UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA
PhD SCHOOL

PhD THESIS

THE STUDY OF THE ACTION OF CYTOTOXIC COMPOUNDS ON TUMOR CELLS USING MATHEMATICAL MODELS

ABSTRACT

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Key words: mathematical model, MATLAB Simulink, synergistic action; cytotoxic compounds, glioblastoma.

STATE OF KNOWLEDGE

In order to increase the effectiveness of chemotherapeutic treatment, based on the association of cytostatic compounds, the drug / tumor cells experiment plays an important role, under strict laboratory conditions. The estimation of dose – effect type reports regarding the action of an oncological drug on tumor cells leads to relevant conclusions in establishing the optimal medication specific to the chemotherapeutic act. The identification of the optimal association between cytotoxic agents is very important, based on experiments on cell lines with regards to cellular kinetics.

In this study, mathematical modeling and the analysis of the action of cytotoxic compounds on tumor cells *in vitro* focuses on:

- the development of analytical models describing the evolution of the proliferation rate (PR) and the doubling time (DT) respectively, in the tumor cell proliferation process; Studies point out that the exposure of tumor cells to a cytotoxic drug should be carried out in the exponential growth phase, (this requirement is not fully fulfilled in medical practice);

- the development of an analytical model for quantifying the dose – response type of action of one or more cytotoxic agents on glioblastoma (GB) tumor cells. The establishment of mathematical models which describe the dose-response tye of action of the association of two or more cytotoxic agents on tumor cells *in vitro*, is very important. In the theoretical analysis of the synergistic action the identification and usage of the combination index (CI) is of high importance.

- the role of the **MATLAB** tool along with the **Simulink** extension in the development of numerical simulation models.

PERSONAL CONTRIBUTIONS (EXPERIMENTAL STUDY)

The study achieved the following objectives:

- **Objective no. 1** – The study of PR and of DT respectively, on GB cell lines and the numerical simulation using mathematical models that describe the evolution of these parameters in the cell proliferation process.

The numerical simulation using mathematical models describing the evolution of PR and DT respectively in the cell proliferation process, on the GB3B, GB4B, GB5B, GB9B and GB10B cell lines represents a major advantage in establishing a tratament scheme based on a cytotoxic drug. The numerical simulation model generates specific graphics and provides access to data sets with statistical significance.

- Objective no. 2 –The investigation of the individual action of tyrosine kinase inhibitors (**SU** 1498, **AG** 1433, Imatinib (**IMT**)) and of the alkylating agent Temozolomide (**TMZ)** on GB cells *in vitro* using a numerical simulation model. The cell line on which the individual action of tyrosine kinase inhibitors and of TMZ was studied is GB10B.

The biomedical experiment that reflects the dynamic evolution of tumor cell fraction depending on the ratio between D/IC_{50} (the dose of a cytotoxic compound / the dose that produces the median effect on GB cells), can lead to the development of a mathematical model, based on the transfer function.

- Objective no. 3 – The evaluation of synergistic dose – response action type *in vitro* between the alkylating agent TMZ and the tyrosine kinase inhibitor IMT with the establishment of an optimum dosage (OP) for the drugs based on numerical simulation model.

The combination of the two therapeutic agents (TMZ + IMT) may allow the use of lower doses of these substances, which induces a cumulatively cytotoxic effect on the tumor cells.

The development of a numerical simulation model allows the evalution of the synergistic action of these compounds based on the D/IC_{50} ratio with the determination of an OP for the drugs.

The above mentioned objectives have been achieved based on the numerical simulation models built using the **MATLAB** specialized tool with the **Simulink** extension.

- Objective no. 4 – Meta-analysis study concerning the survival gain of patients with recurrent malignant glioma following immunotherapeutic treatment with dendritic cells in comparison with combined treatment with bevacizumab and irinotecan.

An analysis of the published data on PubMed, Cochrane Library, Web of Science, ScienceDirect and EMBASE was performed in order to identify studies with patients who received bevacizumab plus irinotecan or therapy with dendritic cells for the treatment of recurrent malignant gliomas.

MATERIALS AND METHODS presents the materials used in the study and their origins and how they are implemented within the *Objectives 1, 2, 3, 4.* Thus, reference is made to the cell culture medium, the cell growth determination kit MTT, the fetal bovine serum (FBS), antibiotics, trypsin, phosphate-buffered saline (PBS) which are used in the study. Also, the tyrosine kinase inhibitors SU 1498, AG 1433, IMT and the alkylating agent TMZ were used in the study.

In order to implement *Objective no.1* cell lines derived from GB were used: GB3B, GB4B, GB5B, GB9B and GB10B. The GB10B cell line was used to implement *Objective no. 2* and *Objective no. 3.*

In this chapter the following steps are presented:

- The development of the mathematical models describing the evolution of the PR and the DT in the tumor cell proliferation process;

- The development of the mathematical model based on the transfer function specific to the dose –response type of action of a cytotoxic agent on tumor cells *in vitro*;

- The development of a mathematic model based on the transfer function specific to the dose – response type of action of two cytotoxic agents on tumor cells *in vitro*;

- *Research methods, Eligibility criteria, Statistical processing* applied to the meta-analysis study that evaluates the patients with recurrent malignant glioma undergoing a treatment with cytotoxic agents compared to patients undergoing immunotherapy with dendritic cells.

RESULTS AND DISCUSSIONS

- *The evolution of PR and DT on GB lines based on the simulation model MS: DT / PR*

The mathematical model describing the DT based on *n* data pairs, $(t_1, N_1); (t_2, N_2), \ldots, (t_n, N_n)$ is given by the ecuation: $DT = \sum_{i=1}^{n-1} DT_i(N_{i+1} - N_i) / \sum_{i=1}^{n-1} (N_{i+1} - N_i)$. The mathematical model describing PR based on *n* data pairs, $(t_1, N_1); (t_2, N_2), \ldots, (t_n, N_n)$ is: $PR = \ln 2 \cdot \left[\sum_{i=1}^{n-1} (N_{i+1} - N_i) \right] / \left[\sum_{i=1}^{n-1} DT_i (N_{i+1} - N_i) \right]$

The **MS: DT/PR** numerical simulation model is based on the mathematical model which describes DT and the mathematical model that describes PR. **MS: DT/PR** includes: Math Function blocks; arithmetic operations blocks; **Scope DT(N/N$_1$)** block; **Scope PR(N/N$_1$)** block, etc..The numerical simulations were performed on a ACER Aspire S3 type PC equipped with an Intel Core i3, 1,8 GHz processor.

For the GB3B, GB4B, GB5B, GB9B and GB10B cell lines the average values for DT and PR were determinated based on the **MS: DT / PR** simulation model and using the online algorithm: http://www.doubling-time.com.

Determining the mean DT values and the mean PR values is important for the implementation of a treatment scheme which is based on the action of a cytostatic drug: $\underline{DT}_{GB9B}$= 2.281< $\underline{DT}_{GB3B}$= 2.3953< $\underline{DT}_{GB4B}$= 2.4820< $\underline{DT}_{GB5B}$= 2.6993 < $\underline{DT}_{GB10B}$ = 3.573. The cells belonging to GB9B line proliferate with a $\underline{DT}$ of 2.281 days ; the GB10B cells proliferate with a $\underline{DT}$ of 3.573 days ; the GB3B cells proliferate with a $\underline{DT}$ of 2.3953 days; GB4B cells proliferate with a DT of 2.482 days $\underline{DT}$; the cells belonging to GB5B line proliferate with a $\underline{DT}$ of 2.6993 days . $\underline{DT}$ determinated for GB10B cells is 21 hours longer than $\underline{DT}$ determinated for GB5B cells, 26.1 hours longer than $\underline{DT}$ determinated for

GB4B cells, 28.3 longer hours than DT determinated for GB3B cells and with 31 hours longer than DT determinated for GB9B cells, respectively.

The study highlights average values of PR for the GB3B, GB4B, GB5B, GB9B and GB10B cell lines.

Low values for DT and high values for PR indicate an intense cell proliferative activity. Medium values for both DT and PR indicate a moderate cell proliferative activity. High values for DT and low values for PR indicate a reduced cellular proliferative activity.

The determination method of DT and of PR based on the **MS: DT / PR** simulation model is fast and does not require a complex laboratory tehnique. This method involves a calculation tehnique using a **PC** with medium specifications and has software specialized in numerical simulations based on mathematical models. The method is addressed to biomedical investigations that monitor cellular kinetics.

- *The investigation of the individual dose – response action type in vitro for cytotoxic compounds on GB10B based on the MS: $f_n(D/IC_{50})$ simulation model.*

The dose – response action type, $f_n(D/IC_{50})$, of certain cytotoxic substances on a tumor cell line can be described based on the mathematical model reformulated in real field: $f_n = L^{-1}\left[\left(K_1 - K_2\right)\cdot\frac{W}{W\cdot s+1}\right] + L^{-1}\left[K_2\cdot\frac{1}{s}\right]$ with the argument (D/IC_{50}-D_0/IC_{50}), ($D \geq D_o$)).

Using **MATLAB Simulink** a numerical simulation model was built: **MS: $f_n(D/IC_{50})$.** The dose – response action type of SU 1498, AG 1433, IMT and of the alkylating agent TMZ on GB10B can be numerically simulated with **MS: $f_n(D/IC_{50})$**, with the implementation of *Objective no. 2*. Numerical simulations were performed on an ACER Aspire S3 type PC.

A semnificative example of application of the **MS: $f_n(D/IC_{50})$** simulation model is the dose - response action type for IMT on the GB10B cell line. The main values resulted from the simulation on **MS: $f_n(D/IC_{50})$** are presented in the **Tables** below.

Table. The D–f_n type values resulted by numerical simulation on MS; D/ IC_{50} € [0.7, 1.5]

MS: fn(D/ IC_{50})	**D – f_n type values**									
(D/ IC_{50})	0.7	0.8	**0.826**	0.9	1	1.1	1.2	1.3	1.4	1.5
*$(D)_{IMT}$ (μM)**	8.48	9.69	**10**	10.9	12.11	13.32	14.53	15.74	16.96	18.16
$(f_n)_{IMT}$	66.88	60.58	**59.06**	54.98	50	45.56	41.63	38.12	35.01	32.24

* common values of the simulation process / experiment are highlighted

Table. The D–f_n type values resulted by numerical simulation on MS; D/ IC_{50} € [1.6, 2.4]

MS: fn(D/ IC_{50})	**D – f_n type values**									
(D/ IC_{50})	1.6	**1.652**	1.7	1.8	1.9	2	2.1	2.2	2.3	2.4
*$(D)_{IMT}$ (μM)**	19.38	**20**	20.59	21.8	23.01	24.22	25.43	26.64	27.85	29.06
$(f_n)_{IMT}$	29.78	**28.61**	27.59	25.64	23.91	22.37	21	19.78	18.7	17.73

* common values of the simulation process / experiment are highlighted

The $\mathbf{f_n}$ series of the values generated by the numerical simulation on the **MS: $\mathbf{f_n(D/IC_{50})}$** model are quite different from the $\mathbf{f_n}$ values resulted in the dose – response action type *in vitro* experiment using a cytotoxic substance on GB10B cells. This observation outlines the need to validate the **MS: $\mathbf{f_n(D/IC_{50})}$** numerical simulation model based on the distribution of residual data (errors) and based on the correlation between the data series.

- *Investigation of the synergistic dose – response action type in vitro for TMZ in association with IMT on the GB10B based on the numerical simulation model MSS: $f_n(D/IC_{50})$.*

Investigation of the dose – response action type of the TMZ and IMT association on GB10B with the implementation of *Objective no. 3*, can be achieved on the **MSS: $\mathbf{f_n(D/IC_{50})}$** numerical simulation model built using the specialized softwear **MATLAB Simulink**.

The **MSS:$\mathbf{f_n(D/IC_{50})}$** simulation model provides significant data for the numerical simulation process specific to the synergistic action of TMZ + IMT on tumor cells derived from the GB10B line. The resulted values are presented in the **Table** below.

Table. D-f_n type values resulted by numerical simulation on MSS; D/ $IC_{50} \in [1; 1.9]$

MSS: fn(D/ IC_{50})	**D-f_n type values**										
(D/ IC_{50})	**1**	1.1	1.2	1.3	1.4	1.5	1.6	**1.608**	1.7	1.8	1.9
$(D)_{IMT}$ (μM)	**12.11**	13.32	14.53	15.74	16.95	18.16	19.38	19.48	20.59	21.8	23.01
$(D)_{TMZ}$ (μM)	**141.58**	155.7	169.9	184.1	198.2	212.4	226.5	227.7	240.7	254.8	269
$(f_n)_{IMT}$	**0.5**	0.456	0.416	0.381	0.35	0.322	0.298	**0.296**	0.276	0.256	0.239
$(f_n)_{TMZ}$	**0.5**	0.445	0.398	0.358	0.322	0.292	0.266	**0.264**	0.243	0.223	0.207
$(f_n)_C$	**0.5**	0.384	0.295	0.226	0.174	0.133	0.102	**0.1**	0.078	0.06	0.046

The study confirms, based on the **MSS: $\mathbf{f_n(D/IC_{50})}$** model using the **CI** values, the *synergistic* action type. In the case of the synergistic effect, **CI** has the following values **CI** = 0.94; 0.83; 0.64, (**CI** < 1) correlated with the $\mathbf{f_n}$ = 0.12; 0.11; 0.1 values. It is considered that ($\mathbf{f_n}$ = 0.1, **CI**=0.64) indicates a strong synergism for TMZ + IMT associaton on **GB10B** line.

Using **MSS: $\mathbf{f_n(D/IC_{50})}$** enables the reduction of the drug dosage by the *synergistic action* of two drugs on a tumor cell line. It is a known fact that a substance becomes toxic for the body if it exceeds its specific critical dose. Because of $(D^*)_{IMT} << (D)_{IMT}$ and $(D^*)_{TMZ} << (D)_{TMZ}$ it is higly probable for the incidence of individual toxic effects for each agent to be greately diminshed in the body while having a reduced incidence for adverse effects in the case of synergistic action of TMZ in association with the IMT inhibitor.

It is a known fact that the standard treatment for advanced cancer is based on the combined therapy of more than one cytotoxic drug belonging to different classes. It is very important to determine the optimum dosage for the drugs, which is based either on medical practice or on calculation algorithms provided by specialized medical clinics.

The conclusions of the study, on one hand reflect the importance that must be given to determining the dosage values of drugs inducing the median effect (IC_{50}) and on the other hand they reflect the need to build numerical simulation models types such as MSS: $f_n(D/IC_{50})$, allowing the establishment of a treatment protocol with the identification of a calculated optimum dosage.

- *The analysis of the survival gain of patients with recurrent malignant glioma after the immunotherapeutic treatment with dendritic cells compared to the combined treament with bevacizumab and irinotecan. Meta-analysis study.*

Regarding the patients with GBM undergoing treament with bevacizumab plus irinotecan seven clinical studies were identified. The total number of patients was 302 with ages ranging between 18 and 79 years. After the treament the values of mOS were between 7 and 16.25 months. The values of predicted mOS were between 4.44 and 13.74 months.

In the case of the patients with GBM undergoing immunotherapy with dendritic cells seven clinical studies were identified. The total number of patients was 80 with ages ranging between 24 and 74 years. After the treatment the mOS values were between 7.5 and 34 months. The values of predicted mOS were between 11.39 and 35.12 months.

CONCLUSIONS

Study 1. The construction of the **MS: DT / PR** numerical simulation model based on the specialized software **MATLAB** with the **Simulink** extension allows the simulation of the evolution of PR and DT on GB3B, GB4B, GB5B, GB9B, GB10B tumor cell lines derived from GB.

The establishment of the specific value sets for DT and PR belonging to the cellular exponential growth phase is important for the development of a treatment protocol that allows the testing of the sensitivity of tumors to different cytostatic compounds *in vitro*.

Study 2; 3; 4. The dose – response action type of a cytotoxic compound on GB cells *in vitro* can be studied on a numerical simulation model built with the specialized software **MATLAB** with the **Simulink** extension.

The simulation model **MS: $f_n(D/IC_{50})$** allows the numerical simulation of the f_n evolution in the dose – response action type for several concentrations of SU 1498, AG 1433, IMT and TMZ on the GB10B cell line.

The **MS: $f_n(D/IC_{50})$** model allows the generation of $f_n(D/IC_{50})$ graphics and the display of numerical data depending on the D/IC_{50} ratio in the case of SU 1498, AG 1433, IMT inhibitors or in the case of the alkylating agent TMZ through the specialized **Scope $f_n(D/IC_{50})$**, **Dy:f_n**, **Dy:D/IC_{50}** blocks.

Study 5. The synergistic dose – response action type of the combination of two drugs TMZ + IMT *in vitro* can be studied based on the **MSS: $f_n(D/IC_{50})$** numerical simulation model built with the **MATLAB Simulink** tool**.**

The **MSS: fn (D / IC50)** model becomes a laboratory tool that allows the generation of graphics specific to f_n depending on the D/IC_{50} ratio results specific to the alkylating agent TMZ, to the inhibitor IMT and to the TMZ + IMT association respectively. **MSS: $f_n(D/IC_{50})$** allows the determination of the **CI** index.

The analytical formula of **CI** leads to the observation that a synergistic action exists for an agent **i** if the dose **(D_i)***, which in combination with another agent produces an **f_n** value in the system**,** it is at most half the dose **(D_i)** which individually produces the same value of **f_n** in the system.

In the case of the used mathematical model importance should be given, on one hand, to the determination of the values of cytotoxic compounds that induce the median effect **(IC_{50})** and on the other hand to the necessity of building numerical simulation type models **MSS: $f_n(D/IC_{50})$**, models that allow the establishment of a dose – response type treatment protocol based on the indentification through simulation of a calculated optimum dosage **$OP[D/IC_{50}; (f_n)_m]$**.

Study 6. The meta-analysis study enabled us to draw the conclusion: for the patients with recurrent malignant gliomas, the immunotherapeutic treatment with dendritic cells did not have a noticeably different effect compared to the combined treatment of bevacizumab and irinotecan, proving once again that combined treatments are efficient for patients with malignant diseases.

SELECTED REFERENCES

Aghi M, Kramm MC, Chou CT, et al. Synergistic Anticancer Effects of Ganciclovir / Thymidine Kinase and 5-Fluorocytosine/Cytosine Deaminase Gene Therapies. J Nat Cancer Inst 1998; 90: 370-380.

Artene ȘA, Turcu-Știolică A, Hartley R, Ciurea ME, Daianu O, Brîndușa C, Alexandru O, Tătăranu LG, Purcaru ȘO, Dricu A. Dendritic cell immunotherapy vs bevacizumab plus irinotecan in recurrent malignant glioma patients: a survival gain analysis. OncoTargets and Therapy 2016; 9: 6669-6677.

Bokstein F, Shpigel S, Blumenthal DT. Treatment with bevacizumab and irinotecan for recurrent high-grade glial tumors. Cancer, 2008; 112(10): 2267-2273.

Brîndușa C, Șerban F, Alexandru O, Georgescu MA, Ene L, Tătăranu GL, Purcaru ȘO. Angiogenic receptor tyrosine kinase inactivation induced apoptosis in glioblastoma cells in vitro. The Revue of Aeronautical Medicine and Psychology 2015; 19: 25-32.

Carapancea M, Alexandru O, Fetea AS, Dragutescu L, Castro J, Georgescu A, Popa-Wagner A, Bäcklund ML, Lewensohn R, Dricu A. Growth factor receptors signaling in glioblastoma cells: therapeutic implications. J Neurooncol. 2009; 92(2): 137-147.

Chou JH. Quantitation of synergism and antagonism of two or more drugs by computerized analysis, in Synergism and Antagonism in Chemotherapy (Chou TC and Rideout DC), Academic Press New York 1991; 223-244.

Chou TC. Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method. Cancer Res 2010; 70: 440-446.

Chou TC, Tan HQ, Sirotnak MF. Quantitation of the synergistic interaction of edatrexate and cisplatin in vitro. Cancer Chemotherapy and Phamacology 1993; 31: 259-264.

Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 1984; 22: 27-55.

Desjardins A, Reardon DA, Herndon JE, 2nd, et al. Bevacizumab plus irinotecan in recurrent WHO grade 3 malignant gliomas. Clinical cancer research : an official journal of the American Association for Cancer Research 2008; 14(21): 7068-7073.

Friedman H, Kerby T, Calvert H. Temozolomide and Treatment of Malignant Glioma. Clin Cancer Res 2000; 6: 2585-2597.

Friedman HS, Prados MD, Wen PY, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2009; 27(28): 4733-4740.

Kanzawa T, Germano IM, Komata T, et al. Role of autophagy in temozolomide-induced cytotoxicity for malignant glioma cells. Cell Death and Differentiation 2004; 11: 448-457.

Louis DN, Ohgaki H, Wiestler OD and Cavenee WK. The 2007 WHO Classifcation of tumours of the central nervous system. Acta Neuropathol 2007; 114: 97-109.

Ohgaki H, Kleihues P. Genetic Pathways to Primary and Secondary Glioblastoma. The American Journal of Pathology 2007; 170: 1445-1453.

Pinto AC, Moreira J, Simões S. Combination Chemotherapy in Cancer: Principles, Evaluation and Drug Delivery Strategies. Current Cancer Treatment 2011; 29: 693-714.

Popescu MA, Alexandru O, Brîndușa C, Purcaru ȘO, Tache ED, Tătăranu GL, Taisescu C, Dricu A. Targeting the VEGF and PDGF signaling pathway in glioblastoma treatment. International Journal of Clinical and Experimental Pathology 2015; 8: 7825-7837.

Reile H, Birnbock H, Bernhardt G, et al. Computerized Determination of Growth Kinetic Curves and Doubling Times from Cells in Microculture. Analytical Biochemistry 1990; 187: 262-267.

Sakai K, Shimodaira S, Maejima S, et al. Dendritic cell-based immunotherapy targeting Wilms' tumor 1 in patients with recurrent malignant glioma. Journal of neurosurgery 2015; 123(4): 989-997.

Schindler T, Bornmann W, Pellicena P, et al. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. Science 2000; 289: 1938-1942.

Vredenburgh JJ, Desjardins A, Herndon JE, 2nd, et al. Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2007; 25(30): 4722-4729.

Zinner RG, Barrett BL, Popova E, Damien P, et al. Algorithmic guided screening of drug combinations of arbitrary size for activity against cancer cells. Mol Cancer Ther 2009; 8: 521-532.