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VIEWPOINT

Can the therapeutic effects of temozolomide be potentiated by stimulating AMP-activated protein kinase with olanzepine and metformin?

Correspondence
RE Kast, Department of Psychiatry, University of Vermont, 22 Church Street, Burlington, VT 05401, USA.
E-Mail: rekast@email.com

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RE Kast[1], G Karpel-Massler[2] and M-E Halatsch[2]

[1]*Department of Psychiatry, University of Vermont, Burlington, VT, USA, and* [2]*Department of Neurosurgery, University of Ulm School of Medicine, Ulm, Germany*

As current treatments for glioblastoma commonly fail to cure, the need for more effective therapeutic options is overwhelming. Here, we summarize experimental evidence in support of the suggestion that metformin and olanzepine have potential to enhance the cytotoxic effects of temozolomide, an alkylating chemotherapeutic agent commonly used to treat glioblastoma. Although the primary path leading to temozolomide-induced cell death is formation of O-6-methylguanine and apoptotic signalling triggered by O-6-methyl G:T mispairs, that apoptotic signalling goes through a step mediated by AMP-activated protein kinase (AMPK). Metformin or olanzapine have been shown independently to enhance AMPK activation. Metformin to treat diabetes and olanzapine to treat psychiatric disorders are well tolerated and have been used clinically for many years. Thus it should be feasible to increase AMPK activation and add to the pro-apoptotic effects of temozolomide, by adding metformin and olanzapine to the therapeutic regimen. Clinical assessment of the potential benefit of such combined therapy against glioblastoma is warranted.

Abbreviations

AMPK, AMP-activated protein kinase; $K_{Ca}3.1$, intermediate conductance Ca^{2+} activated K^+ channel; LKB1-MO25-STRAD, trimolecular kinase that activates AMP kinase

Introduction

Glioblastoma is a cancer with a notoriously poor prognosis. Current treatments commonly use the alkylating drug temozolomide in combination with irradiation after maximal surgical resection (Stupp *et al.*, 2005; Stupp *et al.*, 2009). These interventions are rarely curative and more efficient and effective treatment options are urgently needed. Here, we propose that olanzapine, a drug used for over a decade to treat psychiatric disorders, and metformin, the most commonly used drug for the initial treatment of type 2 diabetes, may enhance the cytotoxic effect of temozolomide against glioblastoma.

Synopsis of current evidence

Temozolomide and AMP-activated protein kinase (AMPK)

AMPK is a heterotrimeric kinase that functions as an intracellular energy sensor, responding to the AMP:ATP ratio (Cantó and Auwerx, 2010). When phosphorylated at Thr^{172}, AMPK activity is increased 1000-fold, leading to the phosphorylation of downstream target proteins that result in a metabolic shift away from ATP consuming processes.

Recent data from Zhang *et al.* (2010) indicate that at least one path of TMZ-induced apoptosis involves an obligatory

AMPK activation step. These authors showed AMPK activation in two glioblastoma cell lines and in explanted primary human glioblastoma cells, after exposure to temozolomide (Zhang *et al.*, 2010). Moreover, they showed that the crucial link between AMPK and glioblastoma cell death was activated AMPK binding to and phosphorylating p53 (Zhang *et al.*, 2010). In their *in vitro* assay, the AMPK inhibitor rapamycin inhibited temozolomide cytotoxicity and an experimental AMPK-activating drug, 5-aminoimidazole-4-carboxamide-1-β-4-ribofuranoside, enhanced apoptosis due to temozolomide. Thus there is experimental evidence for the general proposition that increasing AMPK activity could enhance glioblastoma cytotoxicity mediated by temozolomide.

Olanzapine and AMPK

Kim *et al.*, (2007) in a study of the intracellular correlates of weight gain associated with the use of certain modern antipsychotic medications, showed that two potent inverse agonists of the histamine H_1 receptor, clozapine and olanzapine, also activated AMPK in the murine brain (Kim *et al.*, 2007). Hypothalamic tissue, in particular the arcuate and paraventricular nuclei, showed the greatest increases in AMPK activation, but at higher concentrations, AMPK was activated in other cerebral areas as well (Kim *et al.*, 2007). The clozapine dose needed to raise cerebral levels of activated AMPK, 5 $mg{\cdot}kg^{-1}$, was well within the dose range used in humans (commonly 400 mg once at bedtime).

Evidence for the growth-enhancing role of histamine and its four receptors in cancer generally was recently reviewed (Medina and Rivera, 2010) and includes assays with glioblastoma cells linking histamine's actions to histamine H_1 receptors ((Hishinuma and Young, 1995, Clark and Perkins, 1971, Falus 1993, Li *et al.*, 2003, Fioretti *et al.*, 2009). There are already two possible mechanisms underlying these H_1 receptor-mediated effects on growth of glioblastoma cells. Firstly, activation of histamine H_1 receptors increased interleukin-6 signalling in glioblastoma (Falus, 1993, Altschuler and Kast, 2005, Kast and Altschuler, 2006). Secondly, activation of histamine H_1 receptors opened the intermediate conductance Ca^{2+} activated K^+ channel [$K_{Ca}3.1$], commonly found on glioblastoma cells (Fioretti *et al.*, 2009). Therefore, inverse agonists of H_1 receptors should decrease both the effects of interlukin-6 and the opening of $K_{Ca}3.1$ channels. The $K_{Ca}3.1$ channel is a voltage-insensitive K^+ efflux channel, tending to hyperpolarize cells when open (Chou *et al.*, 2008, Bradding and Wulff, 2009, Kast, 2010) and it opens in response to a local increase in Ca^{2+} concentration.

There is further evidence that this K^+ channel is highly relevant to glioblastoma. Activation of the chemokine receptor CXCR4 enhanced migration and triggered mitosis in glioblastoma cells (Zagzag *et al.*, 2008), signalling through opening of the same $K_{Ca}3.1$ channel (Kast, 2010, Sciaccaluga *et al.*, 2010). Hence the efficacy of CXCR4 activation to open $K_{Ca}3.1$ channels in glioblastoma cells could be diminished by the negative effects on the same channels exerted by H_1 receptor inverse agonists, such as olanzapine.

Olanzapine is simpler than clozapine to use clinically and would be the drug of choice in the role suggested here also due to its effects in normal subjects. Olanzapine has been extensively studied in subjects without psychiatric disorders, where sleep continuity, sleep efficiency and increased stage III/IV sleep are seen (Cohrs, 2008). These attributes would be of benefit to glioblastoma patients. Olanzapine also exhibits potent antagonism at 5-HT_3 receptors resulting in anti-nausea/ anti-emesis effects (Kast and Foley, 2007), as observed with other 5-HT_3 receptor antagonists, such as ondansetron.

Metformin and AMPK

The anti-diabetic drug metformin enhances the formation of LKB1–MO25–STRAD (Shaw *et al.*, 2005) a trimolecular complex protein which is the primary kinase for phosphorylation of Thr^{172} in AMPK(Cantó and Auwerx, 2010). Thus metformin activates AMPK, independently of histamine H_1 receptors.

The proposed combination of metformin and olanzapine with temozolomide would therefore provide three independent ways of increasing AMPK activation, by temozolomide itself, by olanzapine through inverse agonist activity at histamine H_1 receptors and by metformin through enhancing AMPK kinase via the LBK1-MO25-STRAD complex. Such effects should be additive at least and yield enhanced cytotoxic effects against glioblastoma cells.

In clinical terms, the proposed combinations are feasible. For instance, because both clozapine and olanzapine increase appetite and are therefore associated with weight gain and attendant diabetes, metformin is commonly used with these drugs when treating psychiatric illness (Baptista *et al.*, 2007, Chen *et al.*, 2008, Wu *et al.*, 2008, Carrizo *et al.*, 2009) and the combination is well tolerated. Also using metformin as an adjunct to cancer chemotherapy is not new (Ben Sahra *et al.*, 2010, Jalving *et al.*, 2010, Zadra *et al.*, 2010) and, indeed, metformin is in one phase III and six phase II trials in this role.

Discussion

The effects of olanzapine on AMPK are mediated by histamine H_1 receptors and would thus be restricted to those cells expressing such receptors. However, there is good evidence that H_1 receptors are commonly expressed on glioblastoma cells.from studies on glioblastoma cell lines (Hishinuma and Young, 1995, Clark and Perkins, 1971, Falus 1993, Li *et al.*, 2003, Fioretti *et al.*, 2009) and patient biopsies (Weydt *et al.*, 1997, Li *et al.*, 2003).

Temozolomide exerts its cytotoxic effects by methylating the guanine bases in DNA to form O-6-methylguanine and this, as already mentioned, leads to mispairing and consequent defects in DNA replication. (Roos *et al.*, 2007). Such methylation of DNA is normally corrected by O-6-methylguanine-DNA methyltransferase (MGMT), one of several endogenous DNA repair proteins (Vassella *et al.*, 2010, Lai *et al.*, 2011). Levels of this enzyme vary normally because the promoter for the MGMT gene can be methylated and thus become less effective. Individuals with methylated MGMT promoters have less MGMT protein and clinically this translates into somewhat longer survival of glioblastoma patients with the methylated promoter on treatment with TMZ and related alkylating agents. For example, median overall survival was 24.7 months in patients with MGMT promoter methylation and 15.9 months in patients without

promoter methylation (Lai *et al.*, 2011). The corresponding progression-free survival was 17.5 with and 10.5 without promoter methylation (Lai *et al.*, 2011). This endogenous mechanism for enhancing temozolomide action has led to blocking MGMT function with exogenous compounds. However these efforts have been hampered by increased haematopoietic toxicity when bone marrow is fully exposed to this enhanced action of temozolomide (Hegi *et al.*, 2008). Our proposal should provide benefit to all glioblastoma patients irrespective of MGMT status, as it is based on amplification of AMPK activation. Indeed the AMPK amplification proposed here constitutes an independent, previously unexplored path to enhancing temozolomide cytotoxicity.

Some bone marrow cells do express histamine H_1 receptors, at low densities [Pereira *et al.*, 2003], but clinical frequency and intensity of potent H_1 receptor antagonist effects on bone marrow function is low [Rettenbacher *et al.*, 2010]. Unfortunately, many normal cell types expressing histamine H_1 receptors would be at risk for increased TMZ apoptotic actions.

Conclusion

If, as recent work suggests, at least some of the cytotoxic action of temozolomide on glioblastoma goes through an obligatory AMPK activation step, then adding metformin and olanzapine should augment such cytotoxicity. Metformin and olanzapine are already being combined in the treatment of psychotic disorders. Such combinations are already used and well tolerated and would therefore not be expected to add to the side effect burden or patient morbidity of temozolomide.

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This was unfunded research.

Conflicts of interest

All authors report no conflict of interest.

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