In vitro studies on radiation and temozolomide in human glioma

van Nifterik, K.A.

2011

document version
Publisher's PDF, also known as Version of record

Summary

In vitro studies on radiation and temozolomide in human glioma.

In the last decades the median survival of patients with a glioblastoma multiforme (GBM) has only slightly improved, despite the search for new treatment strategies and/or new drugs or combinations of drugs.

The main objective of this thesis was to investigate the potential of 'targeted' therapy in combination with current treatment protocols to improve glioma therapy. Therefore, several molecular targets inside the glioma tumour cell were selected (Chapter 1, Figure 6) as potential therapeutic targets for specific drugs; in particular, the response in combination with radiotherapy was investigated. Two studies even describe trimodality treatments by combining radiotherapy and the alkylating agent temozolomide (TMZ) with the anti-epileptic drug valproic acid (VPA) and the cyclooxygenase-2 (COX-2) inhibitor meloxicam (MLC). Furthermore, two other studies presented in this thesis examine the influence of the enzyme O^6-methylguanine-DNA methyltransferase (MGMT) in predicting the sensitivity of the tumour cells to TZM-induced alkylating DNA damage, and the use of genetics to differentiate recurrence from newly developed tumours.

Radiotherapy combined with TMZ is the standard treatment for GBM patients. Failure analysis suggests that chemoradiotherapy improves local control; moreover, of those patients that have a recurrence, the most recur in the brain at some distance from the original tumour. It was unclear, however, whether such a distant recurrence is a metastasis from the original tumour or a *de novo* GBM. Therefore, in Chapter 2 we discuss two patients that developed a second GBM outside the previously irradiated target area after a relatively long time interval. To discriminate between distant recurrence and second primary GBM the genetic profiles of the tumours were compared. These profiles allowed to conclude that, in both patients, the second GBM was a recurrence rather than a second independent tumour. In the rare cases where patients with a GBM live long enough to manifest a distant recurrence, the radiotherapy protocol appeared to be insufficient, and additional radiotherapy and/or chemotherapy might be necessary to control these relocation problems.

Chapter 3 presents the results of a study that tested whether the cytotoxic response to TMZ was associated with either the expression of the MGMT protein and/or the methylation of the *MGMT* gene promoter. It was concluded that MGMT protein expression, rather than promoter methylation of the *MGMT* gene, predicts the response to TMZ in human tumour cell lines. In all cases, MGMT protein expression by Western blot analysis was able to predict sensitivity to TMZ. Although quantitative real-time methylation-specific PCR (qMSP; 92%) and bisulphite sequencing (100%) were also able to predict TMZ sensitivity, these results should be interpreted with caution. The qMPS results only applied to the glioma cell lines, and bisulphite sequencing only to a selected group of cell lines with a known association between sensitivity to TMZ and MGMT protein status and MGMT promoter methylation status: based on methylation-specific multiplex ligation-dependent probe

amplification (MS-MLPA) and qMSP. Therefore, MGMT protein expression by Western blot analysis is the preferred technique for predicting sensitivity to TMZ in human tumour cell lines.

The radiosensitising potential of TMZ was further investigated and described in Chapter 4 for three long-term primary genetically characterized human GBM cell lines using single-dose and fractionated γ-irradiation. It was shown that TMZ is at least additive and can even enhance the radiation response, both after single dose and fractionated irradiation. However, in one cell line the radiosensitising effect was only seen after single dose and not after fractionated radiation. The three tested cell lines lacked detectable expression of the MGMT protein and showed methylation of the *MGMT* gene promoter, indicating that the effects of TMZ on the radiation response are independent of the MGMT status.

The potential interaction (positive or negative) between the standard clinical treatment regimen of radiotherapy plus TMZ and VPA (a commonly prescribed anti-epileptic drug in glioma patients) was investigated and described in Chapter 5. The concerns about the potential antagonising effect of VPA on TMZ activity were not confirmed. Contrary to the theoretical hypothesis, VPA was found to enhance the cytotoxic effects of TMZ in both a TMZ sensitive cell line and a TMZ resistant cell line. Furthermore, VPA also showed enhancement of the radiation response, which may be even further enhanced by combining VPA with TMZ. As described in Chapter 4, the cytotoxic enhancement by TMZ and the radiation enhancement by VPA plus TMZ was independent of the sensitivity of the cells to TMZ, hence MGMT protein expression.

Next, in Chapter 6, the combination of radiotherapy plus TMZ was investigated with the COX-2 inhibitor MLC, known for its ability to enhance the effect of radiation in glioma tumour cells. Pretreatment with TMZ significantly enhanced the cytotoxic response of the three tested cell lines to MLC. The combination treatment of TMZ plus MLC can also cause considerable enhancement of the radiation response, although only D384 cells benefit from trimodal over bimodal treatment. Again, since all three cell lines were sensitive to TMZ and lacked MGMT protein expression, enhancement of the cytotoxic response to MLC by TMZ and enhancement of the radiation response by TMZ plus MLC were not dependent on TMZ sensitivity of the cell lines.

Although more research is needed on the mechanisms of the pathways involved, it can be concluded that combined modality treatment consisting of radiotherapy and TMZ with one or more ‘targeting’ drugs might have great potential benefit in glioma treatment.