Modelling of Tirapazamine Effects on Solid Tumour Morphology

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Abstract Bioreductive drugs are in clinical practice to exploit the resistance from tumour microenvironments especially in the hypoxic region of tumour. We presented a tumour treatment model to capture the pharmacology of one of the most prominent bioreductive drugs, Tirapazamine (TPZ) which is in clinical trials I and II. We calculated solid tumour mass in our previous work and then integrated that model with TPZ infusion. We calculated TPZ cytotoxicity, concentration, penetration with increasing distance from blood vessel and offered resistance from microenvironments for drug penetration inside the tumour while considering each cell as an individual entity. The impact of these factors on tumour morphology is also showed to see the drug behaviour inside animals/humans tumours. We maintained the heterogeneity factors in presented model as observed in real tumour mass especially in terms of cells proliferation, cell movement, extracellular matrix (ECM) interaction, and the gradients of partial oxygen pressure (pO_2) inside tumour cells during the whole growth and treatment activity. The results suggest that TPZ high concentration in combination with chemotherapy should be given to get maximum abnormal cell killing. This model can be a good choice for oncologists and researchers to explore more about TPZ action inside solid tumour.

***Index Terms*— AQ4N, Extra Cellular Matrix, Hypoxia and Tirapazamine.**

INTRODUCTION

Most common cancer treatments like chemotherapy and radiotherapy are facing a strong resistance from hypoxic regions inside the tumour. When a tumour reaches to a critical size approximately 10^6 cells the nutrients diffusion is insufficient to supply required amount of oxygen to the inner parts of the tumour initiating a situation called hypoxia (Gerlee and Anderson 2007).

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Hypoxia is recognized as a factor that helps the tumour cells survival by giving them more aggressive phenotypes. Majority of tumours with size greater than $1mm^3$ have got hypoxic regions because of irregular blood vessel structure and increased distance from blood vessels. The high rate of glycolysis has been shown in hypoxic regions of most tumours. (Shannon et al. 2003). Hypoxic cells were thought to be present at about 100-150 μm from functional blood vessels but now recent studies showed that hypoxia can be found at about 20-25 μm from blood vessels (Marcu and Olver 2006). Deformed capillary vessels of tumour and increased distance of tumour cells and blood vessels result in poor drug penetration (Marcu and Olver 2006). The hypoxia is a major challenge in the control of tumour while either using radiation or chemotherapy. Chemotherapy's major aim is to decrease down the number of tumour cells with a number of treatment cycles (Algoul et al. 2010). Tumour cells that are at distal locations from blood vessels are difficult to treat with chemotherapy. With increasing distance drug penetration slows down making it less effective. Cells with good distance from blood vessels are deficient in oxygen supply and slow in proliferation rate, show resistance towards effective chemotherapy treatment. Chemotherapy is not an effective way to treat hypoxic regions, as it is developed to kill cells having rapid division cycles (Brown 1999). Radiation, another cancer treatment works against tumour cells by damaging their DNA. This DNA damage remains permanent under the presence of oxygen molecules and results in cell death. So this cancer treatment is also most effective to those cells having sufficient oxygen (Bronwyn et al. 2003). Some bioreductive drugs are under high consideration to exploit these hypoxic regions with no or less harm to normal cells. Tirapazamine (TPZ) and AQ4N are under experiments and are in clinical practice as bioreductive drugs (Patterson and McKeown 2000). TPZ is in clinical phase II and III trials with radiotherapy and anti cancer drug cisplatin respectively. Its effectiveness can be determined by two factors one is the action of reductive enzymes and secondly the extent of hypoxia. Potential cell killing has been measured for both radiation and TPZ combination in three murine tumours. In SCCVII, DNA damage decreased with increasing oxygen concentration and this damage was half of the best possible value at 0.2% pO_2. These in-vitro experiments showed the oxygen dependence of DNA damage when treated with TPZ. In well oxygenated cells back oxidation of radical converts it to parent compound with no toxicity (Shannon et al. 2003). Results showed that less TPZ concentration is highly toxic at low pO_2 regions (0.2% O_2) and more concentration is required if oxygen pressure increases. Current studies still show no confirmed answer for its actions, toxicity and optimal administration.

Mathematical and computational modelling has introduced a new horizon for biologists, scientists and doctors in making hypothesis and experimentations about complex biological phenomenons and in curing diseases. Several attempts have been made to model tumour growth process but mostly considered it as a whole entity at tissue level. An in-silico model was developed to investigate early tumour growth under the influence of Extracellular Matrix (ECM), Cell-Cell and Cell-Matrix adhesion and cell movement as growth constraints while used a po-

werful artificial intelligence decision making tool; the neural network considering each cell as an individual and independent entity (Kazmi et al. 2010). Now we extended our previous computational modeling technique to capture TPZ preferential action towards tumour at cellular level. The initial TPZ concentrations 10 μM, 50 μM and 100 μM were infused to tumour mass from surrounding blood vessel and model calculated drug cytotoxicity, its penetration, metabolism inside each cell. The results section showed the impact of all these factors on tumour morphology. This model with some parameter modifications can be served as a tool in assumptions and experiments for bioreductive drugs in laboratories and in clinical trials by oncologists, researchers and pharmacologists.

The model

We did not consider tumour as a whole entity, our model explored the behavioral characteristics of the tumour at basic cell level. The model is developed using a 2 dimensional plot of size 400 that can simulate tumour of radius 200. Each element shows the availability or absence of a tumour cell. A well known decision making artificial intelligence technique; neural network is used to calculate the response or phenotype of each abnormal cell. The model calculates tumor microenvironments values and passes them to input layer and used one hidden layer of neurons to calculate the middle values and then pass them to the output layer as final phenotype of that specific cell using standard transfer function at each layer. Partial differential equation set PDE (1) was used to calculate nutrients/microenvironments i.e. the consumption of oxygen, glucose and production of hydrogen ions for each cell at specific location *x* and at specific time instance *t,* during tumour growth process (Gerlee and Anderson 2007).

$$\partial c(x,t)/\partial t = D_c \Delta c(x,t) - f_c(x,t)$$

$$\partial g(x,t)/\partial t = D_g \Delta g(x,t) - f_g(x,t)$$

$$\partial h(x,t)/\partial t = D_h \Delta h(x,t) - f_h(x,t) \qquad (1)$$

D_c, D_g and D_h are the diffusion constants for oxygen, glucose and hydrogen. They are given values $D_c=1.8\times10^{-5}cm^2s^{-1}$ (Grote et al. 1997), D_g =$9.1\times10^{-5}cm^2s^{-1}$ and $D_h=1.1\times10^{-5}cm^2s^{-1}$ Crone and Levitt (1984). The actual target was to explore the TPZ effects on tumour morphology and cell killing during the treatment. The whole tumour mass was divided into five hypoxic regions based upon available pO_2 for each cell. These five identified hypoxic regions were hypoxia I, II, III, IV and V based upon pO_2 values; 20.9%, 10%, 2%, 0.2% and 0.02% respectively. Using experimentally measured and published data in the literature, the values of drug concentrations required to kill cells at various oxygen tensions was entered into the model: Above 500 μM TPZ was cytotoxic towards all cells at O_2 tensions

of 20.9% and 10% 50 μM was toxic towards cells at 2% O_2 while 10 μM was enough to kill cells at 0.2% and 0.02% O_2 (Lartigau and Guichard 1994). We introduced 10 μM, 50 μM and 100 μM as initial concentrations of TPZ in our simulations. In the model, blood vessel has surrounded the tumour using boundary condition and the cell that is residing at outer most edge closest to the blood vessel and first to be infused with TPZ. Now the tumour is surrounded by blood vessel, an infused drug penetrates downward passing through the cells at outer boundary of tumour i.e. the proliferating rim moving towards the inner part and reaches to the inner most area i.e. the severe hypoxic region. With increasing distance from blood vessel drug penetration decreases and becomes less effective at distal areas. As shown from literature TPZ is less toxic to rapidly dividing cells, so we assumed it as a nontoxic agent to proliferating rim. We calculated the drug concentration for each cell following (2) (Kevin et al. 2003).

$$\begin{aligned} \partial Tpz/\partial t &= D_{MCL}\partial^2 Tpz/\partial^2 dv - \phi\partial M/\partial t \\ \partial M/\partial t &= k_{met}Tpz + V_{\max}Tpz/K_m + Tpz \end{aligned} \tag{2}$$

Where D_{MCL} is the diffusion coefficient for TPZ *Tpz* gives the individual cell captured tirapazamine concentration at the time instance *t* and at position *x*. Now the drug diffusion is function of time and the distance from blood vessel *dv*. The initial drug concentration was considered as an initial condition for *Tpz*. The metabolism factor of the available drug concentration for the specific position at specific time step has been calculated using (3). The description of the other used parameters is given in table 1.

RESULTS AND DISCUSSION

Main aim was to explore TPZ pharmacology, especially inside hypoxic regions of tumour. The model was executed to calculate tumour mass for continuous 6 days and divided the tumour mass into five different hypoxic regions based upon their pO_2 criteria. Fig. 1 differentiated these five regions using five different colours: cells that fall in hypoxic I region have been shown in blue, hypoxia 2 with yellow, hypoxia 3 with red, hypoxia 4 with black and the most severe and oxygen deprived one i.e. the hypoxia 5 in magenta colour. During the growth of tumours, the oxygen level decreases in gradients as shown in fig 1. The cell closest to the blood vessel was considered to be first infused with TPZ. The drug infusion was modelled in layers, from top to bottom one. First drug penetrates to cells of proliferating region when infused through surrounding blood vessel. Then it penetrates to hypoxia I region underlying the dividing cells and further penetrates to downward areas. We selected 10 μM, 50 μM and 100 μM as three TPZ initial concentrations in separate simulations and continued the treatment for 25 continuous cycles. The model must meets the criteria of supplying drug first to hypoxia layer I completely. Then it comes to hypoxia II, III and so on. Fig. 2 shows the tumour

morphology after 5 days with TPZ 100 µM as an initial concentration. It shows that no cell is alive from hypoxia IV and hypoxia V regions. Because in simulations it was assumed that if the available penetrated drug concentration is greater than 10 µM then it is enough to kill cells of these two regions. This was the reason that on day 5 no cell of this area was alive. Then drug was infused and results were collected on 10th day of treatment in fig. 3. On 10th continuous TPZ cycle all the cells from hypoxia III region were dead. As the cell killing threshold of this region was set at 50 µM and when the cells from this region experienced enough damage equivalent to 50 µM drug exposure, they were got killed. On 16th day of treatment no hypoxic cell was observed, only cells with good amount of oxygen were alive. Only the cells with good amount of oxygen were present because TPZ was nontoxic to these cells (fig. 4). Results showing TPZ toxicity and number of survived hypoxic cells using initial drug concentrations of 10 µM, 50 µM and 100 µM are compared in fig. 5. Total number of hypoxic cells was plotted against the number of days (TPZ cycles). This comparison showed highest toxicity i.e. highest cell death rate against 100 µM concentration. Cell death was observed with first few treatment cycles and on 16th day hypoxic cell survival rate approached zero. Cytotoxicity level was also high at 50 µM concentrations but was bit less than that observed using100 µM. The cell survival approached to zero on the 23rd day of treatment. Cytotoxicity at 10 µM concentration was the lowest and failed to kill all hypoxic cells on 25th day of treatment.

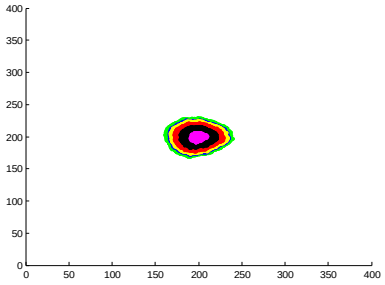

Fig. 1. Tumour mass is divided into 5 different hypoxic regions.

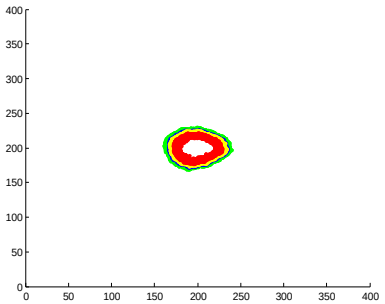

Fig. 2. On 5th day of treatment with TPZ 100 µM as initial concentration.

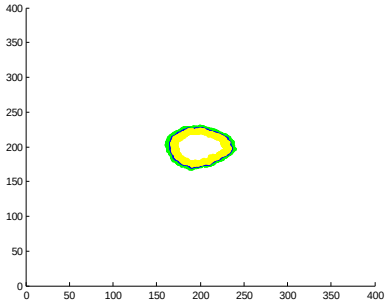

Fig. 3. **S**hows alive cells after 10 days of treatment with 100µM TPZ initial concentration.

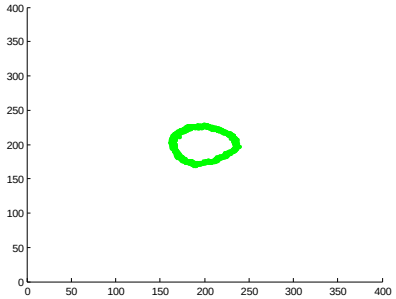

Fig. 4. Shows tumour morphology on 16th day of treatment with TPZ 100µM initial concentration.

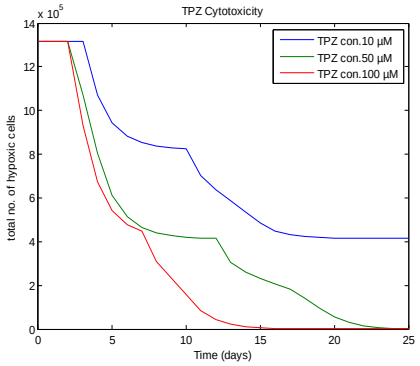

Fig. 5. TPZ cytotoxicity measured in 25 days of treatment with 10µM, 50 µM and 100 µM as initial concentrations.

CONCLUSIONS AND FUTURE WORK

This paper presented an in-silico model to observe the pharmacology of bioreductive drug tirapazamine inside solid tumour. The model calculated the amount of TPZ and its efficient cell killing on each day of continuous drug infusion during the whole treatment cycle. Drug metabolism and drug concentration inside each cell was calculated using PDEs and solved in one dimension inside each cell. Drug resistance and cytotoxicity effects on tumour morphology were also calculated using 10 μM, 50 μM and 100 μM as initial TPZ concentrations. Highest toxicity was measures at 100 μM and lowest at 10 μM while we proposed that its highest amount should be given to solid tumour to exploit hypoxia fully. As TPZ have no or less toxic effects on normal cells. This model with integration of laboratory data and parameters modifications can be used by oncologists and pharmacologist to explore TPZ dynamic behaviour inside multicellular spheroids, animal and human tumours. A quite strange behavior of bioreductive drugs has observed in clinical practice that its considerable amount disappears inside the blood vessel before reaching to the targeted locations. Our next aim is to capture the effects of Tirapazamine inside the blood vessel and then its combination with chemotherapy treatment.

TABLE 1: Parameters used for various experiments

Value	Description	Units	Parameters
8.5	Maximal rate for TPZ metabolism	$\mu M.min^{-1}$	V_{max}
3.5	Michaelis constant of TPZ metabolism	μM	K_m
0.78	First order rate constant of TPZ metabolism	min^{-1}	k_{met}
0.40	TPZ diffusion constant	$cm^{-2}s^{-1}\times10^{6}$	D_{MCL}
0.508	Intracellular volume fraction	___	Ø

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