

Profound Blockage of CXCR4 Signaling at Multiple Points Using the Synergy Between Plerixafor, Mirtazapine, and Clotrimazole as a New Glioblastoma Treatment Adjunct

CXCR4 Sinyal İletişiminde Birden Fazla Noktada Plerixafor, Mirtazapin Arasında Sinerji İle Sinyalizasyon Etkileşimi İle Derin ve Yeni Bir Glioblastoma Tedavi Ajan Olarak Klotrimazol

ABSTRACT

CXCL12 signaling at CXCR4 is important in glioblastoma growth promotion as a migration-directing chemokine and as a mitosis-stimulating cytokine system. Recent developments in other areas of medicine may have made it now possible to comprehensively block glioblastoma's use of CXCL12 signaling. CXCL12 signaling at CXCR4 requires an active intermediate conductance Ca²⁺-activated K⁺ channel to function. Plerixafor (AMD3100) is a new small molecular weight inhibitor of CXCR4, FDA approved to aid in stem cell mobilization. Inhibition of CXCR4 by plerixafor is expected to inhibit particularly the glioblastoma stem cell population by inhibiting that sub-population's homing to the protective hypoxic niche. Histamine signals through the H1 receptor in glioblastoma cells to activate the intermediate conductance Ca²⁺-activated K⁺ channel also, thereby forming a potential bypass for inhibition of CXCR4-initiated signaling. The antidepressant mirtazapine is perhaps the most potent H1 antagonist in common clinical use. By inhibiting H1 stimulation of intermediate conductance Ca²⁺-activated K⁺ channels, it could prevent circumvention of CXCR4 inhibition by that path. The anti-fungal clotrimazole directly inhibits the intermediate conductance Ca²⁺-activated K⁺ channel at clinically achievable and well-tolerated doses. These three drugs used simultaneously are potential low morbidity paths to deeply inhibit CXCR4/CXCL12 signaling during cytotoxic glioblastoma treatment.

KEYWORDS: CXCL12, CXCR4, Glioblastoma, Homing, Hypoxic, Plerixafor

Öz

CXCR4'de CXCL12 sinyalizasyonu glioblastoma gelişimi ve tümör hücrelerinin göç etme sürecinde migrasyon yönlendirici ve bu hücrelerin mitotik aktivitesini artırıcı etki yapan uyarıcı bir sitokin sistemidir. Tıpdaki son gelişmelerle birlikte glioblastoma gelişim sürecinde yer alan CXCL12 sinyal sistemi tam olarak anlaşılmiş ve bloke edilebilir hale gelmiştir. CXCL12'nin CXCR4 üzerinden olan ara aktivasyon basamağı potasyum kanallarının kalsiyum ile aktive edilmesi ile gerçekleşir. Plerixafor (AMD3100) düşük moleküler ağırlığa sahip CXCR4 inhibitörüdür. Bu ajan, FDA tarafından kök hücre mobilizasyonunu kolaylaştırıcı ajan olarak onaylanmıştır. Plerixaforun glioblastoma kök hücrelerinin hipoksik yuvalarda çoğalan alt gruplarının üremesini bloke edebileceği düşünülmektedir. Histaminin, glioblastoma hücrelerindeki histamin (H1) reseptörlerine bağlanması ile kalsiyum ile aktive olan potasyum kanalları uyarılır, bu nedenle bu nokta CXCR4'ün bloke edilmesinde bir kontrol noktası olabilir. Klinikte antidepresan olarak kullanılan mirtazapine muhtemelen bilinen en güçlü H1 reseptör blokörüdür. H1 reseptörlerinin bloke edilmesi kalsiyum ile aktive olan K kanallarının aktive olmasını önler, bu yol ile de CXCR4 sinyali bloke edilebilir. Antifungal bir ajan olan klotrimazol direkt olarak kalsiyum ile aktive olan K kanallarını tolere edilebilir dozlarda bloke eder. Bu üç ilacın eşzamanlı kullanımı ile CXCR4 ile CXCL12 arasındaki sinyal iletimi bozulur ve glioblastoma hücreleri üzerine sitotoksik etki ortaya çıkar.

ANAHTAR SÖZCÜKLER: CXCL12, CXCR4, Glioblastoma, Yer belirleyici, Hipoksik, Plerixafor

Richard E. KAST

University of Vermont, Psychiatry,
Burlington, USA

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Correspondence address:

Richard E. KAST

Phone : 802 863 24 62

E-mail : rekast@email.com

INTRODUCTION

To improve the current poor prognosis of glioblastoma (1), a recent series of papers have appeared outlining some currently available drugs that block cytokines and growth factors that are active in promoting glioblastoma growth (2-13). These studies are summarized in Table I.

Although the drugs in Table I are not generally cytotoxic, they are FDA-approved and marketed around the world for non-glioblastoma indications. Ample research data points to their ability to block relevant cytokines or growth factors, as referenced in the Table. Also ample research data indicates that the listed cytokine or growth factor is indeed active in promoting glioblastoma growth. Table I matches these papers with the relevant glioblastoma cytokine or growth factor discussed, and the drug(s) suggested to inhibit them. To this list the current paper now adds three further drugs acting at three different but intersecting points along a prominent glioblastoma growth factor signaling pathway, that of CXCR4/CXCL12. Together the three drugs may synergistically inhibit this single growth-promoting pathway more completely than any used singly.

Mean glioblastoma survival times are commonly found to be 8 to 16 months, with younger patients, males, those with higher Karnofsky Performance Scores, and a tumor allowing total macroscopic resection having somewhat better survival time (1). Clearly better treatments are needed.

THE SPECIAL NATURE OF CXCR4 SYSTEM IN GLIOBLASTOMA

CXCR4 function is a cornerstone of and a crucial signaling hub in glioblastoma growth. CXCR4 is an outer cell membrane chemokine receptor, found as one of three major species of 87, 67, or 55 kDa (14). After ligation of its only known cognate receptor, 8 to 12 kDa CXCL12 (previously known as SDF-1) multiple signaling events occur (14). A wide variety of cells migrate toward a CXCL12 gradient.

Interesting and important parallels can be drawn between the hypoxic pockets in glioblastoma tissue and the hematopoietic stem cell niche. In both glioblastoma (15-17) and the bone marrow hematopoietic stem cell niche (17-19) stem cells are directed towards the hypoxic niche that is required for maintenance of the stem cell population. The hypoxic glioblastoma niche is therefore an attractive target to destroy in glioblastoma treatment.

Particularly strong CXCR4 expression is seen in the glioblastoma sub-population with stem cell characteristics such as symmetric or asymmetric self-renewal and radio-resistance (16).

CXCL12 ligation by CXCR4 stimulates glioblastoma cells' motility (20, 21), metabolism (22), and proliferation (15, 16, 23). Higher glioblastoma tissue levels of CXCR4 expression are associated with shorter survival (24, 25).

INTERMEDIATE CONDUCTANCE Ca^{++} ACTIVATED K^{+} CHANNEL AND THE CXCR4 SYSTEM IN GLIOBLASTOMA.

An introduction to a particular K^{+} channel is required for understanding the comprehensive inhibition suggested in this paper: The intermediate conductance Ca^{++} activated K^{+} channel, IK(Ca) (also abbreviated KCa3.1) when open allows K^{+} egress, along its concentration gradient but against its electrical gradient, hyperpolarizing cells (20, 26). IK(Ca) is insensitive to voltage changes, opening only in response to exposure to an increase in intracellular Ca^{++} level. Calmodulin is constitutively bound to IK(Ca). It is Ca^{++} binding to that calmodulin that increases the K^{+} conducting pore open probability (26, 27). IK(Ca) is not expressed on CNS neurons but is amply expressed on CNS glia and CNS vasculature (26, 27).

IK(Ca) is richly expressed on glioblastoma cells and the abnormal vasculature within glioblastoma tissue (20, 28-30). Expression on both is not as curious as it seems at first glance since much of the abnormal malformed blood vessels within glioblastoma are in fact blood channels formed by the malignant glioblastoma cells themselves (31), without endothelial lining, smooth muscle tunica media, nor tunica adventitia. This process is called "vasculogenic mimicry" (31). In glioblastoma this process can be extreme, resembling an arteriovenous malformation on initial radiographic study (32).

THE DRUGS: clotrimazole, mirtazapine, plerixafor.

1. Plerixafor

In preclinical research on plerixafor (Mozobil®) it was called AMD3100 (33). It is a potent well-tolerated antagonist at CXCR4, FDA-approved and marketed for assisting in hematopoietic stem cell mobilization (33). By antagonism of CXCR4 plerixafor partially

Table I: Array of currently available drugs that, although FDA approved and marketed for other indications, may be of adjunctive benefit during standard current cytoablative treatment of glioblastoma. Abbreviations: BBB = blood-brain barrier. CCL5 = 8 kDa chemokine ligand for CCR5 and others, also known as RANTES. IK(Ca) = intermediate conductance Ca⁺⁺ activated K⁺ channel. CXCR4 = cognate ligand for the 8 kDa chemokine CXCL12. NK-1R = the cognate receptor for 11 amino acid signaling peptide substance P. Ref. is the reference number where research on the listed glioblastoma cytokine or growth factor is reviewed, along with the drug(s) that have been shown in other contexts to inhibit that system

Cytokine, growth factor, or receptor system	Drug(s)	Ref.
cathepsin B	auranofin	2
substance P at NK-R1	aprepitant	3
aldehyde dehydrogenase	disulfiram, chloramphenicol	4
histamine at H1	mirtazapine	5, current
sigma-1 receptor	donepezil	6, 7
tyrosine kinase	dasatinib + imatinib synergy	8
serotonin receptor-7	pimozide, risperidone	9
CCL5 + NK-1R	miraviroc + aprepitant synergy	10
Src	methylnaltrexone	11
BBB opening	methamphetamine	8, 11, 12
interleukin-8	dapsone (+ cimetidine)	13
IK(Ca)	clotrimazole	current
CXCR4	plerixafor	34, 35, current

defeats the CXCL12 gradient-directed homing within the hypoxic bone marrow niche, exerting thereby a centrifugal force (18) on CD34⁺ hematopoietic stem cells driving them into the periphery for harvest in apheresis prior to autologous transplant procedures. This has a parallel in glioblastoma physiology. Plerixafor is eminently suited for an adjuvant role during glioblastoma treatment and has been suggested previously for this (34, 35). The hypoxic niche is needed for or at least greatly enhances symmetrical self-renewal of glioblastoma stem cells (36-38). By thwarting or inhibiting CXCR4 directed glioblastoma stem cell sub-population homing towards the required hypoxic niche, radiosensitivity may be enhanced and the self-renewing stem cell population made more amenable to cytotoxic ablation by for example temozolomide.

A detail of CXCR4 physiology suggest that the addition of two other drugs to plerixafor will deepen CXCR4 system inhibition, augmenting stem cell sub-population killing ability by cytotoxic drugs and radiation: CXCR4 must signal through IK(Ca) (20). If this single report is correct we can hit this nodal point with two other drugs.

2. Clotrimazole

Clotrimazole is a widely available generic anti-fungal drug that happens to inhibit IK(Ca) in clinically achievable well-tolerated concentrations after oral administration (39-42).

3. Mirtazapine

Histamine signaling through H1 receptors goes as potentially useful during glioblastoma treatment previously (5, 20, 28). Since histamine signaling through H1 receptors going through a required IK(Ca) step also, potent H1 inhibition may further block residual CXCR4-related signaling as well as preventing circumvention. We have many potent H1 blocking drugs. Of drugs in common clinical use, the anti-depressants doxepin and mirtazapine have the tightest antagonistic (technically inverse agonist) binding to H1 (5). Mirtazapine would be preferable by both safety and tolerability standards.

CONCLUSIONS

By augmenting plerixafor with clotrimazole and mirtazapine we may be able to achieve a more profound inhibition of glioblastoma stem cell homing to the protective hypoxic niche than that obtained

by using any of them singly. These three medicines can then be added to the developing list of currently available glioblastoma cytokine and growth factor inhibitors.

Although the complete list (Table 1) of medicines is daunting and ungainly, there is no particular specific physiologic clash or predictable deleterious interaction between them. If all were to be used simultaneously, there would be no evident element of great concern other than the obvious unpleasantness of the number of drugs, the potential for unknown interactions, and the need for daily patient evaluation for the duration of such treatment. Clearly daily patient monitoring for the duration of treatment will be required to address and correct any incipient or developing problems given the unpleasant and untested medicine mix. The simultaneous use of the entire list of medicines in Table 1 would be reflexively repugnant and unprecedented in the treatment of any disease but potentially warranted given the bleak outlook for glioblastoma as things now stand.

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