UNIVERSITY OF MEDICINE AND PHARMACY
"CAROL DAVILA", BUCHAREST
DOCTORAL SCHOOL MEDICINE

EXPERIMENTAL INSIGHTS INTO THE TREATMENT OF HIGH-GRADE GLIOMAS USING INNOVATIVE BIOMATERIALS

PHD THESIS ABSTRACT

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List of published scientific papers

1. **Idu, A.-A.**; Albu Kaya, M.G.; Rău, I.; Radu, N.; Dinu-Pîrvu, C.-E.; Ghica, M.V. **Novel Collagen Membrane Membrane Formulations with Irinotecan or Minocycline for Potential Application in Brain Cancer**. Materials 2024, 17, 3510. https://doi.org/10.3390/ma17143510
   https://www.mdpi.com/2871496 Indexed PubMed, Impact factor 3.1

2. **Idu AA**, Eva L, Covache-Busuioc RA, Glavan LA, Ciurea AV. **Progressive Paraparesis and Spinal Glioblastoma in a Young Female.** Cureus. 2024 Apr 4;16(4):e57593. doi: 10.7759/cureus.57593. PMID: 38707088; PMCID: PMC11069329.
   https://pubmed.ncbi.nlm.nih.gov/38707088/ Indexed PubMed, Impact factor 1.1

3. Onciul R, Toader C, Glavan LA, Covache-Busuioc RA, Bratu BG, Costin HP, Corlatescu AD, Ciurea AV, Grama M, **Idu AA**. **Retrospective Analysis of Glioblastoma Outcomes.** Cureus. 2024 Jun 16;16(6):e62462. doi: 10.7759/cureus.62462. PMID: 38882229; PMCID: PMC11180423.
   https://pubmed.ncbi.nlm.nih.gov/38882229/ Indexed PubMed, Impact Factor 1.1

4. **Idu AA**, Bogaciu NS, Ciurea AV. **Brain imaging and morphological plasticity in glioblastoma**: a literature review. J Med Life. 2023 Mar;16(3):344-347. doi: 10.25122/jml-2022-022-0201. PMID: 37168303; PMCID: PMC1016565525.
   https://pubmed.ncbi.nlm.nih.gov/37168303/ Indexed PubMed, Scopus

Contents

Introduction

Glioblastoma is one of the most aggressive neoplastic entities of the central nervous system, characterized by an overall median survival rate of approximately 15 months post-diagnosis, even with advanced therapeutic protocols. The current standard treatment, known as the Stupp protocol, includes a multimodality approach comprising extensive surgical resection of the tumor mass within safe limits, followed by the synergistic use of radiotherapy and temozolomide chemotherapy. This therapeutic regimen aims to maximize life expectancy [1].

However, glioblastoma is characterized by its capacity for deep infiltration into adjacent tissues, which results in increased difficulty of complete resection and an increased risk of neoplastic recurrence. This essential pathologic feature emphasizes the limitations of this therapeutic approach [17].

The development of new therapeutic protocols is hampered by many factors, including the cellular and molecular heterogeneity of tumors, mechanisms of resistance to alkylating agents, and various physiological and anatomical barriers that limit the efficacy of systemic therapies. In this context, innovative therapeutic options available on the market are limited. A notable example is the use of biodegradable implants, such as biodegradable membranes called Gliadel®, which are applied directly into the tumor site post resection. This method proposes a local, minimally invasive approach that is in line with the principles of standard therapy. However, despite its promising potential, the clinical efficacy of these implants remains controversial due to significant side effects [18].

This thesis aims to explore and develop innovative therapeutic strategies for the treatment of glioblastoma, with a particular focus on the use of biodegradable implants impregnated with alkylating agents. This approach aims to optimize the local delivery of alkylating therapeutic agents in order to maximize the efficacy of the treatment at the tumor tissue level while minimizing systemic effects. Integrating this local treatment strategy with the STUPP protocol is a promising direction [19].

In this summary, the pagination of the table of contents, as well as the numbering of chapters, subchapters, figures, tables and bibliographical references are the same as in the thesis.

4. General working hypothesis and objectives

This doctoral thesis starts from the fact that current treatments for glioblastomas are not curative, but aim to control their natural progression by surgical resection, radiotherapy or chemotherapy; or by prolonging overall survival while maintaining a high quality of life.
Thus the main objective of this thesis was:

- Analyzing and improving multimodal treatment of high-grade glioma.

To achieve the main objective, the following specific objectives were considered:

- Assessing the limitations of current protocols used in the treatment of patients with high grade glioma
- Proposing a treatment option of topical chemotherapy for patients with high-grade glioma
- Development and characterization of novel biodegradable collagen membranes with antineoplastic and antimicrobial activity

- Biochemical membrane testing to identify the optimal concentration o f irinotecan and/or minocycline
- Obtaining and characterization of membranes based on collagen and irinotecan nanoparticles

The current work starts from a thorough literature review. Clinical and paraclinical factors on patient prognosis are analyzed to identify elements in the initial assessment that may predict disease progression. Assess existing options and therapeutic combinations to estimate their impact on survival, functional status and disease progression. Rigorous reviews of existing diagnostic methods shall be performed to determine their efficacy and reliability when specifically applied to high-grade glioblastomas.

The thesis focuses on an in-depth examination of treatments already in clinical use, to identify potential gaps and potential improvements and to provide more optimal treatement management. An in-depth topic is that of biodegradable membranes, where the development of new types of membranes aimed at combating cerebral or medullary glioblastoma is being pursued.

6. Functional Magnetic Resonance Imaging in the Management and Monitoring of Neuroplasticity in High-Grade Gliomas

6.1. Working hypothesis and specific objectives

Glioblastoma is the most common primary tumor of the central nervous system with an aggressive course and a poor prognosis. The majority of patients diagnosed with glioblastoma have a higher prevalence in the age range $65 – 75$ years, with a male predominance ratios of 1.5:1 [71].

MRI imaging of glioblastoma usually reveals hypo- to iso- intense signals on T1-weighted images, while on T2 and FLAIR sequences hyperintense with visible perileional edema appears on T2-weighted and FLAIR sequences. DWI/ADC sequences demonstrate limited diffusion of the solid tumor component [44].

Functional MRI (fMRI) can identify brain areas activated under specific stimulation using the BOLD (blood oxygen level-dependent) mechanism. Specifically, T2* sequences highlight areas of increased cell activity due to increased oxygen supply. Unfortunately, fMRI provides only indirect evidence of neuronal activity and has several limitations that restrict its applicability, limitations that include field heterogeneity at tissue interfaces such as bone, soft tissue and air, large vein interference and susceptibility to metallic artifacts [72, 73].

Neuroplasticity refers to the brain's capacity for adaptability in response to various forms of aggression. This ability is activated by its similar organization network with connected nodal nodes; and neurogenesis has been identified in several areas, including the dentate gyrus of the hippocampus and the lateral subventricular zone [74].

Electroencephalography (EEG), imaging modalities such as computed tomography (CT), PET-CT and magnetic resonance imaging (MRI), especially functional MRI (fMRI), diffusion tensor imaging (DTI) and direct electrical stimulation (DES), are some of the tools developed to study structural and functional aspects of the brain [75].

William R. Gibb et al. conducted a study using direct cortical stimulation and compared its effects between two surgical interventions. To establish neuroplasticity, the evidence included observations such as functional progress or loss when stimulating the same cortical area without changing the neurologic examination. If no response was observed from specific

cortical areas during both surgeries, this was seen as an indicator of pre-existing reorganization that occurred before the initial surgery [76].

Neuroplasticity can be observed on functional MRI (fMRI) by differences in cortical activation when identical actions are performed pre- and postoperatively, as well as unexpected activations of regions during tumor detection. [77].

6.2. Methodology

A search, in English, on PubMed, using the terms "glioblastoma", "plasticity" and "MRI" provided 33 articles. After analyzing the titles and abstracts of each article, five articles related to the research theme were selected.

6.4. Discussions

In the literature we identified only few articles addressing high-grade tumors, and these include both heterogeneous and single pathological series reporting cases of glioblastomas (Table 6.2).

Studies of brain functional changes in different regions use various techniques, such as imaging methods, non-invasive stimulation/recording techniques and invasive stimulation/recording techniques. However, direct cortical stimulation remains the 'gold standard'.

The paucity of literature on glioblastoma-associated neuroplasticity underscores the urgent need for further studies in this area. Understanding how brain structures respond to aggressive tumors, such as glioblastoma, is essential for optimizing treatment strategies and improving patient outcomes.

Table 6.2 A summary of glioblastoma cases using different plasticity assessment techniques and interpretation [78 – 82]

Crt. No.	Used Technique	Patients No	Results	Reference
1	fMRI + PET scan + TMS	1	Activation of the lower limb fronto-medial cortex and motor area additional for hand	[78]
2	fMRI	7	Broca's area - no activation on fMRI in cases studied	[77]
3	nrTMS	1	Contralateral hemisphere activation after surgical resection of a glioblastoma and activation of post-central and temporal areas disable above	[79]
4	DES, fMRI, ptential induced	1	Contralateral hemisphere activation in a lesion of the left superior parietal lobe, confirmed by DES and also described on fMRI	[81]
5	DES	6	Evidence of plasticity in all cases	[76]
6	fMRI	16	Activation of the supplementary motor area (12 cases) and the premotor area (8 cases) contralateral to the lesion, such as visualized on fMRI	[82]

6.5. Partial conclusions

While direct cortical stimulation techniques are more invasive and technically demanding, functional MRI is a widely accessible non-invasive solution. Any 3 Tesla or 1.5

Tesla device can perform functional MRI, making it an easy-to-use method for neurosurgeons, allowing the interpretation of high-resolution, high-contrast anatomical images. However, functional MRI has some disadvantages, as discussed above.

Functional MRI has proven useful in numerous studies, providing a wealth of information. Functional imaging allows the mapping and prediction of functional changes in specific neuronal network nodes, anatomical shifts in function and activation patterns observed in patients [85]. Numerous studies have confirmed the usefulness and efficacy of this technique [72, 86].

Functional imaging can be particularly valuable for preoperative planning in glioblastoma resection surgery, although it should not serve as the sole basis for surgical decisions. It can be argued that follow-up with functional imaging in patients diagnosed with glioblastoma is highly advantageous. The comparison of preoperative activation zone changes with immediate postoperative assessments performed 48 – 72 hours after surgery, as well as longer-term assessments are also crucial components.

Since direct cortical stimulation cannot be performed regularly enough but only during clinic visits, functional imaging is an invaluable means of continuous assessment.

Functional imaging techniques, such as fMRI and nrTMS, provide valuable information and can help in preoperative planning for treating glioblastomas. However, their limitations require the use of complementary methods to ensure a comprehensive evaluation and safe surgical treatment outcomes.

Thus the functional MRI represents an affordable tool for assessing brain functionality that could be integrated into preoperative glioblastoma plans while offering numerous advantages over its non-invasive counterparts, including wide availability, generation of high-resolution anatomic images, and repeated examinations without adverse effects. In addition, its use for follow-up purposes to monitor the development of brain plasticity may be particularly advantageous.

Although functional MRI has its benefits, there are also notable drawbacks such as neurovascular decoupling and lack of reliability when making definitive surgical decisions. The combined use of functional MRI and transcortical stimulation provides more accurate preoperative brain mapping.

There is great potential and opportunity with these techniques and we need to continue to explore and refine them to increase understanding and improve the management of glioblastoma, ultimately improving patient outcomes.

7. Management of high-grade glioma at the spinal level

Primary tumors of the spinal cord account for 2 to 4% of central nervous system tumors and are most often benign, malignant tumors being extremely rare. Intradural spinal tumors account for 5-10% of spinal tumors in adults, and 35-37% of intramedullary tumors in children [87].

7.2. Methodology

The literature search for this investigation consisted of a PubMed search over the last five years using the terms "glioblastoma" and "spinal cord". Articles written in English with confirmed diagnoses of glioblastoma by histopathologic analysis were considered for our inclusion criteria, and exclusion criteria included other neoplasms, secondary cases due to dissemination from cerebral glioblastomas, or incomplete information. Articles that met the exclusion criteria and thus qualified for review are shown in Table 7. 1 [96 – 107].

Table 7.1. Articles on which this case was based

Crt. No.	Authors	Title	Publication year	Reference
1	A. Chanchotisatien, J. Xiong, J. Yu, S. Chu	Exophytic Primary Intramedullary Spinal Cord Glioblastoma: Case Report and Critical Review of Literature	2018	[95]
2	K. Yang, W. Man, L. Jing, Z. Sun, P. Liang, J. Wang, G. Wang	Clinical Features and Outcomes of Primary Spinal Cord Glioblastoma: A Single- Center Experience and Literature Review	2020	[96]
3	U. Alok, K. K. Subhas, B. N. Nandeesh, S. Dhaval	H3K27M-Positive Primary Spinal Glioblastoma	2019	[97]
		Presenting with Hemorrhage-A Rare Clinical Entity		
4	C.-X. Shen, J.-F. Wu, W. Zhao, Z.-W. Cai, R.-Z. Cai, C.-M. Chen	Primary spinal glioblastoma multiforme: A case report and review of the literature	2017	[98]
5	A. Cacchione, A. Mastronuzzi, M. G. Cefalo, G. S. Colafati, F. Diomedi-Camassei, M. Rizzi, A. De Benedictis, A. Carai	Pediatric spinal glioblastoma of the conus medullaris: a case report of long survival	2016	[99]
6	K. Peters, D. Pratt, C. Koschmann, D. Leung	Prolonged survival in a patient with a cervical spine H3K27M-mutant diffuse diffuse midline glioma	2019	[100]
7	A. Nunn, S. Polyzoidis, B. Piechowski-Jozwiak, L. Brazil, K. Ashkan	Primary glioblastoma multiforme of the conus medullaris with leptomeningeal metastasis	2017	[101]
8	E. Caro-Osorio, J. C. Herrera-Castro, A. Barbosa-Quintana, M. Benvenutti-Regato	Primary Spinal Cord Small- Cell Glioblastoma: Case Report and Literature Review	2018	[102]
9	E. E. Cabrera-Aldana, R. De la Garza Ramos, R. Pichardo-Bahena	Multicentric Spinal Cord Glioblastoma	2017	[103]
10	L Dormegny, S Chibbaro, M Ganau, Mdn Santin, L Kremer, F Proust	Biopsying a spinal cord lesion: A diagnostic dilemma. Case report and review of literature	2018	[104]
11	B: J: Delgado, L. Moosavi, E. Rangel, W. Stull, R. Dev Polineni, J. Chen, E. Cobos	An Unusual Presentation of Spinal Giant Cell Glioblastoma in a 21-Year-Old Female	2019	[105]
12	K. Yang, J. Wang, G. Wang	Multicentric Exophytic Primary Spinal Cord Glioblastoma Mimicking Teratoma	2020	[106]

7.3. Results

The patients included in the study were analyzed according to sex: F - female, M - male; lesion level: c - cervical, t - thoracic, l - lumbar; type of surgery: b - biopsy, str - subtotal

resection, pr - partial resection, gtr - gross total resection; and KPS - Karnofsky performance status [108].

In this series, the mean age at diagnosis was 27 years, with a male to female ratio of 1:1.5.

Although the onset of symptoms averaged 10.6 months, the vast majority of cases developed symptoms within a shorter time frame of about 3 months, with only a few exceptions with initial symptoms at very long intervals.

The data obtained showed that out of 20 patients followed up, 11 underwent subtotal resection, 3 biopsies, 1 had macroscopic total resection and 4 had partial resections.

The Karnofsky Performance Score (KPS) for these patients was 64.

9 patients received both adjuvant radiotherapy and chemotherapy (temozolomide), and 4 patients received adjuvant chemotherapy only.

The median progression-free survival (PFS) was 8.8 months, with the majority centered at 5.5 months.

Median overall survival (OS) averaged 12.85 months, with a majority centered at 9 months.

7.5. Partial conclusions

The Kaplan-Meier survival curves in this study showed a correlation between resection grade and overall survival although this did not reach statistical significance. On the other hand, chemotherapy, either alone or in combination with radiotherapy, appears to act as a preventive factor against tumor progression and also to contribute to prolong overall survival.

Glioblastoma of the spinal cord is an extremely rare form of cancer with a poor prognosis, currently limited by the therapeutic resources available to stop its aggressive progression. There have been some cases in the literature where more aggressive treatments have resulted in longer survival rate than expected.

The radiologic diagnosis of this form of tumor depends largely on magnetic resonance imaging, as other imaging modalities cannot effectively differentiate tumor tissue from normal tissue.

There may be some prognostic markers that indicate a better prognosis for patients with spinal cord glioblastomas; however, generally, their prognosis remains poor. The research has shown that radiochemotherapy has significantly extended both progression- free survival and overall survival among patients diagnosed with this form of cancer. However, more effective therapeutic approaches remain crucial for treatment success. Similar conclusions have been obtained in patients with cerebral GBM [109].

8. Treatment of high-grade gliomas at spinal level

The annual incidence of glioma is quantified at an estimated rate of 5.26 cases per 100,000 people. In the spectrum of primary malignant neoplasms of the central nervous system in adults, malignant astrocytomas occur as the most common type, and glioblastomas, account for about 60-70% of all malignant gliomas. Primary glioblastomas of the spinal cord, on the other hand, occur as extremely rare entities.

8.2. Patients and methods

The 20-year-old patient presented to the neurosurgery clinic was revealing a progressive paraparesis with insidious onset for about 1 month with worsening up to 3/5 MRC and urinary retention syndrome with insidious onset and worsening for about a week. On clinical examination she presents T10 sensitivity level, bilateral extensor CPR, bilateral lower extremity

hyperreflexivity, probed urinary retention. From biological point of view there were no pathologic changes. No significant APP or occupational exposures. The native dorsolumbar MRI performed in the territory shows a T10-L1 intramedullary lesion with infiltrative character, which points the diagnosis towards gliomatous lesion (Figure 8.1).

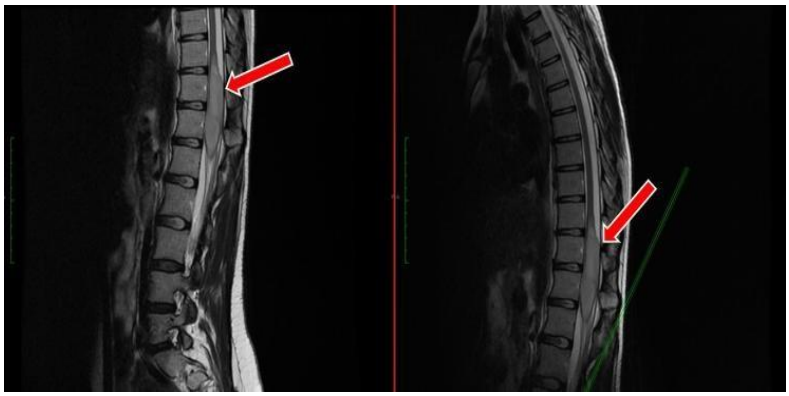

Figure 8.1. Preoperative MRI T2 T2 MRI, thoracic and lumbar spinal cord, sagittal section, T10-L1 intramedullary lesion with infiltrative character.

Surgery was performed by T10-L1 laminectomy, median durotomy and dissection of the posterior median sulcus with evidence of a solid intramedullary lesion without a well demarcated separation plane, with infiltrative areas. The resection was performed with CUSA under electrophysiologic monitoring.

Postoperatively, the patient shows improvement of the motor deficit and the urinary retention syndrome remits. The final anatomo-patologic result is glioblastoma. Subsequently the patient underwent adjuvant chemotherapy and radiotherapy.

The patient is lost to follow-up and returns after 2 years presenting paraplegia and urinary retention with insidious onset and progressive worsening to 0/5 MRC by about 1 month.

Dorsolumbar contrast-enhanced MRI with contrast shows tumor recurrence and extension at the dorsal and lumbar levels.

In view of the strong deficit installed one month ago and the patient's prognosis, we opted for cordectomy up to T6, augmented with gliolan to highlight the tumor infiltrate and to perform a supramarginal resection (Figure 8.4.) Intraoperatively CSF sampled does not reveal tumor cells.

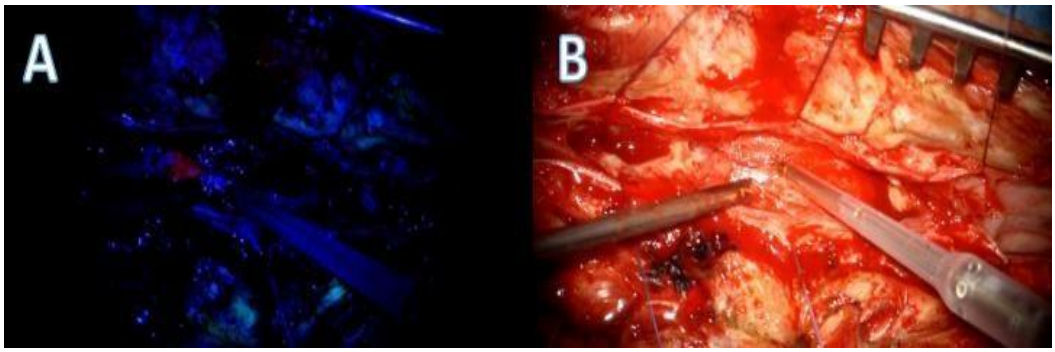

Figure 8.4. Intraoperative view of surgery; A. Fluorescence-guided surgery. B. Microscopically guided surgery.

Postoperative recovery was notable for improvement in motor deficit and resolution of urinary retention syndrome. The final histopathologic diagnosis confirmed IDH1 wild- type

glioblastoma (Figure 8.6), with TP53 mutation and high Ki-67 mitotic index (25%). The patient received adjuvant chemotherapy with a daily dose of 120 mg temozolomide (TMZ) for six weeks and concomitant radiotherapy (RT) with 2Gy fractions daily, five days a week, totaling 60 Gy for six weeks. The tumor had an initial positive response to treatment [114].

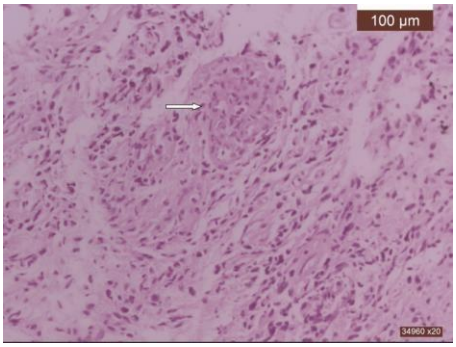

Figure 8.6. Medullary glioblastoma (HE, x40); medullary glioblastoma, IDH1 wild-type, grade 4. Densely cellularized tumor proliferation, composed of diffusely arranged anaplastic astrocytes on a fibrillar background. A vessel with glomeruloid-type endothelio- pericytic proliferation can be identified intratumorally (arrow).

8.5. Partial conclusions

In conclusion, a 20-year-old patient with progressive paraparesis, subsequently diagnosed with spinal glioblastoma, presented a significant challenge given the complexities of interdisciplinary care in the treatment of primary neoplasms of the spinal cord. Glioblastoma, primarily recognized for its supratentorial manifestation, aggressive clinical behavior, and dismal prognosis, when localized to the spinal cord, as demonstrated by this case, introduces a significant therapeutic dilemma.

Thus, meticulous documentation of each case of spinal glioblastoma becomes imperative to enrich the collective understanding of the disease, facilitating a comparative analysis of surgical and adjuvant therapeutic modalities. I believe that, in this case, the use of biodegradable membranes with irinotecan (which are presented in the next chapter) would have induced an eventual remission of the cancer, thus avoiding metastasis.

The patient's favorable initial response to treatment, as evidenced by symptomatic improvement and a prolonged survival period of 34 months post-diagnosis, underscores the potential advantages of comprehensive and multidisciplinary treatment approaches. Although the patient's survival rate does not compare favorably with the outcomes of other oncologic disorders, it is noteworthy that her lifespan was significantly prolonged by the administration of adjuvant chemotherapy and radiotherapy. However, tumor recurrence and the patient's subsequent preference for palliative and supportive care underscores the grim prognosis often faced by patients with this type of diagnosis.

9. Innovative biomembranes with applications in the treatment of high-grade gliomas

9.1 Collagen-based biomembranes, Irinotecan and Minocycline

The bioactive derivative of irinotecan, 7-ethyl-10-hydroxycamptothecin (SN-38), exhibits significantly greater potency - ranging from 100 to 1,000-fold - than its precursor, irinotecan, in inhibiting the topoisomerase enzyme. This enhanced efficacy of SN-38 as a topoisomerase inhibitor is the result of its generation by carboxylesterase-mediated enzymatic

breakdown of irinotecan. Glioma cells have been shown to possess the ability to directly convert irinotecan to SN-38. Subsequent increases in SN-38 concentration are associated with morphologic changes indicative of its cytotoxicity, with a reduction in cell proliferation leading to amplification of cytotoxic effects.

Minocycline is noted for its ability to inhibit the activation of p38 MAP kinase MAP kinase in microglial cells and to suppress chemokine secretion. These actions are implicated in glial cell-mediated regulation of matrix metalloproteinase type 1 (MT1- MMP) in tumor-associated microglia, which use MT1-MMP to promote invasion by degrading the extracellular matrix.

9.1.2.3. Biomembranes obtaining

The manufacture of the biodegradable control membranes involved mixing bovine skin collagen gel with glycerol and distilled water, adjusting the pH of the resulting mixture to 7.2 – 7.4 using 1 M sodium hydroxide, and then introducing a glutaraldehyde solution to facilitate cross-linking of the collagen. Glutaraldehyde was chosen because of its cross-linking efficacy, promoting interactions between its aldehyde groups and the amino groups present in the lysine or hydroxylysine residues in collagen polypeptide chains.

Drug-infused membranes were produced by a similar methodology. The active agents (Irinotecan or Minocycline) were initially dissolved in water. A precise amount of collagen gel was then mixed with glycerol and the respective drug solutions, the pH of the solution was adjusted to 7.2 – 7.4 using 1 M sodium hydroxide, before addition of the cross-linking agent, glutaraldehyde. Membranes were designed to incorporate Irinotecan at five distinct concentrations (10%, 20%, 20%, 30%, 40% and 50%) or Minocycline at two concentrations (40% and 20%) against the collagen base.

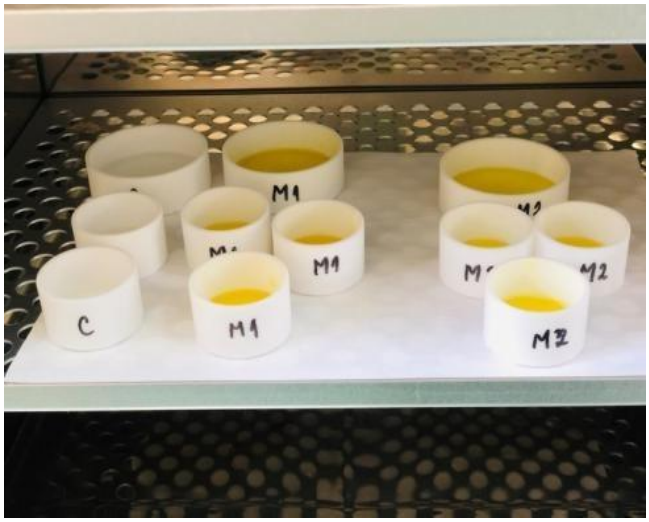

Figure 9.2. Step in membrane manufacture; Addition of cross-linking agent and deposition in Teflon Petri dishes for drying.

These resulted solutions were subsequently deposited in Teflon Petri dishes for drying at room temperature, resulting in the formation of membranes (Figure 9.2). A summary of membrane compositions is provided in Table 9.1.

Table 9.1. Composition of tested membranes - C - collagen membrane, M1, M2 - membrane with collagen and minocycline, IR1-5 - membrane with collagen and irinotecan

Membrane tested	Minocycline (%)	Irinotecan (%)
C	0	0
M1	20	-
M2	40	
IR1	-	10
IR2	-	20
IR3	-	30
IR4	-	40
IR5	-	50

From the experimental study it can be stated that the minocycline impregnated membranes showed a slight tendency to embrittlement.

9.1.3.1. The ability of membranes to absorb water

The results of the water uptake analysis for the Minocycline impregnated membranes are shown in Figure 9.4. The results indicate that a low concentration of Minocycline does not significantly affect the water uptake capacity of the collagen, while a higher concentration of Minocycline results in a decrease in the water content of the absorbed water compared to the control collagen membrane. This behavior may be due to an interaction between Minocycline and collagen during membrane formation.

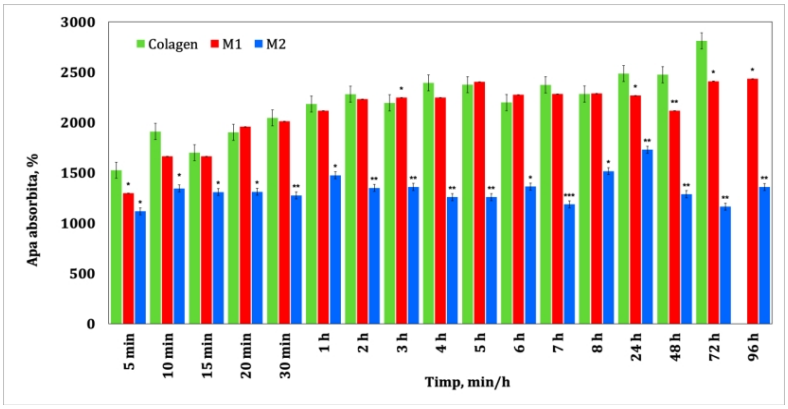

Figure 9.4. Water absorption for collagen membranes with different minocycline concentration; M1- 20% minocycline; M2- 40% minocycline

A p-value > 0.5 indicates that the results obtained are not statistically significant. '*' corresponds to results with statistical significance ($0.5 < p < 0.1$),

"**" corresponds to statistically distinctly significant results ($0.1 \leq p < 0.01$) and '***' corresponds to highly statistically significant results ($p \leq 0.01$).

The water absorption capacity was also evaluated for membranes containing Irinotecan, as shown in Figure 9.5. In both cases, the maximum water absorption capacity was approximately 2500%.

Typically, a glioblastoma measures about 4 cm in diameter. Following surgical resection, the resulting cavity could be filled with a membrane of equivalent size. The ability of this membrane to retain water could offer advantages in the context of treatment management, especially since cerebrospinal fluid is 99% water.

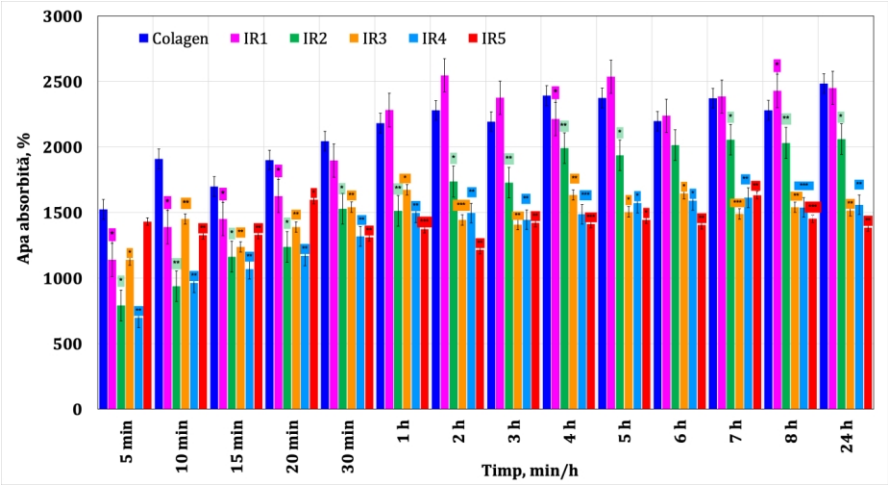

Figure 9.5. Water uptake for collagen-based membranes with different concentrations of irinotecan; IR1-10%; IR2-20% irinotecan; IR3-30% irinotecan; IR4-40%; IR5- 50%
A p value > 0.5 indicates that the results obtained are not statistically significant.
"*" corresponds to results with significance statistical significance (0,5 < p < 0,1),
'**' corresponds to statistically distinct significant results ($0.1 \leq p < 0.01$) and
'***' corresponds to highly statistically significant results ($p \leq 0.01$).

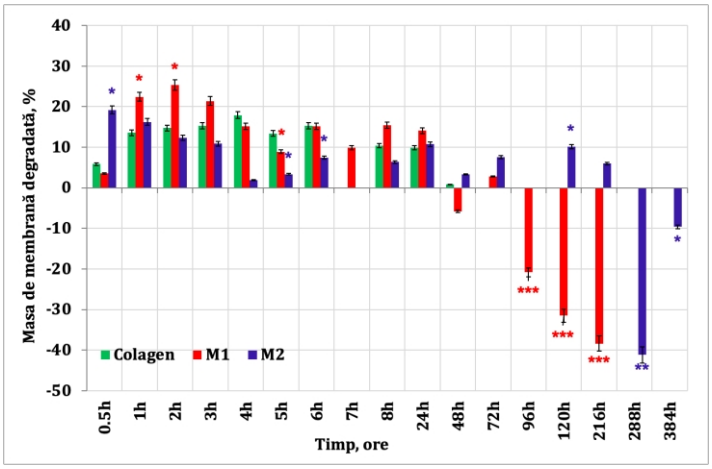

Figure 9.6. Influence of collagenase on collagen membranes with minocycline A p-value > 0.5 indicates that the results obtained are not statistically significant.
'*' corresponds to results with statistical significance (0.5 < p < 0.1),
"**" corresponds to statistically distinctly significant results ($0.1 \leq p < 0.01$) and
'***' corresponds to highly statistically significant results ($p \leq 0.01$).

9.1.3.2. Degradation of bimembrane by collagenase

Figures 9.6. and 9.5. show the results of the enzymatic degradation analysis. Initial observations indicate that the membranes undergo degradation within the first 4 hours. This process subsequently ceases when the membranes begin to absorb the liquid. This behavior suggests that Minocycline, once released from the membrane, inhibits enzyme activity. In addition, the obtained data also suggest that Irinotecan, upon its release from the membrane after a period of 24 hours, similarly exerts inhibitory effects on the enzyme.

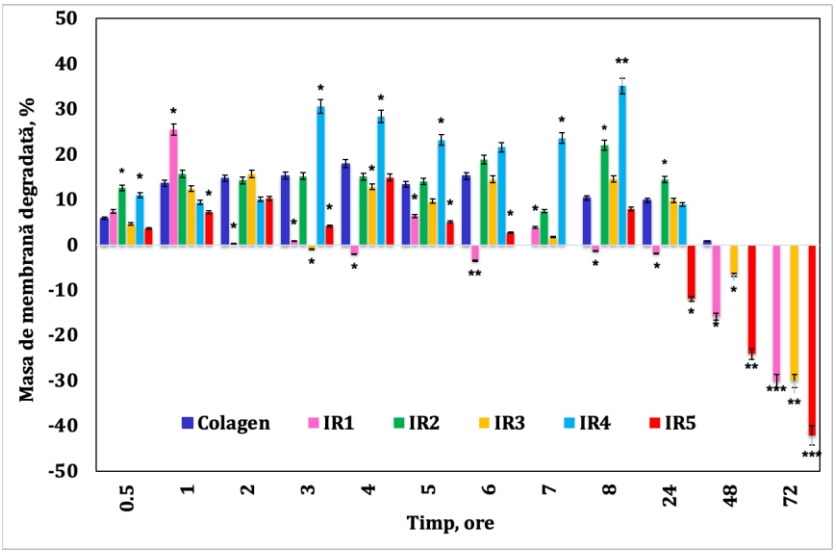

Figure 9.7. Influence of collagenase on collagen membranes with irinotecan
A p- value > 0.5 indicates that the results obtained are not statistically significant.
'*' corresponds to results with statistical significance (0.5 < p < 0.1),
"**" corresponds to statistically distinctly significant results (0.1 ≤ p < 0.01) and
'***' corresponds to highly statistically significant results (p ≤ 0.01).

9.1.3.3. Drug release

From Figure 9.8 it can be seen that the minocycline-loaded samples show consistent release profiles, with a pronounced initial release observed within the first 15 minutes (48.57% for M2 and 59.35% for M1). This abrupt release is advantageous for establishing an aseptic environment post-operatively, subsequently transitioning to sustained drug release within 24 hours to inhibit further bacterial proliferation. Total minocycline released within 24 hours was 71.95% for sample M1 with a lower drug concentration and 65.81% for sample M2 with a higher drug concentration [151, 152]

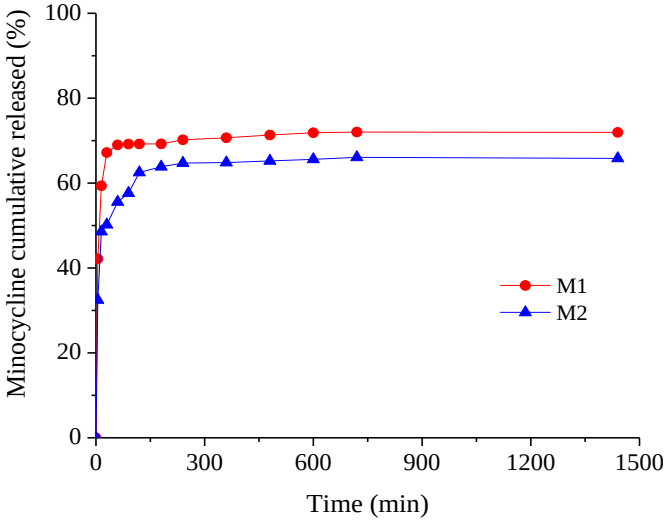

Figure 9.8. Time dependence of minocycline release from collagen membranes

The analysis of Figure 9.9 shows that for irinotecan-loaded samples, an enhanced initial release is observed for samples with lower drug concentrations, reaching 74.45% (IR1) and 73.61% (IR2), while samples with higher drug content showed a significant reduction in initial

release, about 2-fold for IR3 and five to six-fold for IR4 and IR5, respectively. During the 48-hour experiment, the cumulative percentage of irinotecan released ranged from 50.25% (IR5) to 89.85% (IR1). The rapid release of high local concentrations of irinotecan facilitates immediate regression of tumor cells, while the gradual and continuous release that follows maintains effective concentrations for sustained therapeutic action and tumor resolution [153].

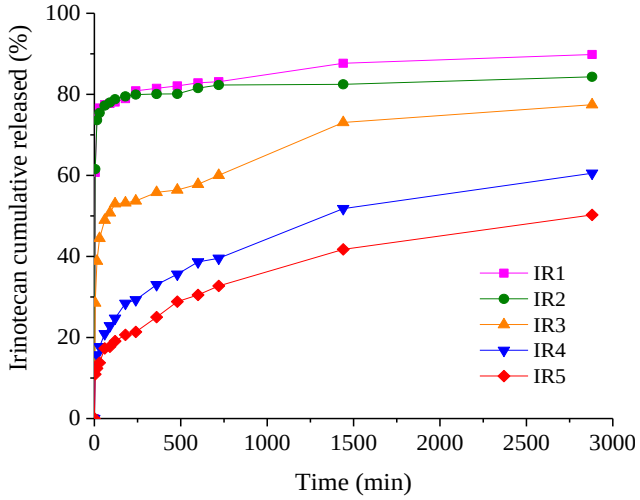

Figure 9.9. Time dependence of irinotecan release from collagen membranes.

9.1.3.4. Antimicrobial activity

Tests performed on *E. coli (G- microorganism)* showed that minocycline almost completely inhibited the growth of this microorganism (d=41.7 ± 2.89mm). As for the tested biomaterials, they behaved somewhat differently, probably depending on the concentration of the antibiotic in the material (fig. 9.11 – 9.12). The best inhibition diameters are obtained for the biomaterial M2 (fig.9.12, d=21.67 ± 1.15mm) followed by the biomaterial M1 (fig.9.11, d = 20.33 ± 0.58mm).

In the case of *S. aureus* (G+ microorganism), minocycline totally inhibited the growth of this microorganism (d=50 ± 0.00mm). Regarding the membrane with monocycline this resulted in an inhibition diameter of 29 ± 5.41 mm, membrane M1, Figure 9.14.

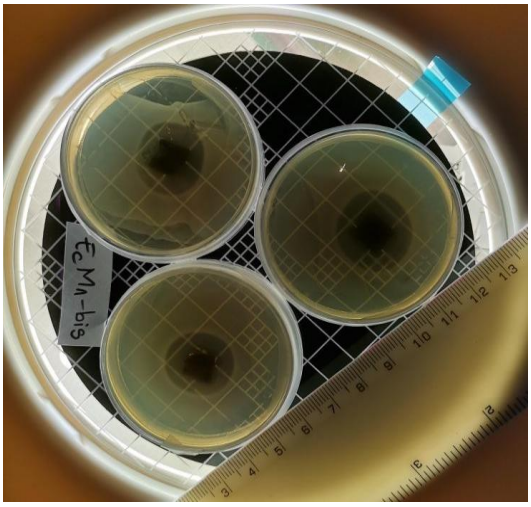

Figure 9.11. Effect of biomaterial M1 on *E. coli*.
DI = (20.33 ± 0.58)mm

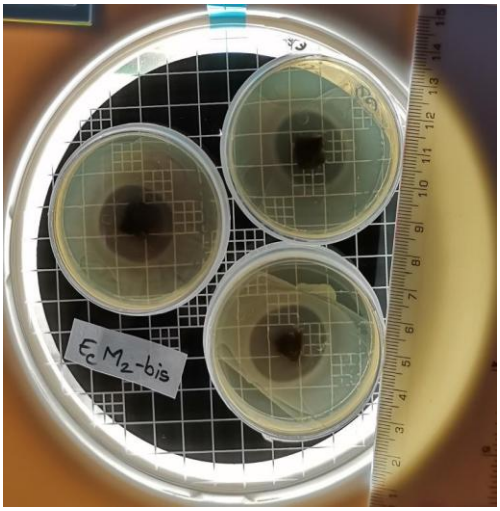

Figure 9.12. Effect of biomaterial M2 on *E. coli*.
DI = (21.67 ± 1.15) mm

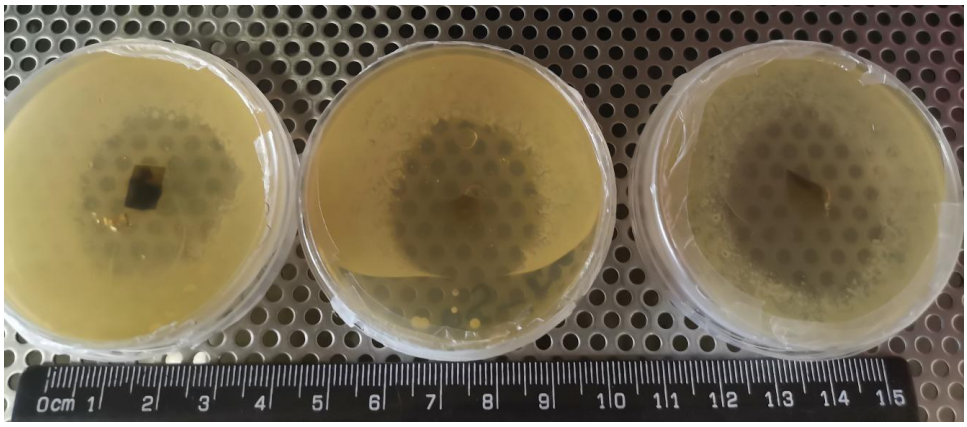

Figure 9.14. Effect of M1 bis biomaterial on *S. aureus*
Mean inhibition diameter (28 ± 2.65) mm

9.1.4. Partial conclusions

Collagen membranes, characterized by their distinct physical, chemical and biological attributes, have attracted considerable attention in the fields of tissue engineering and drug delivery. The focus has been on cross-linking collagen gels using glutaraldehyde, incorporating Minocycline and Irinotecan, which serve as antibiotic and chemotherapeutic agents, respectively.

The collagen-based membranes proposed in this research represent a promising therapeutic pathway for targeting glioblastomas that traditionally show poor responses to medication. Specifically for glioblastoma, advances in nanoengineering have emerged as viable strategies. However, the use of membranes incorporating minocycline and irinotecan against high-grade glioma has remained unexplored, despite the fact that these agents are recognized for their therapeutic efficacy in such malignancies.

The results obtained on water uptake, enzymatic degradation, antimicrobial efficacy and drug release dynamics from the designed membranes emphasize the significant role of drug type and concentration. Each membrane variant demonstrates a biphasic release profile, advantageous for localized prophylaxis and treatment strategies in the management of glioblastoma.

This research supports that a membrane with a 30% concentration of irinotecan continues drug release for more than two days, while a membrane containing 40% minocycline maintains drug presence for more than five hours, with sustained release extending over 24 hours. This gradual release mechanism ensures a stable and effective drug concentration at the treatment site, maximizing therapeutic potential and decreasing the likelihood of tumor recurrence. Accordingly, these membranes could substantially improve the efficacy of localized treatment by optimizing initial release with extended drug availability, particularly in the context of antibiotic administration and the presence of chemotherapeutics. Therefore, the development of these membranes marks a significant step forward towards the formulation of a comprehensive treatment modality for the particular challenge of glioblastoma.

9.2. Collagen and nanoparticles based biomembranes with Irinotecan

This chapter reviews advances in nanotechnology-based drug delivery systems (nanoDDS), focusing on the bioengineering of irinotecan-loaded nanostructured lipid

frameworks (NLCs) in collagen matrices with potential uses in tissue engineering and drug delivery applications. Irinotecan, a potent chemotherapeutic agent, is combined with Minocycline, an antibiotic that additionally causes apoptosis of glioblastoma cells, this combination harnessing the effective therapeutic potential on high-grade glioblastoma. The manufacturing process involved the use of type I collagen, derived from bovine skin, refined by previously described fibril-forming techniques. After fibril formation, collagen matrices were cross-linked using glutaraldehyde, resulting in the creation of bioresorbable membranes. These engineered membranes were doped with varying concentrations of NLC suspension to achieve therapeutic agent (irinotecan) loadings of 10%, 20%, 30%, 40% and 50% relative to pure collagen matrix, respectively 20% Minocycline.

The bioresorbable membranes, impregnated with different drug concentrations, were subjected to rigorous evaluations, including water absorbency and enzymatic degradation rates. This methodology highlights the substantial advantages of localized drug delivery systems, particularly in the context of treating high-grade gliomas. The targeted delivery mechanism significantly diminishes systemic side effects while simultaneously amplifying drug concentration in the tumor bed.

Furthermore, this research critically examines the impediments associated with the systemic administration of chemotherapeutic agents, emphasizing in particular the challenges imposed by the restrictive properties of the blood-brain barrier. We advocate the use of localized delivery methods as a possible effective strategy in the management of glioblastoma. The incorporation of bioresorbable matrices in the delivery systems of drug delivery presents itself as a particularly promising avenue, providing a platform for targeted delivery of therapeutic agents. The thesis aligns itself among innovations in the efficacy of nanotechnology-based interventions in medical treatments, proposing novel solutions to the complexities of drug delivery in the context of cancer therapy.

Table 9.2. Membranes tested and their composition

Sample Code	NLC IR 1	NLC IR 2	NLC IR 3	NLC IR 4	NLC IR 5	NLC IR 3 MC
Irinotecan (%)	10	20	30	40	50	30
Minocycline (%)	-	-	-	-	-	20

9.2.6. Partial conclusions

The use of nano-lipid-carriers (NLC) for the delivery of irinotecan is a highly efficient modality in view of the long-term release of the chemotherapeutic agent. By comparing membranes with irinotecan that manage to deliver the chemotherapeutic agent over a duration of 3 days, and nanoparticle-enhanced membranes that provide a pre-released release for at least 7 days, a significant increase of about 60% in drug release time is observed.

The incorporation of minocycline into the collagen-based membrane, together with irinotecan, gives the material strong local antibacterial properties, effectively targeting pathogenic microorganisms such as *Escherichia coli* (E. coli) and *Staphylococcus aureus* (S. aureus). This characteristic is crucial for applications where prevention of post-surgical infections is important, providing a proactive measure against common bacterial pathogens.

Beyond the antimicrobial capabilities, the combination of minocycline and irinotecan in

these biomaterials is promising for enhancing the antiproliferative effects on cancer cells, particularly those characteristic of glioblastoma. The synergism between irinotecan, a known chemotherapeutic agent, and minocycline extends beyond simple additive effects, suggesting a potentiated interaction that may amplify the therapeutic impact against glioblastoma. This synergistic mechanism may involve concomitant disruption of cell replication processes by irinotecan and modulation of the tumor microenvironment or inhibition of mechanisms contributing to chemoresistance by minocycline.

Such a dual-functional approach - combining antimicrobial protection with enhanced antitumor activity - highlights the innovative potential of these biomaterials [183 - 188]. Thus the strategic advantage of incorporating multiple therapeutic agents into a single delivery platform to address both the immediate risk of infection and the long-term limitation of cancer proliferation is emphasized. This multi-planar strategy could significantly improve clinical outcomes, particularly in treating conditions where risk of infection and tumor resistance to conventional therapies are major concerns .

General conclusions and personal contributions

In this thesis, I conducted a comprehensive and detailed analysis of central nervous system glioblastoma, including both cerebral and medullary glioblastoma, and I explored multiple facets of this devastating pathology. This study has been developed on many levels, from diagnosis and imaging to current treatments and proposed therapeutic innovations.

Cerebral and Medullary Glioblastoma

Glioblastoma, either cerebral or medullary, is one of the most aggressive forms of central nervous system tumor, with an extremely poor prognosis. In the case of medullary glioblastoma, this rare pathology is characterized by a rapid and aggressive progression and current therapeutic options are insufficient to halt disease progression. There are some exceptional cases in the literature where aggressive treatments have significantly prolonged patient survival. Diagnosis for these tumors is only possible by magnetic resonance imaging (MRI), highlighting the importance of advanced imaging technologies in the management of this disease. Confirmation of pathology is based on pathology studies performed on intraoperatively sampled tissue specimens.

Role of Functional Imaging

Functional imaging, especially functional MRI, plays a crucial role in the diagnosis and management of glioblastoma. I demonstrated that functional MRI is a valid tool for the assessment of brain functionality and should be systematically included in the preoperative planning of glioblastoma, in case of localizations in both functional eloquent areas such as motor, pre-central and post-central areas, deep brain areas such as basal ganglia or brainstem, and areas responsible for language such as Wernicke's area or Broca's area. The main advantage of this technique is its non-invasiveness, followed by its wide availability, the ability to provide detailed anatomical images and the possibility to repeat the examination without the risk of radiation to the patient.

Functional MRI is a useful tool for assessing brain functionality, thus it can be routinely included in the preoperative planning of glioblastomas in functional areas, allowing the most complete resection to be achieved; the quality of resection being directly correlated with

survival and recurrence rate.

I have shown in the present work that by concomitant use of functional MRI and navigated TMS I obtained a more detailed and descriptive preoperative planning.

I also emphasized the importance of functional MRI in monitoring postoperative brain plasticity. However, there are disadvantages, such as neurovascular decoupling phenomenon and unreliability in surgical decision making. Through the case study, I have shown that the use of MRI in combination with navigated transcranial magnetic stimulation (TMS) can provide more detailed and accurate preoperative planning.

In conclusion I believe that there is still much unexplored potential in the use and development of both functional MRI and navigated TMS.

Prognostic Assessment and Current Treatments

Assessing prognosis in glioblastoma remains a major challenge. Although there are some favorable prognostic factors, overall, the future of patients diagnosed with glioblastoma is limited. Among the good prognostic factors I identified:

- younger patients have a more favorable prognosis,
- a longer course of symptoms before diagnosis is suggestive of a more favorable prognosis,
- macroscopic resection may improve the mortality.

As a negative prognostic factor, I identified histopathologic Ki-67 index with values >40% and P53 mutation as associated with a limited prognosis.

I have shown that radiochemotherapy can significantly prolong progression-free survival and overall survival in patients diagnosed with medullary glioblastoma.

I have shown by means of graphs how the Kaplan-Meier survival curve is correlated with the degree of exeresis, but the association has no statistical significance. In the study I observed how chemotherapy alone or in association with radiotherapy is a protective factor against tumor progression, thus allowing prolongation of overall survival or progression-free survival.

However, these treatments remain insufficient to ensure a complete cure or long- term survival.

Innovations in topical chemotherapy treatments with chemotherapeutic and antimicrobial collagen-based membranes

A recent study, published in February 2024, demonstrates that implants are a promising new treatment for glioblastoma that could be rapidly implemented in the clinic. Nanoengineering has shown huge potential in the treatment of glioblastoma, and the collagen-based membranes proposed in this thesis represent an innovative therapeutic opportunity for treating drug-resistant brain cancers.

Membranes utilizing minocycline and irinotecan have been developed to respond specifically to high-grade glioma, and these substances are proven therapeutic agents for this type of central nervous system malignancy. The results of this study showed that drug type and concentration significantly influence the water uptake, enzymatic degradation, antimicrobial activity, and drug release characteristics of the created membranes. The biphasic kinetic profiles are favorable for prophylaxis and local treatment of glioblastoma, and the drug release results suggest that the membrane containing 30-50% irinotecan continues to release drug even after two days, whereas the membrane with 40% minocycline provides substantial drug

concentrations within the first five hours and lower concentrations up to 24 hours.

Release experiments of irinotecan encapsulated in nanocarriers have shown that its release occurs, as expected, following a multi-step mechanism. In the first step, in the first 100 minutes, more rapidly, irinotecan that is not encapsulated in nanoparticles is rapidly released. This is beneficial for annihilating existing cancer cells. A plateau stage follows, the stage where the encapsulated irinotecan crosses the lipid barrier of the nanocapsule. In the next step, between about 600 min and 4000 min, the irinotecan that has left the capsule is released. The last step is extremely slow and is the step in which the remaining irinotecan in the membrane is released. This extremely slow release is extremely beneficial because in this way precancerous cells that were not killed initially can be annihilated. The nanoparticles are designed to gradually release irinotecan directly into tumor tissue, providing sustained therapeutic levels for at least 66 hours. By using nanoparticles as carriers for irinotecan, its distribution and efficacy are improved, making it an effective localized chemotherapy treatment for glioblastoma.

The slow release of irinotecan ensures a constant and efficient therapeutic concentration of the drug, maximizing the therapeutic effect and reducing the risk of tumor recurrence. Thus, these membranes could be effective in local treatment due to the balance in the prolonged release of the anticancer chemotherapeutic drug.

The study of the antimicrobial efficacy of the biomembranes doped with irinotecan based nano-lipid-carriers in 30% concentration and minocycline 20%, showed significant antibacterial effects against Gram-negative and Gram-positive bacteria. Tests demonstrated a mean inhibition diameter of 17.33 mm for *Escherichia coli* and 16.5 mm for *Staphylococcus aureus*. This moderate activity emphasizes the importance of the composition of the biomaterial in its antimicrobial efficacy.

Collagen biocomposites offer advantages in medical applications, including biodegradability and preventive antimicrobial activity.

The composite biomaterial combining irinotecan and minocycline has not only antimicrobial effects but also synergistic antiproliferative potential against cancer cells, reducing chemoresistance and enhancing the efficacy of chemotherapy. Research suggests that minocycline contributes to tumor cell sensitization, improving therapeutic outcomes. Developing these membranes is an important step forward in the treatment of brain cancer.

Future Directions

Exploring functional imaging, assessing prognosis and innovating treatments are essential steps in the fight against this devastating pathology.

Chemotherapeutic agent-encapsulated nanoparticles offer a promising approach for the effective use of irinotecan in the local treatment of glioblastoma after surgical resection, as it manages to bypass the blood-brain barrier, thus that effective doses are lower, with increased bioavailability directly at the operating bed, and with limited side effects in terms of systemic toxicity.

This thesis emphasizes the promising potential of chemotherapeutic-encapsulated nanoparticles, and the importance of further research and development of new therapies to improve the prognosis and quality of life of patients diagnosed with glioblastoma, whether cerebral or medullary.

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