., Cao G., 2010, Experimental analysis and modelling of in vitro HUVECs proliferation in the presence of various types of drugs, Cell. Prolif, 43, 617-628.

Pisu M., Lai N., Cincotti A., Delogu F., Cao G., 2003. A simulation model for the growth of tissue engineered cartilage on polymeric scaffolds, Chemical Engineering Transactions, 3, 1307-1312.

Appendix

$$\begin{array}{ccc} R_{in} & \xleftrightarrow{K_{ai}} & R_{ac} \\ + & & + \\ D & & D \\ K_{in}\uparrow\downarrow & & \downarrow\uparrow K_{ac} \\ R_{in}D & \xleftrightarrow{W_{ai}} & R_{ac}D \end{array} \tag{A1}$$

$$K_{ai}=\frac{[R_{in}]}{[R_{ac}]},\quad K_{ac}=\frac{[R_{ac}][D]}{[R_{ac}D]},\quad W_{ai}=\frac{[R_{in}D]}{[R_{ac}D]},\quad K_{in}=\frac{[R_{in}][D]}{[R_{in}D]}\qquad W_{ai}=K_{ai}\frac{K_{ac}}{K_{in}} \tag{A2}$$

$$IE=\frac{E_C-E}{E_C-E_\infty}=\left(1+\frac{EC50}{[D]}\right)^{-1} \tag{A3}$$

$$E=\frac{[R_{ac}]+[R_{ac}D]}{[R_{ac}]+[R_{ac}D]+[R_{in}]+[R_{in}D]};\ E_C=\frac{[R_{ac}]}{[R_{ac}]+[R_{in}]}=\frac{1}{1+K_{ai}};\ E_\infty=\frac{[R_{ac}D]}{[R_{ac}D]+[R_{in}D]}=\frac{1}{1+K_{ai}\dfrac{K_{ac}}{K_{in}}} \tag{A4}$$

$$EC50=\frac{1+K_{ai}}{\dfrac{1}{K_{ac}}+\dfrac{K_{ai}}{K_{in}}};\ K_{aff}=\frac{[RD]}{[R][D]}=\frac{([R_{ac}D]+[R_{in}D])}{([R_{ac}]+[R_{in}])[D]}=\frac{\dfrac{1}{K_{ac}}+\dfrac{K_{ai}}{K_{in}}}{1+K_{ai}}=\frac{1}{EC50} \tag{A5}$$

$$EC50=\frac{1+K_{ai}}{K_{ai}}K_{in};\ K^{I}_{aff}=\frac{[R_{in}D]}{[R_{in}][D]}=\frac{1}{K_{in}}=\frac{1}{EC50}\left(\frac{1+K_{ai}}{K_{ai}}\right) \tag{A6}$$