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Mini-Review

Pharmacology and scheduling of Temozolomide

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ABSTRACT

Temozolomide (TMZ) is an alkylating drug, belonging to the family of derivatives of Imidazotetrazinone. Pre-clinical studies showed that TMZ has a good bioavailability after oral administration and is able to cross the blood-brain barrier. Due to these peculiar pharmaco-dynamic features, TMZ has been first adopted in the treatment of malignant brain tumors. The clinical use of TMZ has shown that the drug has a negligible toxicity and its antitumor activity is "schedule-dependent". Therefore, many schedules of TMZ administration have been developed in order to investigate and ameliorate its antitumoral efficacy. A higher response rate was detected when the TMZ total dose is administered over 5 days. Recently, the activity of TMZ in combination with different antineoplastic agents is under investigation in children and adults in order to test its activity in malignant tumours other than brain tumors.

KEYWORDS: temozolomide, brain tumors, toxicity.

Introduction

Temozolomide (TMZ) is an alkylating drug, belonging to the family of derivatives of Imidazotetrazinone, synthesized for the first time at the University of Aston in 1984 [1]. One of the first compounds studied, belonging to this category, was Mitozolomide, with a broad spectrum of antitumor activity in preclinical models [2]. However, studies on the clinical activity of this drug were abandoned following the results of the first clinical trials which showed a long-lasting haematological toxicity not foreseeable. The analysis of the correlation between molecular structure and pharmacological activity revealed the formation, starting from structural Mitozolomide, such as TMZ, of the same active metabolite, 3-methyl-(triazen-1-yl)imidazole-4-carboxamide (MTIC) [3]. Preclinical data confirmed that TMZ has a very different toxicity profile from Mitozolomide in various tumor models, including astrocytoma, glioblastoma and ependymoma [4, 5]. Unlike Dacarbazine, which requires liver-level enzymatic demethylation to be converted into active metabolites, TMZ is spontaneously activated to MTIC, at a physiological pH, in aqueous solution.

Pre-clinical studies have shown that TMZ has a good bioavailability after oral administration, antitumor activity of "schedule-dependent" type and good tissue penetration also at the level of the central nervous system [3].

Pharmacokinetic

The TMZ administered by mouth is absorbed through the gastrointestinal system in a rapid and complete way with modest inter-individual variability [6, 7]. If taken simultaneously with food, absorption is delayed, maximum plasma concentration is reduced and area under the curve (AUC) decreased by 10% [8, 9]. Maximum plasma concentrations are reached 30-90 minutes after intake and are dose-dependent. Plasma clearance, distribution volume and half-life, on the other hand, are independent of

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the dose administered. With regard to the spread of the drug within the cerebrospinal fluid, the measurements carried out show that the fluid concentration is equal to 40% of the plasma concentration [10]. The excretion of TMZ is essentially renal: the drug is eliminated renally as unchanged drug (5-10%), and inactive metabolites (90-95%). There are however no recommendations for dose reduction in patients with kidney failure [11]. The half-life of the drug is 1.8 hours.

Mechanism of action

Unlike Dacarbazine (to which TMZ is structurally related) which requires liver-level enzymatic demethylation to be activated, TMZ is spontaneously activated to MITC at a physiological pH (Figure 1) [12]. The reaction of water with the electropositive atom in TMZ C4 opens the heterocyclic ring, releasing MTIC and carbon dioxide. MTIC is unstable and degrades to methyldiazonium, a reactive DNA-methylating compound [13]. Imidazotetrazines are selective for some DNA bases and bind preferentially with the central guanine of the GGG sequence. Methylation sites on DNA are: guanine N7 atom, adenine O3 atom and guanine O6 atom [12]. Although 70% of the methylating action affects the N7 atom of guanine, the cytotoxic activity of the drug is related to O6-methylguanine. O6-methylguanine itself is not lethal to the cancer cell: it does not inhibit the process of replication and transcription of DNA. However, its presence determines the insertion of a thiamine base in place of the cytosine in the conjugated helix during the replication phase. The DNA repair mechanism recognizes the error of insertion of thiamine residue on the second helix and removes it. However, by persisting O6-methylguanine, thymine is reinserted into the conjugate helix. The commissioning of the "mismatch-repair" several times in a row in the same cell giving rise to futile reparative cycles, leads to cell death through the activation of a process of apoptosis [14].

Resistance mechanism

The presence of some drug-resistance mechanisms appears to be responsible for the therapeutic failure of TMZ. Three mechanisms have mainly been associated with ineffective TMZ treatment: 1) O6-methylguanine-DNA methyltransferase (MGMT);

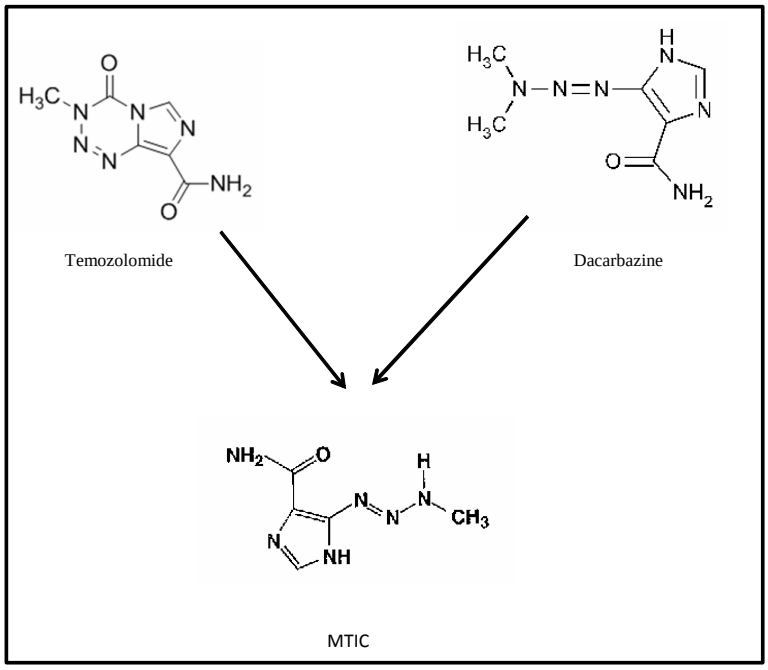

Figure 1. Conversion mechanism of Temozolomide and Dacarbazine to the active compound 3-methyl-(triazen-1-yl)imidazole-4-carboxamide (MTIC).

2) DNA mismatch repair (MMR) pathway proteins; 3) Poly enzyme(ADP)-ribose polymerase (PARP).

MGMT acts by transferring the methyl group from O6-methylguanine onto a cysteine residue of the enzyme itself, thereby repairing the DNA. The concentration of this enzyme was found to be the main determinant of the cytotoxic effect of TMZ *in vitro* [15, 16]. In fact, it was observed that the chemo-resistant central nervous system (CNS) tumours (meningiomas and neurinomas) had higher concentrations of O6-methylguanine-DNA alkyltransferase than chemo-sensible tumours (anaplastic oligodendroglioma) [17].

Although MGMT is important in cell resistance to TMZ, some cell lines expressing low levels of MGMT are nevertheless resistant. This indicates that other resistance mechanisms are involved [18, 19].

As for the second mechanism of resistance to TMZ, it seems that mutations that interfere with the proper functioning of the proteins "mismatch repair" give resistance to the action of the drug. The damage induced by the alkylating agent, in fact, would be "tolerated" without starting the apoptotic process, as it is not recognized and repaired [20-22].

Another mechanism of resistance to TMZ is the system of repair of the excision of the bases and, in particular the enzyme poly-ADP-ribose polymerase (PARP), which seems to be involved in the repair of nucleotides N7-methylguanine and O3-methyladenine [23, 24].

The PARP, in fact, binds closely to the breaking point of the DNA strand, probably to avoid incorrect transcription or recombination. Enzymes that repair DNA later access the lesion, making the cell resistant to the action of the drug [25].

It was noted that PARP inhibitors, by preventing the synthesis of ADP-ribose polymers, enhance the cytotoxic activity of TMZ [26-31].

The importance of these allegations in the anticancer activity of the drug, however, could be secondary to that of the adduct O6-MG, except in those tumors that are deficient in the system of repair of excision of the bases [32-34].

The final effect of the cytotoxic action of TMZ will be cellular apoptosis through induction of p53 and p21 and activation of caspase-3. The absence of any of these mechanisms could lead to the development of drug resistance [35].

Schedules of administration of TMZ

Preclinical studies have shown that TMZ has a good bioavailability after oral administration, good tissue penetration, also at the level of the central nervous system and an antitumor activity of the "schedule-dependent" type [36-38]. At present, TMZ is utilized for the treatment of different tumors (i.e., brain tumors, neuroblastoma, sarcoma, etc.) [39-48].

The first treatment schedule developed on the basis of preclinical models confirmed the antitumoral schedule-dependency of the drug: a higher response rate was detected when the total dose was administered over 5 consecutive days compared with a single-day dose [36]. A daily dose of 200 mg/m2/day (150 mg/m2/day at first cycle in pre-treated patients with chemotherapy) was defined as the maximum tolerated dose and recommended for clinical phase II studies [49, 50]. The dose-limiting toxic effect was thrombocytopenia. At the recommended dose of 200 mg/m2/day, IV-grade thrombocytopenia were found in 10% of cases and III- and IV-grade neutropenia in less than 5%. Nausea and vomiting were easily controlled with common antiemetic drugs [51-54].

Recent studies based on the mechanism of action of the drug and its resistance to cytotoxic activity have led to the elaboration of schedules intensive with TMZ administration to patients with metastatic malignant melanomas every 4 hours, having been verified in preclinical studies that the lowest level of intracellular concentration of MGMT is reached every 4-8 hours after administration of the drug, with recovery of 23% of enzyme activity within 24 hours. It was observed that patients undergoing this type of schedule had a significant haematological toxicity: 68% thrombocytopenia and 54% neutropenia of III and IV degree. Administration of TMZ every 8 hours, however, allowed a better tolerability (34% of patients had thrombocytopenia and 28% neutropenia of III or IV degree) [55, 56]. Although, with intensive schedules a higher percentage of response could theoretically be obtained, the relevant haematological toxicity seems to be strongly limiting. This toxicity seems to be even more important in patients previously treated with

chemotherapy, for whom the maximum tolerated dose of TMZ is lower [57-60].

Studies have been started to evaluate the action of TMZ administered with extended schedules, in view of the progressive depletion of MGMT following prolonged exposure to the drug [5, 61-65]. It has been confirmed by several clinical studies that the extended schedules allow for the administration of a higher dose rate, potentially providing greater clinical efficacy not burdened by significant toxicity and therefore appears to be well tolerated and guarantor of a higher clinical efficacy.

The extended dosing schedules developed are as follows (Table 1):

- For 7 days every 14 days. Dose limiting toxicity (DLT = Dose limiting toxicity) was achieved with 175 mg/m2/day. The recommended dose for phase II studies was 150 mg/m2/day [61].
- For 21 days every 28 days. The recommended dose for phase II studies is 100 mg/m2/day [62].
- For 42 days every 70 days. DLT was achieved with 100 mg/m2/day. The recommended dose for phase II studies is 75 mg/m2/day [63].

According to these extended schedules, the total dose administered is equal to 2.1 g/m2 in four weeks, which is above 750-1000 mg/m2/4 weeks of the 5-day schedules (150 and 200 mg/m2/day, respectively) (ratio of 2.8 and 2.1).

This prolonged exposure with higher overall intensity could lead to an advantage for the therapeutic efficacy of TMZ.

Combined therapies with TMZ

The effects of TMZ administered in combination with other drugs have also been studied.

Various studies have tested the possibility of using combinations of different alkylating agents such as BCNU (Carmustine) to deplete MGMT levels [66]. Additional anticancer effects and complementary toxicity profiles characterise these combinations. Other preclinical studies have shown that O6-benzylguanine (O6-BG) increases the therapeutic index of TMZ. O6-BG is a low molecular weight substrate of MGMT and a powerful DNA resistance inhibitor [67-69]. Pre-treatment with O6-BG increases TMZ activity *in vitro* and *in vivo* especially in cells with high levels of MGMT [15, 27, 70-72]. Extended treatments with O6-BG were more effective than single treatments [70-73]. Other studies have shown that bone marrow cells have low MGMT activity and that depletion of MGMT with O6-BGBG substantially increases the sensitivity of these cells to O6-alkylating agents, such as Carmustine and the TMZ [74-76], and hence this haematological toxicity could limit the use of O6-BG in clinical practice.

It has also been shown that inhibition of PARP may sensitise the tumor to the cytotoxic effects of TMZ. In one study it was observed that systemic administration of the PARP inhibitor significantly increased the anticancer activity of TMZ in xenograft models of intracranial melanomas, gliomas and lymphomas [77]. Susceptibility to TMZ in otherwise resistant cells therefore appears to be restored through combined administration with a PARP inhibitor.

Studies have also been initiated on the combination of TMZ with a range of anti-neoplastic agents, both chemotherapeutic and immunotherapeutic. The combination of TMZ with topoisomerase inhibitors, Topotecan or Irinotecan, in solid tumors and Procarbazine in gliomas has been investigated [78, 79]. Studies on the administration of TMZ

Table 1. TMZ schedules and doses.

Schedule	Dose (mg/m2/day)	Dose rate* (mg/m2/week)	Reference
Daily for 5 days every 28days	200	250	36
Daily for 7 days every 14 days	150	525	38
Daily for 21 days every 28 days	100	525	39
Daily for 42 days every 70 days	75	315	40

*Dose rate is calculated over the total cycle length.

combined with traditional chemotherapeutics reported an increased incidence of toxicity, particularly haematological, limiting the dose rate.

The combination with Interferon and Thalidomide seems to be better tolerated although there is currently no evidence of increased clinical efficacy [55].

Finally, the combination of TMZ and radiation therapy for survival is of particular importance. Since the first studies it has been seen that this association is well tolerated and has provided encouraging results in patients with glioma of high degree treated from the beginning with TMZ in conjunction with radiotherapy [80-82]. TMZ showed promising responses in a subset of adults with recurrent high-grade glioma or newly diagnosed glioma [6-12]. In children and adolescents with high-grade glioma, TMZ showed promising activity and a profile of moderate toxicity although it proved to be ineffective in children with brainstem glioma [13-14].

Conclusion

TMZ represents a new class of second-generation of imidazotetrazine prodrugs that are rapidly degraded at physiologic pH to the cytotoxic monomethyl 5-triazeno imidazole carboxamide (MTIC).

The TMZ is administered orally, is rapidly absorbed and shows approximately 100% bioavailability within 1-2 h of administration. Its demonstrated ability to cross the blood-brain barrier is of special interest with respect to its activity in patients with central nervous system (CNS) tumors. So, a large number of patients with cerebral tumors, mainly high grade gliomas, were enrolled in early clinical trials.

The antitumour activity of TMZ has been shown to be schedule-dependent and a higher response rate was found when the total dose was administered over 5 days. Recently, the activity of TMZ in combination with different antineoplastic agents is under investigation in order to potentially increase the therapeutic index by these drugs in treating children and adults with malignant tumours.

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CONFLICT OF INTEREST STATEMENT

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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