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Evaluating the Effectiveness of Gabapentin and Ginkgo Biloba in the Neuroinflammatory Process within a Co-Culture Model of GBM and Neurons

GBM ve Nöron Ko-Kültür Modelinde Gabapentin ve Ginkgo Biloba'nın Nöroinflamatuar Süreçteki Etkinliğinin Değerlendirilmesi

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ABSTRACT

This study aimed to investigate the anti-inflammatory effects of Ginkgo Biloba extract and Gabapentin on a neuron-Glioblastoma cell line. Utilizing advanced co-culture techniques, we simulated a tumor environment that closely mimics in vivo conditions and performed a detailed genetic analysis of cytokine expression. Testing a range of doses from 20 to 80 µg/ml, we found that the combination of Gabapentin (80 µg/ml) and Ginkgo Biloba extract (80 µg/ml) did not alter levels of proinflammatory cytokines such as IL1β, IL6, IL8, and TNF-α. However, it significantly increased the levels of the anti-inflammatory cytokine IL10 compared to other groups. These findings suggest that the co-administration of Gabapentin and Ginkgo Biloba can modulate the inflammatory response, maintaining it at levels similar to the control group. Determining the precise effective dosage range and understanding the mechanisms to halt neuroinflammation will be crucial for advancing therapeutic options for glioblastoma multiforme. This research provides a promising foundation for developing new treatment strategies aimed at reducing inflammation in glioblastoma patients.

Key Words
Cell culture, proinflammatory cytokines, glioblastoma multiforme, interleukin.

ÖZ

Bu çalışma, Ginkgo Biloba ekstresi ve Gabapentin'in nöron-Glioblastoma hücre hattı üzerindeki anti-inflamatuar etkilerini araştırmayı amaçladı. Gelişmiş ko-kültür teknikleri kullanarak in vivo koşulları yakından taklit eden bir tümör ortamı simüle ettik ve sitokin ekspresyonunun detaylı genetik analizini gerçekleştirdik. 20 ila 80 µg/ml aralığında farklı dozları test ettik ve Gabapentin (80 µg/ml) ile Ginkgo Biloba ekstresinin (80 µg/ml) kombinasyonunun, IL1β, IL6, IL8 ve TNF-α gibi proinflamatuar sitokin seviyelerini değiştirmediğini, ancak diğer gruplara kıyasla anti-inflamatuar sitokin IL10 seviyelerini önemli ölçüde artırdığını bulduk. Bu bulgular, Gabapentin ve Ginkgo Biloba'nın birlikte uygulanmasının inflamatuar yanıtı düzenleyebileceğini ve bunu kontrol grubuna benzer seviyelerde tutabileceğini göstermektedir. Kesin etkili doz aralığının belirlenmesi ve nöroinflamasyonun durdurulma mekanizmalarının anlaşılması, glioblastoma multiforme için terapötik seçeneklerin ilerletilmesi açısından kritik olacaktır. Bu araştırma, glioblastoma hastalarında inflamasyonu azaltmayı hedefleyen yeni tedavi stratejilerinin geliştirilmesi için umut verici bir temel sağlamaktadır.

Anahtar Kelimeler
Hücre kültürü, proinflamatuar sitokin, glioblastoma multiforme, interlökin.

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INTRODUCTION

Gliomas, which are primary brain tumors originating from glial cells, are highly lethal and incurable. The development of these tumors is caused by the malicious deregulation of glial cells [1,2]. Among diffuse gliomas, the most common and deadliest malignancy is Glioblastoma multiforme (GBM), a type of tumor found in the brain's parenchyma. GBM accounts for approximately 54% of all gliomas and and occurs at an annual rate of 3-5 cases per 100,000 individuals and is predominantly found in the frontal lobe. Its highly heterogeneous structure prevents complete surgical resection, significantly reducing patient survival rates. The aggressive nature of GBM is further amplified by its rapid infiltration into surrounding tissues. Both the tumor's anatomical positioning and molecular characteristics are pivotal in influencing not only the disease's prognosis but also the strategies for surgical intervention and treatment planning [2–4]. Consequently, a combination of radiotherapy and chemotherapy is typically administered after surgery. However, challenges arise in the effectiveness of these treatments due to the emergence of inherent and acquired resistance in tumor cells. The current treatment protocol is surgical resection plus radiotherapy and adjuvant temozolamide or carmustine administration [2,3, 5].

The primary aim of the present study is to provide insight into the micro-environment surrounding tumors, which comprises of inflammatory chemokines and cytokines [6]. This micro-environment is known to have a significant impact on the initiation and progression of cancer. Inflammation, regarded as the seventh hallmark of cancer, is proposed to have a connection with acquired chemo-resistance in GBM [7]. GBM is encompassed by growth factors, chemokines, and pro-inflammatory cytokines. Many studies have highlighted the role of inflammation in the development of chemo-resistance [8]. Research has also indicated that TMZ treatment can enhance the expression of pro-inflammatory cytokines that promote oncogenesis. Activating inflammatory chemokines, long-term treatment with TMZ has been found to induce resistance in astroglia cells. Therefore, it is plausible to suspect that the production of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α by TMZ may contribute to drug resistance [2,9].

Gabapentin (GBP), initially developed as an epilepsy treatment, finds widespread use in clinical practice for managing neuropathic pain [10,11]. While it bears a structural resemblance to the neurotransmitter γ-aminobutyric acid (GABA), its precise mode of action remains unknown. Unlike GABA's metabolites, GBP's metabolites do not engage with GABAA or GABAB receptors, nor do they influence the decay or uptake of GABA [11,12]. Research suggests that GBP may form bindings with glutamatergic receptors and α2δ subtype voltage-dependent calcium channels. As a result, it curbs neuronal excitability by reducing the creation of excitatory presynaptic vesicles [11,13]. Furthermore, GBP's pleiotropic effects enable it to counter gastric inflammatory damage caused by indomethacin and ethanol [11,14]. In vivo studies have demonstrated the systemic anti-inflammatory actions of GBP, showcasing its ability to curb cytokine release and diminish oxidative stress [11,15].

In a similar study, it was shown that gabapentin decreased IL-6 production by inhibiting NFκB in human neuroblastoma (SH-SY5Y) and rat glioma C6 cell lines [11,16].

Comprehensive analysis suggests substances with chemopreventive properties, including polyphenols (e.g., flavonoids), terpenoids, carotenoids, tocopherols, thiols, and trace metals, may offer protection against cancer through multiple biochemical mechanisms [17,18]. Since the same principle can be applied to other plant extracts and their chemical components, it becomes imperative to explore the use of such agents in the pursuit of cancer control. This is crucial because most forms of cancer are believed to originate from various factors, involving alterations in the expression of multiple genes and disruptions in cellular signal transduction occurring at both the genetic and epigenetic levels [19]. An alternative approach to cancer prevention, distinct from chemotherapy, may involve the advancement of plant extracts (and/or their chemical components). Certain plant extracts have already demonstrated their ability to impact the advancement of cancer, leading to a potential foundation for chemopreventive therapy. An analysis conducted recently, encompassing studies carried out with both animals and humans in controlled environments and within living organisms, has shed light on the potential anticancer properties of an extract obtained from Ginkgo biloba leaves, commonly known as 'EGb 761', along with certain elements found within it [20]. EGb 761 stands out as one of the extensively employed preparations among the diverse range of Ginkgo leaf extracts. This specific extract (at a 50:1 ratio) con-

tains approximately 24% flavonoid glycosides, around 6% terpene trilactones exclusive to Ginkgo (namely, ginkgolides, classified as diterpenoids, and bilobalide, categorized as a sesquiterpene), roughly 7% proanthocyanidins, as well as select low molecular weight organic acids. These constituents collectively contribute to the wide range of effects observed from its use. Several countries have authorized the prescription of EGb 761 and similar products for the treatment of conditions such as cerebrovascular and peripheral vascular insufficiency, neurosensory disorders, and issues associated with impairment in alertness, short-term memory, and other cognitive functions that commonly arise in the context of aging, dementias, and senility [20,21].

We aimed to investigate the anti-inflammatory activities of Gingko Biloba extract (GBe) and Gabapentin in neuron-Glioblastoma cell line. In the co-culture we created, a suitable microenvironment environment was created for the tumor and the expression of pro-inflammatory and anti-inflammatory cytokines were analyzed genetically when given alone and in combination at dose ranges from 20 to 80 µg/ml.

MATERIALS and METHODS

Chemicals

Neurobasal Medium (NBM), Fetal Bovine Serum (FBS), B27 Supplement, and an antibiotic solution containing penicillin, streptomycin, and amphotericin B were purchased from Gibco, USA. Dulbecco's Modified Eagle Medium (DMEM) was also sourced from Gibco, USA. Gabapentin (Neurontin) was acquired from Pfizer Inc., New York, USA, while Ginkgo Biloba extract (Tebonin EGb 761) was obtained from Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany. The cell culture flasks with a surface area of 150 cm^2 were purchased from Corning Inc., Corning, NY, USA.

Preparation of Neurons

The cortical neuron cells used in this study were obtained from the Department of Pharmacology at Atatürk University. Upon receiving the cells in cryofalcons, they were thawed at room temperature and then centrifuged for 5 minutes at 1200 rpm, using a +4 °C centrifuge (Bachmann, Germany). Subsequently, 2 cc of NBM medium (Neurobasal Medium, Gibco, USA) was added to the centrifuged cells. The resulting mixture was transferred to new medium containing neurobasal medium, 1/10 FBS, 1/50 B27, and 0.1% antibiotic (penicillin-streptomycin-amphotericin B).

Glioblastoma Cell Culture

The T98G GBM cell lines were generously provided by the researchers from the Department of Medical Pharmacology at Ataturk University in Erzurum, Turkey. In a concise process, the cells were re-suspended in a fresh medium consisting of Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS; 15%), and a 1% antibiotic solution (comprising penicillin, streptomycin, and amphotericin B). Subsequently, the cells were placed into 150 cm^2 cell culture flasks from Corning, located in Corning, NY, USA, and cultivated within a CO_2 incubator set at the appropriate conditions (5% CO_2; 37 °C).

Co-cultures and treatment

In the experiments, both T98G GBM cell lines and cortical neuron cells were utilized. To facilitate co-culture studies, a transwell culture system was employed. T98G cells were cultivated in six well plates until they reached 80% confluency. The inserts, which were placed in the upper parts of the culture dishes, contained the neuron cells. The insert membrane had pores with a diameter of 0.4 µm, preventing physical interaction between the co-cultured cells. Various concentrations of gabapentin (20, 40, 60, and 80 µg/ml) and Gingko Biloba (20, 40, 60, and 80 µg/ml) were administered to the co-cultured cells [22,23] (Table 1).

Real-Time PCR Analysis

We extracted total RNA from GBM cells through the utilization of the RNeasy Mini Kit from Qiagen. These RNAs were subsequently subjected to reverse transcription, transforming them into complementary DNA, a process executed with the cDNA Reverse Transcription Kit provided by Applied Biosystems in the USA. For the evaluation of TNFα, IL1β, IL6, IL8, and IL10 expression, we harnessed the cutting-edge technology of the StepOnePlus Real-Time PCR System, again a product of Applied Biosystems. In this analysis, we employed the cDNA synthesized from co-culture RNA, a procedure previously detailed. Notably, all the primers and probes applied in this study were procured from TaqMan Gene Expression Assays, which are available through Applied Biosystems. To ensure the accuracy of our findings, we also incorporated expression data related to β-actin as an endogenous control [10].

Table 1. In vitro experimental groups and doses.

Groups	Dose	Substance
Control	-	-
GAB 20	20 µg/ml	Gabapentin
GAB 40	40 µg/ml	Gabapentin
GAB 60	60 µg/ml	Gabapentin
GAB 80	80 µg/ml	Gabapentin
GKB 20	20 µg/ml	Gingko Biloba
GKB 40	40 µg/ml	Gingko Biloba
GKB 60	60 µg/ml	Gingko Biloba
GKB 80	80 µg/ml	Gingko Biloba
GAB 60 + GKB 60	60 µg/ml	Gabapentin + Gingko Biloba
GAB 60 + GKB 80	60 µg/ml +80 µg/ml	Gabapentin + Gingko Biloba
GAB 80 + GKB 60	80 µg/ml +60 µg/ml	Gabapentin + Gingko Biloba
GAB 80 + GKB 80	80 µg/ml +80 µg/ml	Gabapentin + Gingko Biloba

Statistical analysis

In order to compare the molecular data across different groups, we employed the one-way analysis of variance (ANOVA) using IBM SPSS 23.0, a statistical software. To determine the equality of variances among the groups, we conducted Levene's test, and we evaluated the normal distribution within each group using the Shapiro-Wilk test. Group distinctions were established by applying One-way ANOVA, followed by a Duncan multiple range test (DMRT) with a significance level of $p<0.05$. The mean±SD represents the results for each group.

RESULTS and DISCUSSION

The tumor microenvironment (TME) serves as a conducive environment for tumor growth and invasion, aids in neoplastic transformation, shields the tumor from host immunity, and provides supportive conditions for dormant metastases to flourish. The TME arises from the interactions between cancerous and non-cancerous cells, contributing to the disappointing outcomes observed in GBM treatment alongside the high tumor heterogeneity. GBM tumor cells possess the capability to convert the immune response into persistent inflammation, thereby facilitating tumor relapse by nurturing GBM stem cells (GSCs), resulting in epithelial-mesenchymal transition and resistance to multiple drugs. Additionally, GBM expansion induces angiogenesis and inflammatory cytokines, which can lead to the development of an aberrant blood brain barrier (BBB) or blood tumor barrier (BTB), further hindering the infiltration of functional immune cells into the brain. In addition to tumor cells, glioma-associated microglia/macrophages (GAMs) constitute the primary infiltrating immune cell population, accounting for roughly 30% of the GBM cell population. Consequently, the autocrine and reciprocal paracrine interactions between GBM tumor cells and GAMs foster an inflammatory TME that aids tumor promotion. Furthermore, inflammation synergizes with the Warburg effect in GBM, amplifying the inflammatory response in the TME through increased production of inflammatory cytokines, lactate, and factors that suppress the immune system and promote angiogenesis. This creates a more favorable and beneficial environment for GBM progression. Therefore, an appealing therapeutic strategy for GBM lies in targeting the inflammatory TME [24].

The GAB 20 group saw the most substantial increase in IL1β expression. In contrast, the GAB 80+ GKB 80 group showed levels similar to the control group, indicating no significant change. This suggests that lower doses of Gabapentin may elevate IL1β levels more than higher doses or combinations with Ginkgo Biloba extract (Figure 1).

Similar to IL1β, the GAB 20 group recorded the highest increase in IL6 expression. The GAB 80+ GKB 80 group did not show any significant change compared to the control, highlighting that the combination at higher doses maintains IL6 levels within normal ranges (Figure 2).

The TNF-α levels also followed the same pattern, with the GAB 20 group showing the highest expression. The GAB 80+ GKB 80 group again exhibited levels similar to the control group, suggesting the combination of Gabapentin and Ginkgo Biloba at higher doses does not promote TNF-α production (Figure 3).

In the case of IL8 expression, it remained unaltered in the GKB 80 group when compared to the control group, indicating that Ginkgo Biloba extract alone at this concentration does not affect IL8 levels. However, there was a notable increase in IL8 expression in the GAB 80+ GKB 80 group compared to the control. The GAB 20 group recorded the highest IL8 expression levels among all groups, pointing to a dose-dependent response (Figure 4). These findings suggest that the combination of Gabapentin and Ginkgo Biloba extract at higher doses (80 µg/ml each) can modulate the inflammatory response, maintaining it at levels similar to the control group.

In terms of IL10 expression, the GAB 20 group showed no significant change when compared to the control group. This suggests that lower doses of Gabapentin alone may not be effective in modulating IL10 levels. However, the GAB 80+ GKB 80 group displayed the most significant increase in IL10 expression when compared to the control ($P < 0.001$). This indicates a strong anti-inflammatory effect when higher doses of Gabapentin are combined with Ginkgo Biloba extract. Additionally, there was a slight but significant increase in IL10 levels in the GKB 20 group compared to the control ($P < 0.05$),

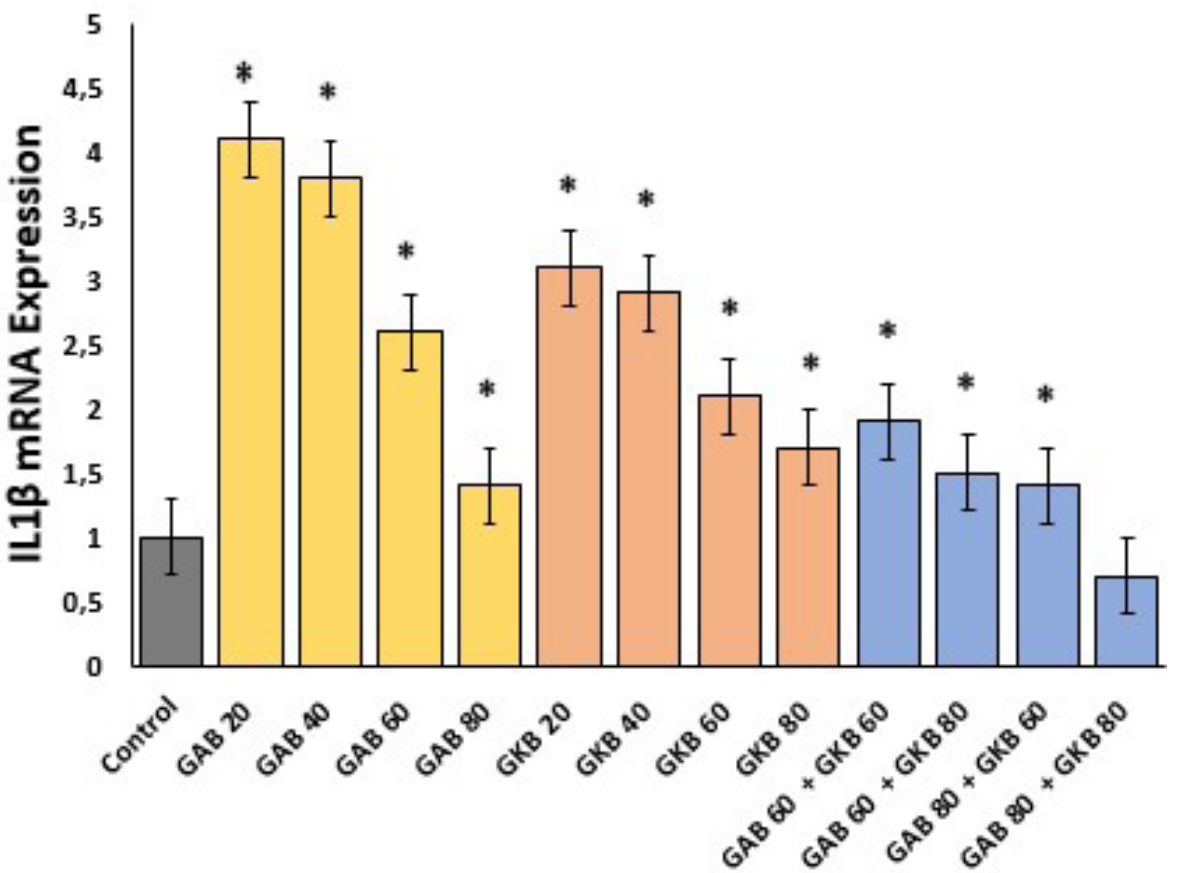

Figure 1. IL1β expression levels in T98G *p<0.001.

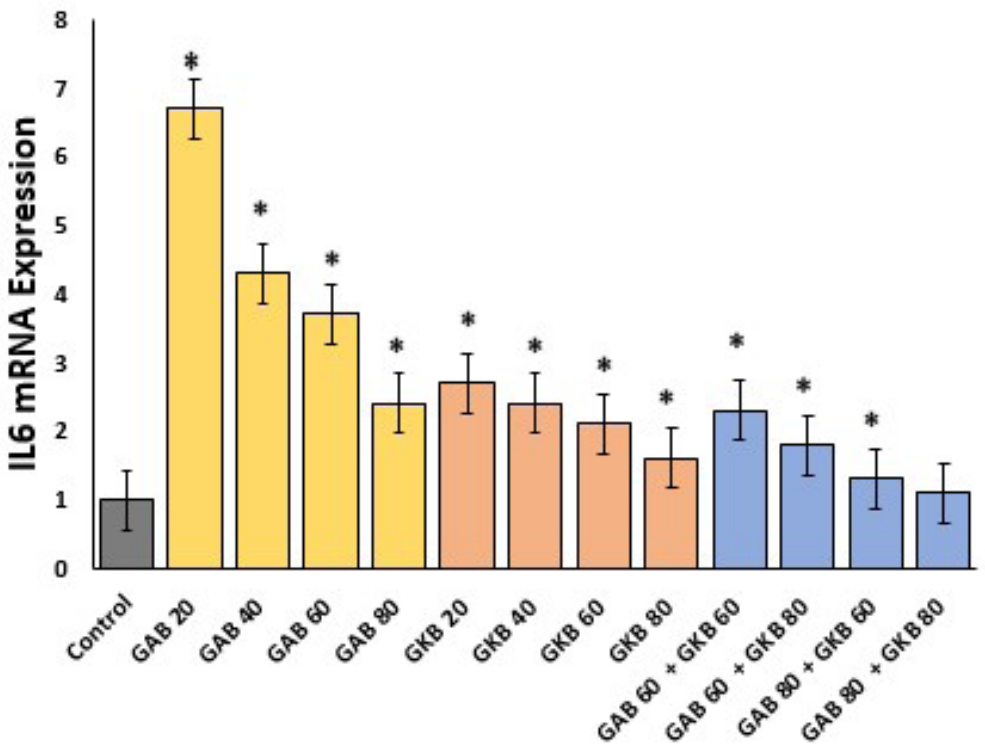

Figure 2. IL-6 expression levels in T98G *p<0.001.

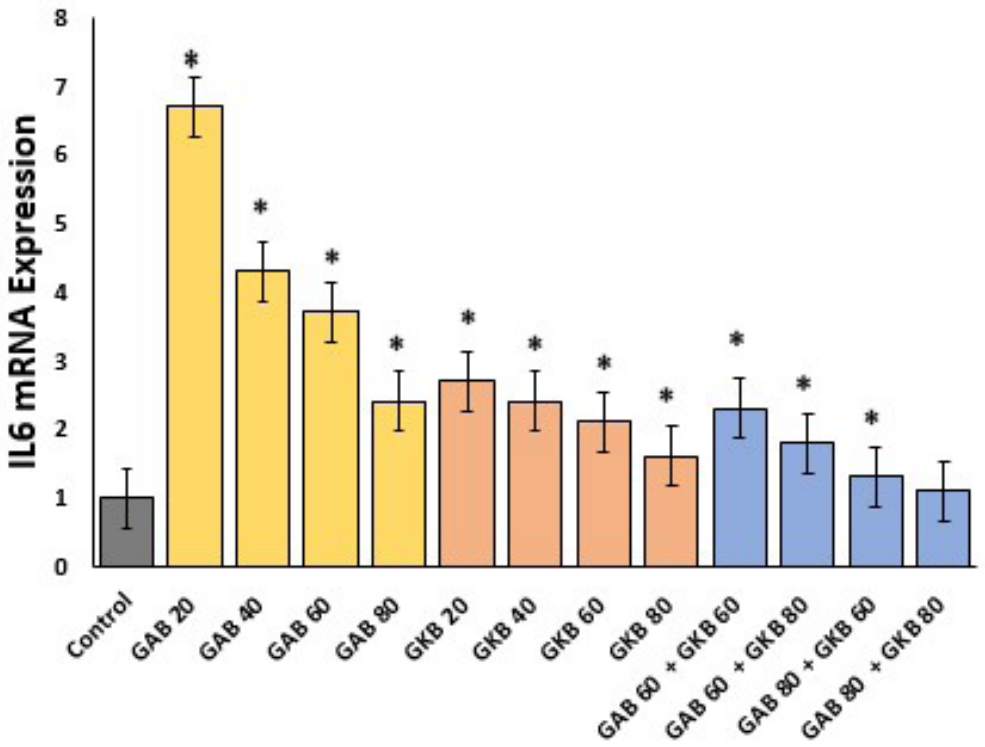

Figure 3. TNF-α expression levels in T98G *p<0.001.

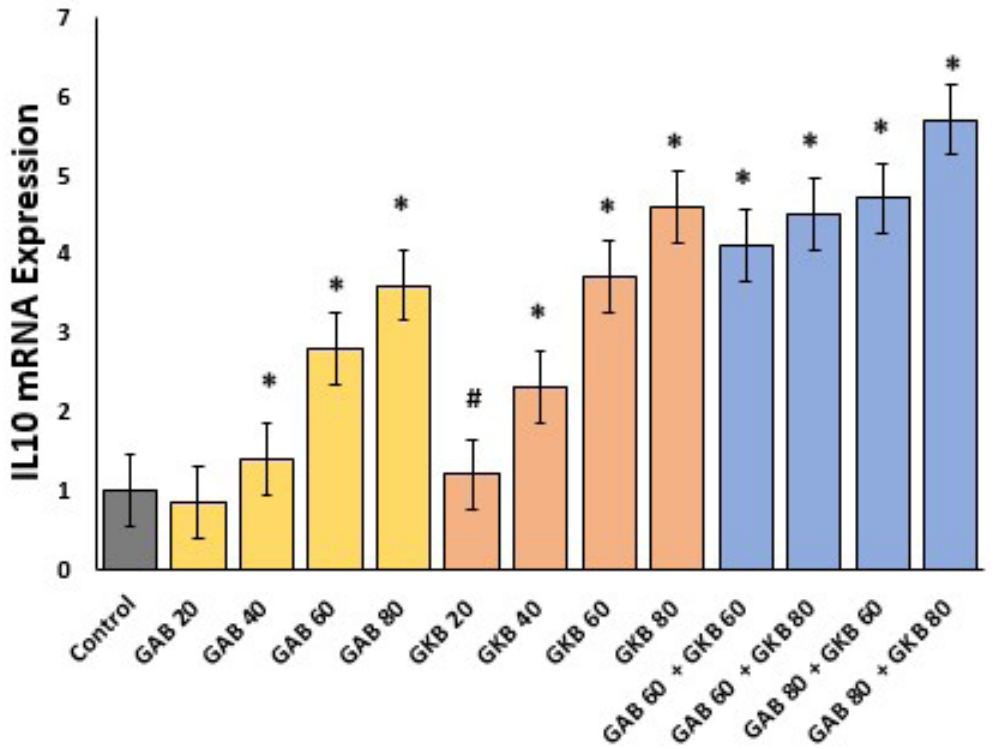

Figure 4. IL-8 expression levels in T98G * p <0.001.

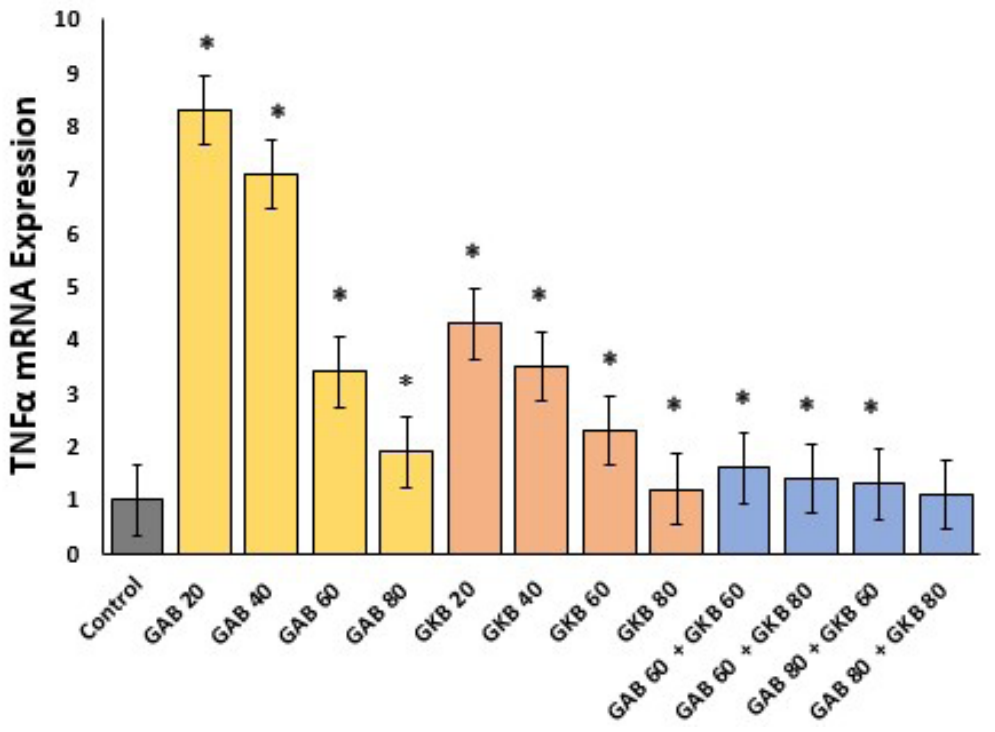

Figure 5. IL-10 expression levels in T98G *p<0.001.

suggesting that even lower doses of Ginkgo Biloba extract can enhance IL10 expression to some extent (Figure 5).

Alteration of the blood brain barrier integrity in the areas of inflammation causes infiltration of immune cells, which leads to an increase in reactive glial cell mediators and strengthening of the response [25]. Among cytokines, especially IL-1 is involved in different physiological processes including sleep, synaptic plasticity, and its levels have been found to increase in trauma, stroke or neurodegenerative disease processes. After acute trauma, this cytokine is mainly secreted from microglia, and although astrocytes cannot respond as rapidly as microglia, they also produce IL-1β and support its production [26]. Inflammation occurs rapidly in acute brain injuries (stroke, bleeding, etc.) and continues in diseases (multiple sclerosis, Parkinson's diseases, etc.). In experimental studies conducted with rat microglia and astrocytes, it was found that increasing IL-1β levels also increased IL-6 synthesis [26–28]. Studies have shown that cytokines are associated with neuroinflammation, astrogliosis, and chronic central nervous system diseases [26–29]. In a study conducted on mice with astrocytoma, these mice were crossed with GFAP-positive transgenic mice in which the IL-6 gene locus was deleted, and the role of IL6 was investigated. It has been shown that malignancy increases with STAT3 activation, and that IL-6 causes an increase in tumor malignancy [30].

Studies with GBM cell lines and patient samples have shown that there is a correlation between tumor aggressiveness and survival and that inflammatory cytokines (IL-1β, IL-6 and IL-8) may have prognostic potential [31,32]. The current understanding is that if these inflammatory mediators can be controlled, the aggression of GBM can be prevented to some extent. The development of new anti-inflammatory agents that can be combined with the cytotoxic agents currently used in treatment may contribute to this understanding [33]. In our study, IL-1β, IL6 and IL8 levels in the 20 µgr/ml and 40 µgr/ml treatment groups were statistically significantly higher than in the control group. It is seen that the most effective dose among the treatment groups is 80 µgr/ml. Therefore, 60 µgr/ml and 80 µgr/ml dose groups were preferred in combination therapy. Within the combination groups, it was observed that 80 µgr/ml dose combination did not cause an increase compared to the control group. IL10 levels, which is an anti-inflammatory cytokine, increased slightly in the 20 µgr/ml dose groups and reached a higher level in the 80 µgr/ml dose groups. Among the combination groups, the highest statistically significant increase was observed in the 80 µgr/ml dose combination compared to the control group.

Experimental studies have shown that GBE increases cerebral blood flow when used in cerebral circulatory disorders, minimizes hippocampal neuronal loss following ischemia and exerts these effects through anti-inflammatory and anti-oxidative mechanisms [34,35]. In a clinical study, patients with dementia were given GBE and serum levels of inflammatory markers were investigated. The results showed that IL-6 and TNF-α levels decreased in a dose-dependent manner [36,37]. In a study in cultured primary rat microglial cells, Ginkgo biloba extract EGb 761 was found to have anti-inflammatory activity, strongly suppressing TNFα, IL-1β and IL-6 [38].

The combination of Gabapentin and Ginkgo Biloba emerges as a promising approach in glioblastoma multiforme (GBM) treatment. This combination effectively regulates pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α while significantly increasing IL-10 expression, fostering a robust anti-inflammatory response. By suppressing chronic inflammation in the GBM tumor microenvironment, it shows potential to inhibit glioblastoma stem cell survival, angiogenesis, and immune evasion. Ginkgo Biloba's antioxidant and anti-inflammatory properties, combined with Gabapentin's modulatory effects on cytokine expression, target inflammatory pathways and the Warburg effect, which are critical for tumor progression.

In vitro studies have demonstrated that this combination can control neuroinflammation, potentially enhancing the effectiveness of radiotherapy and restoring balance in the immune-suppressive environment of GBM. Furthermore, Ginkgo Biloba's ability to improve cerebral circulation and provide neuroprotection synergizes with Gabapentin to mitigate both the adverse effects of the tumor microenvironment and the systemic impacts of GBM. These findings suggest that this combination could offer an innovative option for overcoming inflammation-driven resistance and improving patient outcomes. Further in vivo studies are needed to validate these promising results and establish their clinical applicability.

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In our study, we found that the combination of GAB and GB in co-cultured cells can keep inflammation at the level of the control group. It is important to better determine the analysis of the pathways by modeling this in vitro study in vivo, where we also found effective dose ranges, and to stop the neuroinflammation process is a major contribution to the treatment in terms of tumor radiotherapy.

CONCLUSION

The aim of our research was to establish a specialized microenvironment for co-culturing Glioblastoma cells and neurons. Additionally, we sought to investigate the effectiveness of GAB and GKB in mitigating neuroinflammation. Our findings indicated that the GAB80+ GKB80 µgr/ml group exhibited no alterations in pro-inflammatory cytokine levels, while significantly elevating the concentration of anti-inflammatory cytokines, particularly IL10, when compared to other groups. To further enhance our understanding, we plan to conduct animal studies and analyze changes at the genetic level, with a specific focus on mRNA levels. This will provide valuable insights into the potential of GAB and GKB as anti-inflammatory agents for Glioblastoma.

References

1. D. Laug, S.M. Glasgow, B. Deneen, A glial blueprint for gliomagenesis. Nat Rev Neurosci., 19 (2018) 393-403.
2. S. Nasir, S. Nazir, R. Hanif , A. Javed , Glioblastoma Multiforme: Probing Solutions to Systemic Toxicity towards High-Dose Chemotherapy and Inflammatory Influence in Resistance against Temozolomide. Pharmaceutics, 15 (2023) 687.
3. A.F. Tamimi, M. Juweid, Epidemiology and outcome of glioblastoma. Exon Publications, (2017)143-153.
4. N.T. Cini, M. Pennisi, S. Genc, D.A. Spandidos, L. Falzone, P.D. Mitsias, A. Tsatsakis, A. Taghizadehghalehjoughi. Glioblastoma multiforme: probing solutions to systemic toxicity towards high-dose chemotherapy and inflammatory influence in resistance. Pharmaceutics, 2023.
5. A. Seidlitz, T. Siepmann, S. Löck, T. Juratli, M. Baumann, M. Krause, Impact of waiting time after surgery and overall time of postoperative radiochemotherapy on treatment outcome in glioblastoma multiforme. Radiat. Oncol., 10 (2015), 1-10.
6. Z. Tan, H. Xue, Y. Sun, C. Zhang, Y. Song, Y. Qi, The role of tumor inflammatory microenvironment in lung cancer. Front. Pharmacol., 12 (2021), 688625.
7. F. Colotta, P. Allavena, A. Sica, C. Garlanda, A. Mantovani, Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis., 30(7) (2009), 1073-81.
8. G. Multhoff, M. Molls, J. Radons, Chronic inflammation in cancer development. Front. Immunol., 2 (2012), 98.
9. D.W. Edwardson, J. Boudreau, J. Mapletoft, C. Lanner, A.T. Kovala, A.M. Parissenti, Inflammatory cytokine production in tumor cells upon chemotherapy drug exposure or upon selection for drug resistance. PLoS One., 12(9) (2017), e0183662.
10. L. Arendt-Nielsen, J.B. Frøkjær, C. Staahl, T. Graven-Nielsen, J.P. Huggins, T.S. Smart, et al., Effects of gabapentin on experimental somatic pain and temporal summation. Reg. Anesth. Pain Med., 32(5) (2007), 382-8.
11. T.V. de Brito, G.J.D. Júnior, J.S. da Cruz Júnior, R.O. Silva, C.E. da Silva Monteiro, A.X. Franco, et al., Gabapentin attenuates intestinal inflammation: role of PPAR-gamma receptor. Eur. J. Pharmacol., 873 (2020), 172974.
12. C.E. Hamm, R.D. Gary, I.M. McIntyre, Gabapentin concentrations and postmortem distribution. Forensic Sci. Int., 262 (2016), 201-3.
13. G.C. Quintero, Review about gabapentin misuse, interactions, contraindications and side effects. J. Exp. Pharmacol., (2017), 13-21.
14. O.M. Abdel-Salam, A.A. Sleem, Study of the analgesic, anti-inflammatory, and gastric effects of gabapentin. Drug Discov. Ther., 3(1) (2009), 1-10.
15. J.M. Dias, T.V. de Brito, D. de Aguiar Magalhães, P.W. da Silva Santos, J.A. Batista, E.G. do Nascimento Dias, et al., Gabapentin, a synthetic analogue of gamma aminobutyric acid, reverses systemic acute inflammation and oxidative stress in mice. Inflammation., 37 (2014), 1826-36.

16. X. Li, B. Wang, N. Yu, L. Yang, C. Nan, Z. Sun, et al., Gabapentin alleviates brain injury in intracerebral hemorrhage through suppressing neuroinflammation and apoptosis. Neurochem. Res., 47(10) (2022), 3063-75.
17. Y. Ong, E. Yong, Panax (ginseng)--panacea or placebo? Molecular and cellular basis of its pharmacological activity. Ann. Acad. Med. Singapore., 29(1) (2000), 42-6.
18. L.O. Dragsted, M. Strube, J. Larsen, Cancer-protective factors in fruits and vegetables: biochemical and biological background. Pharmacol. Toxicol., 72 (1993), 116-35.
19. F. Marks, K. Müller-Decker, G. Fürstenberger, A causal relationship between unscheduled eicosanoid signaling and tumor development: cancer chemoprevention by inhibitors of arachidonic acid metabolism. Toxicology., 153(1-3) (2000), 11-26.
20. F.V. DeFeudis, Ginkgo biloba extract (EGb 761): from chemistry to the clinic. Ullstein Med. Wiesbaden., (1998).
21. F.V. DeFeudis, V. Papadopoulos, K. Drieu, Ginkgo biloba extracts and cancer: a research area in its infancy. Fundam. Clin. Pharmacol., 17(4) (2003), 405-17.
22. C.Y. Lee, H. Y. Lai, A. Chiu, S.H. Chan, L.P. Hsiao, S.T. Lee, The effects of antiepileptic drugs on the growth of glioblastoma cell lines. Journal of neuro-oncology, 127 (2016) 445–453.
23. S.H. Hsu, C.J. Chang, C.M. Tang, F.T. Lin, In vitro and in vivo effects of Ginkgo biloba extract EGb 761 on seeded Schwann cells within poly(DL-lactic acid-co-glycolic acid) conduits for peripheral nerve regeneration. J. Biomat. Appl., 19 (2004) 163–182.
24. N. Chen, C. Peng, D. Li, Epigenetic underpinnings of inflammation: a key to unlock the tumor microenvironment in glioblastoma. Front. Immunol., 13 (2022), 869307.
25. R. Roesler, S.A. Dini, G.R. Isolan, Neuroinflammation and immunoregulation in glioblastoma and brain metastases: recent developments in imaging approaches. Clin. Exp. Immunol., 206(3) (2021), 314-24.
26. S.M. Allan, P.J. Tyrrell, N.J. Rothwell, Interleukin-1 and neuronal injury. Nat. Rev. Immunol., 5(8) (2005), 629-40.
27. K. Tanabe, R. Matsushima-Nishiwaki, S. Dohi, O. Kozawa, Phosphorylation status of heat shock protein 27 regulates the interleukin-1β-induced interleukin-6 synthesis in C6 glioma cells. Neuroscience., 170(4) (2010), 1028-34.
28. Simi, N. Tsakiri, P. Wang, N. Rothwell, Interleukin-1 and inflammatory neurodegeneration. Biochem. Soc. Trans., 35(5) (2007), 1122-6.
29. K. Tanabe, O. Kozawa, H. Iida, Midazolam suppresses interleukin-1β-induced interleukin-6 release from rat glial cells. J. Neuroinflammation., 8 (2011), 1-8.
30. J. Weissenberger, S. Loeffler, A. Kappeler, M. Kopf, A. Lukes, T.A. Afanasieva, et al., IL-6 is required for glioma development in a mouse model. Oncogene., 23(19) (2004), 3308-16.
31. C. Rolhion, F. Penault-Llorca, J.L. Kémény, J.J. Lemaire, C. Jullien, C. Labit-Bouvier, et al., Interleukin-6 overexpression as a marker of malignancy in human gliomas. J. Neurosurg., 94(1) (2001), 97-101.
32. Tchirkov, T. Khalil, E. Chautard, K. Mokhtari, L. Veronese, B. Irthum, et al., Interleukin-6 gene amplification and shortened survival in glioblastoma patients. Br. J. Cancer., 96(3) (2007), 474-6.
33. Y. Yeung, K. McDonald, T. Grewal, L. Munoz, Interleukins in glioblastoma pathophysiology: implications for therapy. Br. J. Pharmacol., 168(3) (2013), 591-606.
34. Z. Zhang, D. Peng, H. Zhu, X. Wang, Experimental evidence of Ginkgo biloba extract EGB as a neuroprotective agent in ischemia stroke rats. Brain Res. Bull., 87(2-3) (2012), 193-8.
35. J. Tulsulkar, Z.A. Shah, Ginkgo biloba prevents transient global ischemia-induced delayed hippocampal neuronal death through antioxidant and anti-inflammatory mechanism. Neurochem. Int., 62(2) (2013), 189-97.
36. M.S. Tan, J.T. Yu, C.C. Tan, H.F. Wang, X.F. Meng, C. Wang, et al., Efficacy and adverse effects of Ginkgo biloba for cognitive impairment and dementia: a systematic review and meta-analysis. J. Alzheimers Dis., 43(2) (2015), 589-603.
37. F. Leng, P. Edison, Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here? Nat. Rev. Neurol., 17(3) (2021), 157-72.
38. B. Gargouri, J. Carstensen, H.S. Bhatia, M. Huell, G.P. Dietz, B.L. Fiebich, Anti-neuroinflammatory effects of Ginkgo biloba extract EGb761 in LPS-activated primary microglial cells. Phytomedicine., 44 (2018), 45-55.