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Exploration of the intercellular heterogeneity of topotecan uptake into human breast cancer cells through compartmental modelling

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ABSTRACT

A mathematical multi-cell model for the *in vitro* kinetics of the anti-cancer agent topotecan (TPT) following administration into a culture medium containing a population of human breast cancer cells (MCF-7 cell line) is described. This non-linear compartmental model is an extension of an earlier single-cell type model and has been validated using experimental data obtained using two-photon laser scanning microscopy (TPLSM).

A structural identifiability analysis is performed prior to parameter estimation to test whether the unknown parameters within the model are uniquely determined by the model outputs. The full model has 43 compartments, with 107 unknown parameters, and it was found that the structural identifiability result could not be established even when using the latest version of the symbolic computation software MATHEMATICA. However, by assuming that *a priori* knowledge is available for certain parameters, it was possible to reduce the number of parameters to 81, and it was found that this (Stage Two) model was globally (uniquely) structurally identifiable. The identifiability analysis demonstrated how valuable symbolic computation is in this context, as the analysis is far too lengthy and difficult to be performed by hand.

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1. Introduction

In recent years, anti-cancer drugs with the ability to inhibit DNA topoisomerase I, have become one of the main foci in drug discovery. Topotecan (TPT, Hycamtin®), a cytotoxin alkaloid chemotherapy drug, is a semi-synthetic, water-soluble derivative of the drug camptothecin (CPT) [1]. CPT was originally isolated from a Chinese tree, *Campthotheca acuminata* which is widely found in South East Asia where it is known as Xi Shu. The main drawbacks of CPT are: (i) its poor water solubility; (ii) its lactone instability; (iii) the reversibility of the drug–target interaction; (iv) its drug resistance; and (v) its high toxicity [1,2]. In contrast, the CPT analogue TPT has a more stable structure [3], and clinical evidence for the cytotoxic properties of TPT against lung, breast and ovarian cancers is well known [1].

Topoisomerase I, a monomeric protein, is an enzyme that acts to relax negative and positive super coils that accumulate in DNA during DNA replication [4] and transcription (S-phase), chromosome segregation and the efficient traverse of mitosis [5]. Topoisomerase I is necessary within the nucleus for cell proliferation and its level is constant throughout the cell cycle [6].

Acquired (external factors) or inherited gene mutations lead to the alteration of normal cell proliferation signals and hence a loss of cell cycle controls [7]. This leads to uncontrolled cell proliferation and, as a consequence, tumours develop and become established. The cancer cells are characterised by a rapid proliferation rate which is paralleled by topoisomerase I activity. Since TPT activity is sensitive to proliferating cells and is S-phase specific [8], cancer cells are more likely to be targeted by the drug due to the increased levels of topoisomerase I.

TPT undergoes rapid, reversible, pH-sensitive, non-enzymatic hydrolysis [9] from a ring-closed lactone form (TPT_L) that predominates at low pH (<4) to an open-ringed hydroxyl form (TPT_H), which predominates at high pH (>10). The pharmacologically active lactone form, TPT_L, is a DNA topoisomerase I inhibitor which binds to the nuclear DNA. It disturbs the process of replication resulting in DNA breaks (S-phase) by trapping topoisomerase I and DNA in a covalently bound ternary complex. It also interferes with the catalytic function of the enzyme without trapping the covalent complex. As a result, cell division is prohibited, and cell arrest is caused in the target area [10].

Identification of dissimilitude in the uptake kinetics into human breast cancer cells (MCF-7 cell line) of the topoisomerase I inhibitor topotecan (Hycamtin®; SmithKline Beecham, Pharmaceutics) is essential for the development of therapeutic agents in oncology.

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The search for cell heterogeneity corresponds to a search for knowledge of specific factors involved in the differentiation of subpopulations in most neoplasms. Evidence of heterogeneous characteristics expressed in clonal cancer cells has been recorded in the literature [11,12]. This heterogeneity is further supported in this paper by compartmental modelling of drug uptake.

In this work, a multi-cell compartmental model is derived to describe the *in vitro* kinetics of TPT following administration into a culture medium containing a population of human breast cancer cells (MCF-7 cell line). The proposed model is an extension of that presented in [13], which did not differentiate between the behaviours of different cells. Thus, in the previous model, a heterogeneous population of cells was treated as a single averaged homogeneous type. A similar approach was used in [14,15] for compartmental models that used high performance liquid chromatography (HPLC) data during parameter estimation.

Although the population of 10^5 cells modelled in this study initially originated from a single cloning stream, evidence of the intercellular heterogeneity of breast cancer cells has been found in *in vitro* experiments [16,17].

The previous model [13] did not seek to address intercellular heterogeneity, but rather sought to represent the average population response. This established a firm foundation for the development of a more complex model that contains more compartments and parameters. Therefore, rather than treating the cells as a homogeneous population, the possible features of intercellular heterogeneity in TPT drug uptake is investigated through a model that includes different cell types. The source and effects of heterogeneity in cellular uptake kinetics is sought from estimates for the unknown parameters in the compartmental model. The experimental data used for the parameter estimation were collected from an *in vitro* study of the interaction of a culture of human breast cancer (MCF-7) cells and TPT using two-photon laser scanning microscopy (TPLSM).

Topotecan is a UV-excitable camptothecin and these auto-fluorescent properties have been exploited to evaluate drug resistance in differently derived cell lines using confocal microscopy [18]. The binding characteristics of the drug to DNA were observed in spectroscopy studies by using the high two-photon absorption cross-section properties demonstrated by TPT [19].

The experimental data were collected for the model using TPLSM; investigations [20] showed that TPT is UV light excitable, so that TPT will also be infrared two-photon excitable. The principle of two-photon excitation of fluorescence [21] is that two photons of longer wavelength light are simultaneously absorbed by a fluorochrome that would normally be excited by a single photon with a shorter wavelength. Hence, the fluorochrome excitation is limited to the point of focus by the non-linear optical absorption property of two-photon excitation.

The advantages given by the features of non-linear excitation are that the images obtained have high contrast and are free of out-of-focus light. Also, this method is particularly beneficial to live cell imaging as the light is only restricted in the focal plane, thereby decreasing photo-bleaching of the indicator and photo-damage to the cells. Although this method is not able to differentiate the active form (TPT_L) from the inactive form of drug (TPT_H), as shown in Fig. 1, it has been considered to be a reliable method for the collection of high quality data. The experimental data only provide the total concentration of drug within the extracellular location, cytoplasm and nucleus (see Fig. 2).

The experiment performed to collect data for the purposes of parameter estimation imposes an output structure on the model. This output structure comprises the functions of the model variables that are directly measured in the proposed experiment (the output, or measurement, vector). Prior to the parameter estimation for the new multi-cell model, a structural identifiability analysis [22] is vital to test whether the output structure corresponding to the TPLSM experiment uniquely determines the unknown parameters. A globally identifiable model indicates that the estimated parameters are unique for a given model output vector. Structural identifiability is independent of the experimental data and the analysis of it is a fundamental prerequisite to experiment design, system identification and parameter estimation.

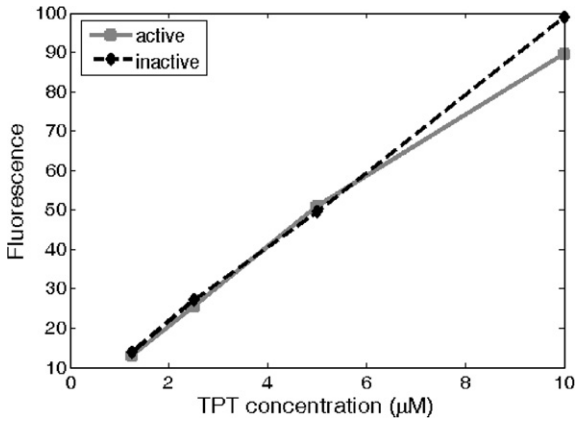

Fig. 1. The fluorescence intensity of topotecan in MCF-7 breast cancer cells, plotted against the concentration of the active and inactive form of the drug.

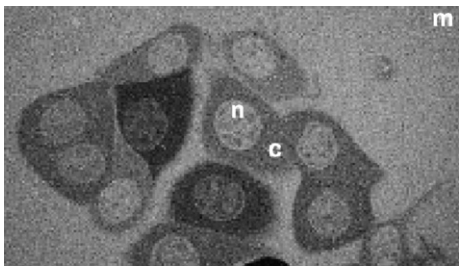

Fig. 2. A TPLSM image of topotecan localisation in MCF-7 breast cancer cells; where **n** denotes the nucleus, **c** the cytoplasm and **m** the medium.

The ultimate goal of this paper is the use of compartmental modelling techniques to implement a valid model to seek for the identification and verification of the importance of heterogeneity in the uptake kinetics of the drug in human cancer cells.

2. The multi-cell model

For a single tumour, not all the cells are homologous; rather there is a range of cells in a population expressing many different phenotypes. Cells might vary according to their structure (e.g., growth rate and morphology) and/or behaviour (e.g., invasion and metastasis) [23]. The verification and importance of heterogeneity of these cells is therefore explored using a new mathematical multi-cell compartmental model [24,25] of the interaction between TPT and a population of MCF-7 breast cancer cells [8].

The experiment, using TPLSM, to track the delivery of TPT to the sub-cellular compartments of a population of MCF-7 cells, was performed in a culture of 10^5 adherent cells. It was not feasible to measure all 10^5 cells, and 104 cells were physically measured. From these, data from 13 individual cells were selected as being representative of the full range of heterogeneity observed in response to the drug; these data are used to validate the multi-cell model proposed in this paper. The heterogeneous population of

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