Agomelatine or ramelteon as treatment adjuncts in glioblastoma and other M1- or M2-expressing cancers

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The impressive but sad list of over forty clinical studies using various cytotoxic chemotherapies published in the last few years has failed to increase median survival of glioblastoma beyond two years after diagnosis. In view of this apparent brick wall, adjunctive non-cytotoxic growth factor blocking drugs are being tried, as in the CUSP9* protocol. A related theme is searching for agonists at growth inhibiting receptors. One such dataset is that of melatonin agonism at M1 or M2 receptors found on glioblastoma cells, being a negative regulator of these cells' growth. Melatonin itself is an endogenous hormone, but when used as an exogenously administered drug it has many disadvantages. Agomelatine, marketed as an antidepressant, and ramelteon, marketed as a treatment for insomnia, are currently-available melatonin receptor agonists. These melatonin receptor agonists have significant advantages over the natural ligand: longer half-life, better oral absorption, and higher affinity to melatonin receptors. They have an eminently benign side effect profile. As full agonists they should function to inhibit glioblastoma growth, as demonstrated for melatonin. A potentially helpful ancillary attribute of melatonergic agonists in glioblastoma treatment is an increase in interleukin-2 synthesis, expected, at least partially, to reverse some of the immunosuppression associated with glioblastoma.

Key words: agomelatine, CUSP9*, glioblastoma, interleukin-2, melatonin, ramelteon, temozolamide.

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Preamble: an orthopaedic aphorism:
If a little force doesn't work, maybe more force will.

Introduction

This paper presents some physiological data supporting adjunctive use of agomelatine or ramelteon in our ongoing effort to improve glioblastoma treatment, given that the current median overall survival remains less than two years [1]. An impressive but sad list of over forty clinical studies, using various cytotoxic chemotherapies and published in the last few years [1, 2], has failed to increase the median survival of glioblastoma beyond two years after initial diagnosis. Many of these studies imposed high side-effect burdens on patients. To redress this situation we took a new approach with the CUSP9* protocol [1, 2]. In CUSP9*, repositioned older drugs, drugs that have not traditionally been used in cancer chemotherapy, are used to block 17 different survival paths used by glioblastoma cells to survive, grow, and avoid cytotoxicity from traditional cytotoxic chemotherapy drugs. We now present past data and explain the rationale to indicate the potential for the benefit of the addition of currently marketed melatonin agonists agomelatine or ramelteon as adjuncts to a compatible, coordinated polypharmaceutical regimen during glioblastoma treatment.

Nile Distributary Problem

Recent oncology studies and thinking, both generally and regarding glioblastoma specifically [1–3], have drawn the conclusion that simultaneous blocking of multiple, cross-covering growth paths will be required for meaningful clinical benefit. This has been termed the Nile Distributary Problem (Fig. 1) [expanded in refs. 1 and 2]. Multiple cross-covering growth stimulating or growth enabling pathways imply a] non-existence of a single or crucial nodal flaw, and b] the consequent need of wide-net polypharmacy. A growth stimulating signalling system that we should inhibit and a growth inhibiting system that we should stimulate are considered functionally equivalent for the purposes of this discussion.

It is a widely implemented principle of modern engineering that if a single part or function of a well-engineered machine were to fail and result in catastrophic or significant harm, that part or function must be made redundant, often several times redundant. For example, many modern jet airliners would become unflyable without their computer's help. Thus they have four redundant computers, any one of which can safely allow the plane to fly. After a thousand million years mammalian cells have become well-engineered entities and therefore have multiple redundant systems for crucial functions. Many of these go simultaneously awry in cancer, implying a need for multiple simultaneous interventions. The Nile Distributary Problem.

Fig. 1. The Nile Distributary Problem. Above is a drawing of the major Nile River distributaries. There are dozens of minor distributaries (not shown) that could become major ones and take up the extra flow created by a blockage in a major distributary. One can imagine that blocking one or a few of the many distributaries would have little effect on total flow to the sea. In the same way, we can view the many recent failures to clinically benefit from profound pharmacological blocking of a single growth path, even when that path had been known to be active in, and a major driver of, glioblastoma growth [1]

In light of and in recognition of the Nile Distributary Problem, agomelatine or ramelteon as treatment adjuncts during glioblastoma treatment are suggested only as a component of any larger CUSP-like regimen, not as a stand-alone option. According to this understanding, clinical control or cure will come when enough of the cross-covering growth paths are blocked so that glioblastoma cell survival is seriously impaired. To that end, this paper now reviews evidence for the role of agonism at the two currently recognised, widely expressed melatonin receptors, M1 and M2, in glioblastoma growth inhibition, and the potential for either of these two commercially approved and marketed M1/M2 agonists to enhance M1/M2-mediated growth inhibition – a counterweight to growth-stimulating paths.

Melatonin as a negative regulator of glioblastoma growth

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is a 232 Da, short half-life hormone [4, 5] synthesised downstream from serotonin [6, 7] and found universally in mammals [8]. Melatonin signals through two outer surface membrane receptors, termed M1 and M2 [9]. Dietary tryptophan is used either i) in protein synthesis, ii) shunted along the kynurenine pathway, or iii) shunted to serotonin synthesis [10]. From the serotonin path melatonin is synthesised [11]. Melatonin as a pharmacological agent has several drawbacks, very short circulating half-life (Table 1) and erratic absorption [12] among them. Pharmacological melatonergic agonists were developed to circumvent these drawbacks and marketed to help initiate sleep during diurnal dysregulation states or to alleviate depression [13, 14].

Table 1. Circulating half-life data for melatonergic drugs. All three drugs are available for use in humans. T1/2 is the circulating half-life

Molecule	T ½	Animal	Reference
Agomelatine	2 hours	human	13
Ramelteon	~1–2 hours	human	67
Ramelteon	~1–2 hours	human	52
Melatonin	23 min	rat	68
Melatonin	< 20 min	hamster	69
Melatonin	40 min	human	42
Melatonin	~30 min	human	70

Traditionally, melatonin has been considered a pineal-synthesised hormone. Melatonin is found throughout vertebrates' body and brain [15], but also in the brain of ancient, primitive life forms such as planaria [16] and marine zooplankton [17]. More recent evidence shows that lymphocytes can synthesise melatonin [18–20], as do other cells involved with innate immunity [21] and inflammation [22]. Normal brain astrocytes and glia also synthesise melatonin [23], as does the gut [24] and other organs [5].

Although best known for diurnal sleep-wake regulation and synthesis in the pineal gland [5], melatonin has significant physiological functions other than sleep-wake cycling [9, 25].

Multiple potential areas for melatonin's anti-cancer action have been postulated by others [25–29]. Detailed below are data on melatonin function specifically in glioblastoma growth.

Melatonin and glioblastoma

The concept of glioblastoma stem cells does not have a precise definition yet, and it may never have one single such definition if, as many now suspect, stemness comprises a range of somewhat differing attributes in subsets of cells, not a unitary entity [30]. Within various

current concepts of glioblastoma, stem cells generally encompass four core cell attributes: i) relative quiescence, ii) a sub-population from which post-chemotherapy (irradiation, resection) glioblastoma preferentially re-grows, iii) enhanced *in vitro* clonogenicity where, relative to the non-stem population, fewer cells are required to establish three dimensional growth in soft agar, and iv] they can divide symmetrically or asymmetrically to both stem or non-stem daughter cells [3, 31–33].

Melatonin-whether by agonism at M1 or M2 or by both is unclear-empirically lowers glioblastoma cell proliferation generally and the stem sub-population plating efficiency (clonogenicity) specifically [34]. The mode of glioblastoma stem sub-population death is autophagy [34]. Melatonin alone shows cytotoxicity to two glioma cell lines that also lower IC_{50} of temozolamide by 3 to 6 fold [35]. Temozolamide is currently the mainstay cytotoxic drug in treating glioblastoma [3, 36]. Under either hypoxia or normoxia, melatonin slows centrifugal migration of glioblastoma cell lines [37]. This would be of considerable benefit were this to hold *in vivo* and in human disease, given that it is precisely these post-primary resection centrifugally migrating cells that go on to kill patients.

A remarkable study from 1996 that does not seem to have been followed up showed that of glioblastoma patients given 60 Gy irradiation plus 20 mg melatonin orally daily for 1 year, 6 of 14 were alive, while in the control group, given 60 Gy alone, only 1 of 16 was alive at 1 year [38]. Two years later (in 1998) this same group published a study in a group of various advanced stage cancers claiming survival benefit from melatonin 20 mg orally per day plus an aloe extract [39]. Procedural study weaknesses and non-replication by others prevent analysis of this data. Still, miraculous and unrefuted results, over a decade old and neither replicated nor followed up, neither widely instituted or acknowledged, should not, cannot, be assumed just on that basis to be incorrect.

Agomelatine

Agomelatine is a 243 Da melatonin agonist licensed by the EMA (but not the FDA) for treating depression [40]. It has a higher affinity to both M1 and M2, with a pKi of about 10 nM, than does the natural ligand and longer half-life - about two hours [4, 14, 41], see Table 1. The evanescent nature of any circulating melatonin has been previously noted as a clinical problem [4], an impediment to effective treatment using exogenous melatonin itself. Added disadvantages include melatonin's poor and erratic oral bioavailability [12] with correspondingly highly variable blood levels in subjects given identical doses [42].

Agomelatine is marketed as an antidepressant with dual modes of action: a) agonism at M1 and M2 melatonin receptors and and b) antagonism at serotonin 2 C receptors (5-HT2C) [43–46]. It readily penetrates the blood-brain barrier. The status or efficacy of agomelatine as an antidepressant is uncertain as of spring 2015, but its excellent tolerability is unequivocal [41] as is its ability to improve sleep during a major depression [47, 48], indicative of potent M1/M2 agonism.

Ramelteon

Ramelteon is a 259 Da. brain-penetrant melatonin agonist, FDA approved [but not approved by the EMA] to help sleep initiation [49, 50]. It is remarkably free of side effects [49–51], in part because of receptor agonism limited to M1 and M2 melatonin receptors. Also of note, ramelteon affinity to M1 and M2 exceeds that of melatonin itself by an order of magnitude, and oral absorption is good. Circulating half-life of ramelteon is several times longer than that of melatonin [52], see Table 1.

Augmenting the augmenter

Increasing ramelteon exposure

Ramelteon is metabolised primarily by hepatic CYP 1A2 [53, 54]. The antidepressant serotonin reuptake inhibitor fluvoxamine is one of the most potent inhibitors known of CYP 1A2 and empirically is found to increase circulating ramelteon levels > 100 fold [54, 55], thus giving us the potential to strongly stimulate M1 and M2 receptors on glioblastoma cells, augmenting the augmentation ramelteon might offer when added to other glioblastoma treatments.

Interleukin 2 and melatonin receptors

Interleukin-2 (IL-2) is a 14 kDa protein that contributes to driving T lymphocyte clonal expansion [56, 57]. Exogenous IL-2 is the first safe and effective pharmacological agent augmenting immunological anti-cancer effects, often giving complete regression in metastatic melanoma and renal cancer [56–58]. Interleukin-2 is in active study for use in other cancers [59].

The wide array of proposed mechanisms by which melatonin might exert its anti-cancer effects have been reviewed elsewhere [60]. Increasing IL-2 might be one of them. Lymphocytes bear melatonin receptors [61]. Of central importance, melatonin exposure enhances lymphocyte's IL-2 synthesis [62, 63]. Empirically, oral melatonin, 20 mg per day given to hepatocellular patients along with transarterial catheter embolisation, increased circulating IL-2 compared to those receiving embolisation alone [64]. We would expect agomelatine and ramelteon to be even more potent and effective in this regard, given these drugs' higher affinity to melatonin receptors and longer half-life than the natural ligand.

Caveats

Cancers have multiple dysregulated physiological systems that act together as an ensemble to mediate malignant behaviour. These dysregulated systems are different over time and space and are different in different parts of the same tumour, so single biopsy study will not give an accurate picture of the complete array of dysregulated systems active over time in a particular tumour. Growth driving systems are not stable; they shift. These systems cross-cover for each other when a pathway is blocked by our drug treatment, others easily take over, compensating for the inhibited pathway, implying a requirement for extensive polypharmacy for effective treatment, as in Nile Distributary Problem.

Several non-oncology drugs with ancillary attributes that inhibit these pathways can be used simultaneously to block enough pathways to stop growth. This requires an admittedly unpleasant extensive polypharmacy. CUSP9* [1] is the ten-drug treatment protocol for recurrent glioblastoma resulting from these considerations.

There is debate as to what degree the antioxidant effect of melatonin is mediated by the molecule itself or intracellular physiological events consequent to M1 or M2 agonism [65, 66]. Since several elements of CUSP9* increase intracellular reactive oxygen species (ROS), and by design such an ROS increase contributes to anti-glioblastoma action, melatonin agonists like agomelatine or ramelteon might best be combined with polypharmaceutical regimens that do not rely on or mediate an increase in intracellular ROS as part of their mechanism of anti-cancer action, until this matter is settled. In other words, melatonin agonists might not be suitable for combination with CUSP9* itself. Given the data on melatonin's potent enhancing of temozolamide cytotoxicity to glioma cell lines [35], adjuvant agomelatine or ramelteon might be best used during initial Stupp Protocol treatment (temozolamide with 60 Gy irradiation) [3].

Conclusions

The limited progress in treating glioblastoma evident over the last decades prompts us to look beyond the traditional array of cytotoxic drugs for new paths to attack this hardy cancer. To augment the current standard cytotoxic drug in glioblastoma, temozolamide, we have briefly outlined past data showing how glioblastoma cells express both melatonin receptors M1 and M2, the stimulation of which seems to be a negative regulator of glioblastoma cell growth. We have readily available, potent, and well-tolerated M1 and M2 agonist drugs agomelatine [EMA approved, marketed in EU] and ramelteon [FDA approved, marketed in the USA]. These melatonin receptor agonists have significant advantages over the natural ligand: longer half-life, better oral absorption, and higher affinity to melatonin receptors.

The Stupp Protocol, maximal feasible resection, irradiation, and classic cytotoxic chemotherapy with temozolamide [3] is the current standard initial treatment for glioblastoma. As outlined, ideally agomelatine or ramelteon would be part of a coordinated polypharmacy. However, at the least, given three prominent facts: i) survival more than two years after diagnosis is still unusual, ii) agomelatine and ramelteon are exceptionally well-tolerated, very low risk medicines, and iii) the outlined data showing melatonergic agonism augments temozolamide cytotoxicity to glioblastoma cells, the risk/benefit of adding agomelatine or ramelteon to standard Stupp Protocol is highly skewed to proceeding to study such addition.

As in the Preamble, adjunctive agomelatine and ramelteon added during an appropriate phase of glioblastoma treatment might be an effective way to apply more force, making a dent in this heretofore intractable disease.

The author declares no conflict of interest.

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