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Treating gliomas with glucocorticoids: from bedside to bench

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Abstract Glucocorticoids are used in the treatment of gliomas to decrease tumour-associated oedema and to reduce the risk of acute encephalopathy associated with radiotherapy. However, the mechanisms by which glucocorticoids work are still largely unknown. In this paper, we survey the experimental and clinical evidence for the effects of glucocorticoids on tumour cell proliferation, apoptosis and sensitivity to chemotherapy, angiogenesis and vascular permeability. We then review current guidelines on the choice of molecule, dose and duration of glucocorticoid treatment for gliomas.

Keywords: glucocorticosteroid ; glioma ; cell culture ; oedema ; angiogenesis

Introduction

Since 1961, glucocorticoids have been used in the treatment of gliomas as they markedly improve clinical status and reduce surgical mortality and morbidity [27]. Their beneficial effects are attributed to a reduction of tumour-associated oedema. Glucocorticoids are therefore among the first drugs given to patients presenting with glioma and clinical or radiological signs of cerebral oedema, resulting in a dramatic, albeit transient, improvement of the patient's condition [25]. Preventive corticotherapy is also used to reduce the risk of acute encephalopathy associated with radiotherapy [25] although this indication has been challenged [75]. Interestingly, glucocorticoids interfere with chemotherapy and seem to reduce its haematopoietic toxicity [72]. As glucocorticoids reduce the permeability of the capillary bed inside the tumour [80], they may also prevent access of chemotherapeutic drugs to the tumour [98]. Despite their widespread clinical use, glucocorticoids operate by mechanisms which are still largely unknown. In this context, the number of experimental studies on glucocorticoids and gliomas appears surprisingly small with only 222 reports found on PubMed when searching for glucocorticoids and gliomas over the last 15 years (www.ncbi.nlm.nih.gov/entrez).

In this paper, we survey the basic mechanisms of action of glucocorticoids and the main pathways involved. We examine the evidence from in vitro experiments, dealing mainly with glioma cell proliferation. We also review the experimental studies on animal models pertaining to the control of cerebral oedema. We then review the information gained from clinical and neuro-imaging studies in patients with gliomas. Finally, we present the current indications and recommendations for the therapeutic use of glucocorticoids in this context.

Basic mechanisms of action of glucocorticoids

Molecular mechanisms

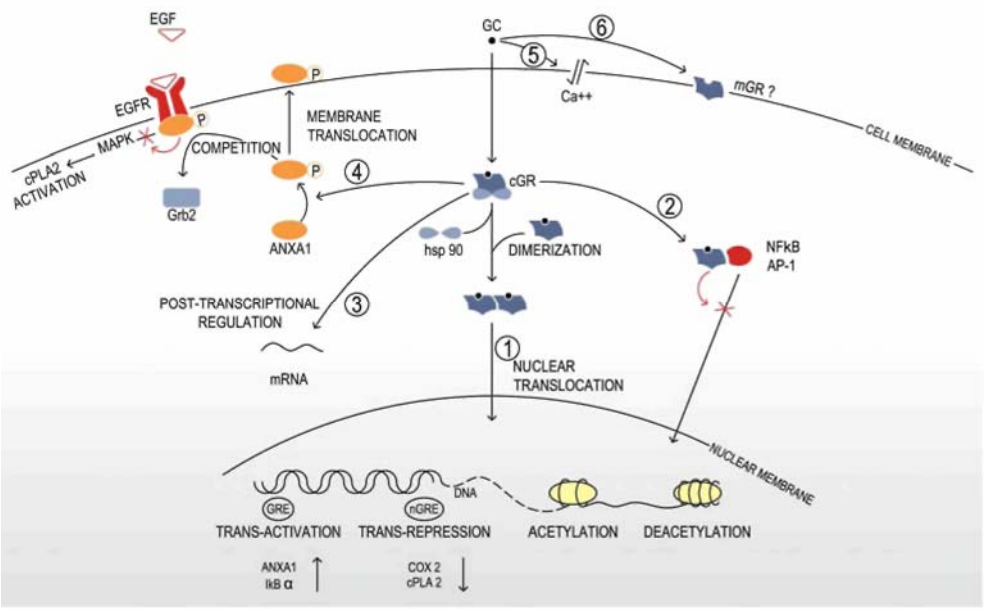
The mechanisms of action of glucocorticoids have been best studied in inflammatory diseases, such as asthma or rheumatoid arthritis [37]. Currently, glucocorticoid effects are classified as genomic or non-genomic (Fig. 1). Genomic effects are characterized by sensitivity to transcription and transduction inhibitors [86] and operate through three main mechanisms: trans-activation (which accounts for most major secondary effects of glucocorticoids); trans-repression, mediation of therapeutic anti-inflammatory effects; and post-transcriptional regulation. Non-genomic mechanisms drive more rapid effects such as activation of signalling cascades [51].

The genomic effects of glucocorticoids are mediated by a cytoplasmic glucocorticoid receptor (GR). Multiple isoforms of GR are generated by alternative RNA splicing, alternative translation initiation of the mRNA and post-translational modifications. GR-β is the best-characterized splice variant. It does not bind to glucocorticoids, but binds to DNA, potentially interfering with the action of glucocorticoids [105]. The inactivated GR is bound to a protein complex, including two molecules of 90 kDa heat shock protein (hsp90), a 59 kDa immunophilin protein, and various other inhibitory proteins. Glucocorticoids are thought to diffuse freely across the cell membrane because of their lipophilicity. After glucocorticoid-GR binding, hsp90 dissociates, exposing two nuclear localization signals [5].

Activation of gene transcription, or trans-activation, occurs after nuclear translocation of the glucocorticoid-GR complex, which forms a homodimer and binds to DNA at consensus sites named glucocorticoid responsive elements (GRE), inducing an enhancement of gene transcription. However, several genes have been shown to be trans-activated by glucocorticoids in the absence of a GRE consensus sequence in their promoter region. Target genes include, for example, annexin A1 (ANXA1), an inhibitor of the cytoplasmic phospholipase A2 (cPLA2),

IκB-α, an inhibitor of nuclear factor-κB (NF-κB) (reviewed in [5]) and mitogen-activated protein kinase phosphatase-1 (MKP-1), which inhibits ERK, JNK and p38 mitogen-activated protein kinase (MAPK) activities [74]. Glucocorticoids can also indirectly enhance gene transcription by inducing acetylation of the core histones of the nucleosomes. This leads to the unwinding of DNA, allowing the transcription factors to gain access to their binding sites and leading to enhanced gene transcription (reviewed in Hayashi [37]).

Fig. 1 *Molecular mechanisms of glucocorticoids.* Genomic mechanisms: glucocorticoids diffuse freely across the cell membrane. In the cytoplasm, the inactivated GR is linked to a protein complex, including two molecules of 90 kDa heat shock protein (hsp90). After glucocorticoid-GR binding, the protein complex dissociates, exposing two nuclear localization signals. The activated GR translocates into the nucleus as a homodimer. Activated GR may either enhance gene transcription via direct transactivation and acetylation, or decrease gene transcription via direct trans-repression and deacetylation (*1*). Activated GR may inhibit the activity of AP-1 or NF-κB by direct protein-protein interaction (indirect trans-repression) (*2*). Activated GR may exert post-transcriptional gene regulation via alterations of the mRNA turnover or translation (*3*). Non-genomic mechanisms: activated GR induces phosphorylation and activation of ANXA1. Activated ANXA1 translocates to the cell membrane and inhibits the EGF-dependent activation of cPLA2 by blocking the recruitment of Grb2 to the activated EGFR (*4*). Glucocorticoids induce alterations of the physicochemical properties of the cell membrane (*5*). Some non-genomic effects of glucocorticoids are thought to be mediated by a non-classical membrane-associated GR (*6*)



It is currently admitted that the major anti-inflammatory effects of glucocorticoids are linked to direct and indirect repression of the transcription of key genes of the inflammatory and immune response. Direct repression of gene transcription (trans-repression) occurs when the homodimeric-activated GR binds to negative glucocorticoid responsive elements (nGRE), "composite GREs" or less well-defined regions of DNA regulating the transcription of target genes, as demonstrated for cyclooxygenase 2 (COX2) and cPLA2 (reviewed in Barnes [5]). Indirect repression of gene transcription results from inhibition of the activity of pro-inflammatory transcription factors, such as activator protein-1 (AP-1) and NF-κB [56]. The negative regulation of AP-1 and NF-κB seems to be a key mechanism of glucocorticoid anti-inflammatory effects and occurs at different levels. First, activated GR may bind to AP-1 or NF-κB via a direct protein-protein interaction, resulting in mutual repression of the transcriptional activity [55]. Glucocorticoids may also antagonize the tumour necrosis factor alpha (TNF-α)-induced phosphorylation and activation of AP-1 [31]. Finally, the glucocorticoid-GR complex, probably through binding to a co-repressor molecule, may deacetylate histones, increasing DNA coiling and thus preventing the binding of transcription factors and leading to gene repression (reviewed in Hayashi [37]).

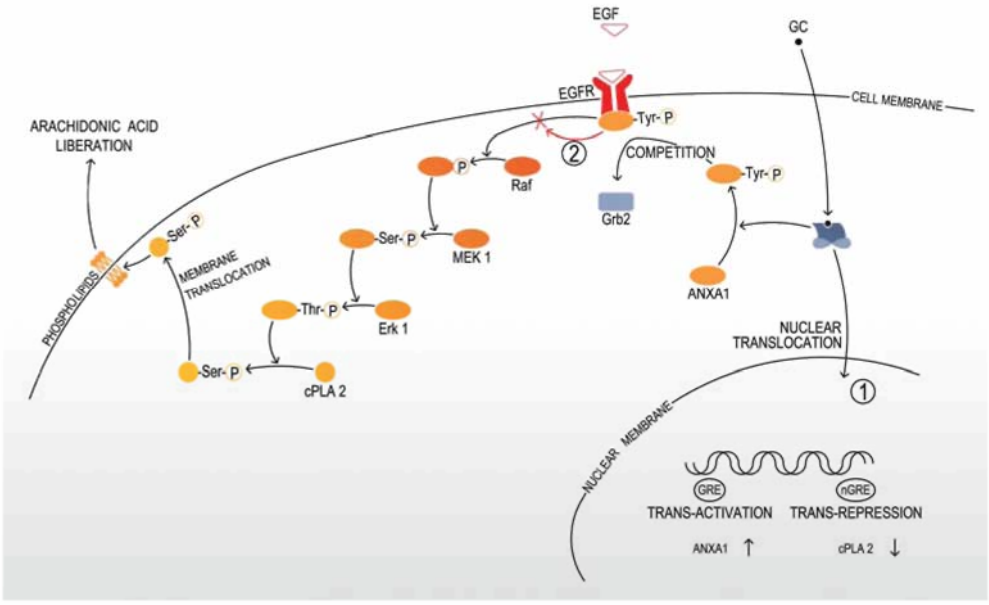
Post-transcriptional gene regulation is included among the genomic effects of glucocorticoids because it affects gene expression and is sensitive to protein synthesis inhibitors (reviewed in Stellato [86]). This mechanism consists of alterations of the mRNA turnover or translation and has been demonstrated for IL-1α, IL-6, IL-8, IFN-β and granulocyte-macrophage colony-stimulating factor (GM-CSF) [86] and TNF [74].

The classical genomic modes of action do not explain some of the rapid effects of glucocorticoids. These rapid effects are mediated by non-genomic pathways and are insensitive to inhibitors of transcription and of protein synthesis (reviewed in Falkenstein [22] and Stellato [86]). Some of these effects do not require GR transduction and are based on alterations of the physicochemical properties of the cell membrane that modify levels of intracellular calcium [94]. Other rapid effects of glucocorticoids involve activation of the GR. This is the case in the A549 human adenocarcinoma cell line, where, after epidermal growth factor receptor (EGFR) activation,

dexamethasone inhibits the MAPK-dependent activation of cPLA2 and the subsequent release of arachidonic acid (Fig. 2). This effect is mediated through dexamethasone-induced phosphorylation of ANXA1 which blocks the recruitment of the growth factor receptor-bound protein 2 (Grb2) to the activated EGF receptor (EGFR) [17]. This may be particularly relevant in gliomas where aberrant EGFR signalling has been recognized as an important step in malignant progression [62]. In a human folliculo-stellate cell line, dexamethasone has been shown to induce a rapid serine phosphorylation and membrane translocation of ANXA1 via a mechanism that requires MAPK, phosphatidylinositol 3-kinase and calcium-dependent PKC pathways [83]. Finally, some non-genomic effects of glucocorticoids are thought to be mediated by a non-classical membrane-associated GR. So far, two putative membrane receptors have been identified: a 63 kD acidic glycoprotein found in the amphibian *Taricha granulosa* [65] and a modified form of the classical GR, which could be linked to glucocorticoid-induced lysis of lymphoma cells and in thymic involution and apoptosis [28, 29].

Glucocorticoid receptor-mediated effects can be experimentally modulated by RU486 (mifepristone), a GR antagonist which also shows anti-progesterone activity [11]. RU486 binds to GR with high affinity and impedes the dissociation of the protein complex, subsequently decreasing or slowing the formation of the activated receptor. Once linked to GR, RU486 may bind to DNA but is unable to directly modulate gene transcription. RU486 may also modulate the glucocorticoid-induced indirect repression of gene transcription. Interestingly, RU486 has been shown to possess GR agonistic activities depending on the intervention of other signalling pathways [11].

Fig. 2 *Glucocorticoid inhibition of the MAPK-dependent release of arachidonic acid.* The recruitment of the adapter protein Grb2 to the activated EGFR initiates the activation of the kinase Raf, MAPK/Erk kinase (MEK) and Erk. Activated Erk phosphorylates specific serine residues within cPLA2, leading to membrane translocation of the enzyme and arachidonic acid liberation. Glucocorticoids inhibit the arachidonic acid liberation through genomic mechanisms (*1*), by direct trans-activation of ANXA1 and direct trans-repression of cPLA2 and GR-dependent non-genomic mechanisms (*2*) by inducing rapid phosphorylation of ANXA1, which blocks the recruitment of Grb2 to the activated EGFR. *Tyr* tyrosine, *Ser* serine, *Thr* threonine



Glucocorticoid-mediated apoptosis and cell survival

Glucocorticoids are able to influence cell survival in a type-specific manner and may either induce apoptosis (as in lymphoid cells, bone, hippocampus, eosinophils, fibroblasts and some small cell lung cancer cells), or promote cell survival (in erythroblasts, neutrophils, mammary gland, liver and fibroblasts) [77].

The mechanisms by which glucocorticoids induce apoptosis are not yet fully understood (reviewed in Schmidt [77] and Greenstein [35]). It is well established that their pro-apoptotic activity is strictly dependent upon the presence of GR. Nevertheless, it is still unclear if glucocorticoids act through transactivation, trans-repression or both. In an effort to identify glucocorticoid-targeted genes involved in apoptosis, Schmidt et al. [77] did a bioinformatic meta-analysis of eight expression microarray datasets and divided the candidate pro-apoptotic genes into three groups. The first group included genes directly involved in cell death (e.g. IκB-α and GILZ, an AP-1 inhibitor) and survival (e.g. the proto-oncogene c-myc). The second group comprised genes whose regulation might lead to cellular distress and subsequent activation of the apoptotic machinery (e.g. thioredoxin

interacting protein, which contributes to the oxidative stress). The last group was formed by genes that are not involved in cell death, such as genes counteracting the apoptotic response (e.g. receptors for TGFβ and for IL-7). Parallel to these direct transcriptional effects, indirect trans-repression of NF-κB or AP-1 is thought to be an important pathway of glucocorticoid-induced apoptosis.

In contrast, glucocorticoids promote survival in several cell types. They induce the proliferation of chicken erythroid progenitors, an effect which may be due to a GR-dependent transcriptional activation of c-Myb, a major protein of haematopoiesis [99]. Glucocorticoids also enhance neutrophil survival, probably via up-regulation of the expression of leukotriene B_4 Receptor-1 (BLT1), leading to enhanced cell response to the antiapoptotic effects of leukotriene B_4 [85]. Glucocorticoids inhibit involution and programmed cell death of mouse mammary gland, by inducing impairment of AP-1 activity [23]. In human granulosa cells, glucocorticoids almost completely inhibit the apoptosis triggered by serum deprivation, cyclic adenosine 3',5'-monophosphate (cAMP) and activation of the tumour suppressor gene p53, by up-regulating the antiapoptotic protein Bcl-2. Interestingly, the same authors showed that in myeloid leukaemia cells, glucocorticoids induced apoptosis via down-regulation of Bcl-2 [76]. Costas et al. [14] showed that glucocorticoids protect fibroblasts from TNF-induced apoptosis, by a pathway that does not involve NF-κB trans-repression. In rat hepatoma cells, Evans-Storms et al. [21] demonstrated that glucocorticoids suppress apoptosis— induced by serum deprivation—by increasing NF-κB activity in a GR-dependent manner. Finally, in thymocytes, Jondal et al. [43] suggested that basal levels of glucocorticoids might promote cell growth while high levels might induce apoptosis.

In the brain, glucocorticoids may also reduce neuron survival by amplifying glutamate excito-toxicity. Indeed, glucocorticoids have been shown to alter glutamate uptake by modulating the levels of excitatory amino acid transporters (EAAT) on astrocytes and microglia through TNF-α inhibition [41].

Therefore, it appears that depending on cell type, glucocorticoids can either induce apoptosis or promote cell survival. This paradoxical effect is probably related to cell-specific patterns of transcription factors, co-activators, co-repressors and panels of GR isoforms.

Effects of glucocorticoids on glioma: evidence from the laboratory

Glioma cell proliferation: in vitro studies

The proliferative activity of glucocorticoids on glioma cell lines has been studied for more than 30 years and both stimulatory [46, 57, 66, 68, 106] and inhibitory [30, 44, 57, 59, 66] effects have been observed. A careful survey of these reports suggests that at least some of the discrepancies between studies result from the use of non-comparable experimental conditions, most notably regarding cell line type, cell culture density, glucocorticoid dose and culture medium (presence or absence of serum) (Table 1).

Cell density in glioma culture markedly affects the response to glucocorticoids as first demonstrated by Freshney et al. [26]. Using a primary culture derived from a human anaplastic astrocytoma in a 20% serum medium, they found that low-density cultures (below 50 cells per colony) proliferate in response to betamethasone at 25 μM, whilst cell proliferation is inhibited at higher cell density. This suggests that cell-cell interactions alter the proliferative response of glioma cells to glucocorticoids. Using dexamethasone at 1 μM in a D384 human cell line 10 % serum model, Langeveld et al. [46] made a similar observation and noticed that the proliferative effect of glucocorticoids decreases with higher inoculation cell density. They suggested that increasing cellular density could lower GR expression and consequently reduce the growth stimulatory effects of glucocorticoids. Unfortunately, the authors could not confirm their hypothesis as the number of cells necessary to perform receptor-binding studies could only be obtained from confluent monolayers [46]. Interestingly though, in a model of rat C6 cell line cultured in the presence of serum, Vielkind et al. [96] showed that GR level was influenced by cell density and was higher in areas where cells had reached confluency. In short, it seems that glucocorticoids have stimulatory effects on cell proliferation at low cell density but tend to become inhibitory at higher cell density, possibly in relation to modifications of GR expression.

The dose at which glucocorticoids are given also orients the cell culture response towards proliferation or inhibition. Glucocorticoid concentrations used in experiments range from 0.01 nM to 250 μM. Both stimulatory and inhibitory effects have been described. In primary cultures of human glioblastoma and rat C6 glioma cell lines, dexamethasone exerts an inhibitory effect at high concentrations [58, 32]. When exposed to dexamethasone or methylprednisolone at doses of 250 μM, glioma cells develop significant toxic manifestations (swelling and vacuolization of the cytoplasm and cell processes with disruption of the cell membranes) while their cytology is not altered at concentrations between 0.625 and 6.25 μM [58]. Accordingly, Paoletti et al. [66] also observed an inhibition of primary glioma cultures at dexamethasone 125 μM, produced by GR-independent mechanisms, such as membrane alterations. By contrast, at low concentrations, dexamethasone usually has a proliferative effect in cultures supplemented with serum. D384 cell line grown in a 10% serum medium proliferates in response to dexamethasone. This stimulatory effect is maximal at 0.1 μM and disappears for

concentrations below 0.1 nM [46]. Using U-87 MG cells complemented with serum, Pinski et al. [68] observed that dexamethasone stimulates cell proliferation for concentrations between 10 nM and 10 μM, with a maximal effect at 10 μM. Thus, it appears that in the presence of serum, dexamethasone exerts a physiological dose-dependent proliferative effect on glioma cultures for concentrations between 0.1 nM and 10 μM; concentration above 125 μM result in cell growth inhibition due to non-specific cytotoxic effects.

The proliferative response to glucocorticoids also depends on the expression of GR in the cell culture. GR has been detected both in glioma cell lines [61] and in human GBM samples [20]. Its level of expression depends on the cellular density and is higher in areas of cell confluency [96]. Using the U-87 MG cell line in the presence of serum, Pinski et al. [68] demonstrated that the proliferative response to dexamethasone between 10 nM and 10 μM is significantly reduced in the presence of the GR antagonist RU 486, at concentrations of 1 μM and 10 μM. Zibera et al. [106] obtained similar results with the HU-197 cell line in the presence of serum. They showed that the 17 beta-carboxamide steroid DXB, a GR antagonist that competes with dexamethasone for binding to the GR but does not trigger the glucocorticoid effect, used at 16 μM, completely inhibits the proliferative effect of dexamethasone at 40 nM. Paoletti et al. [66] characterized the GR status of nine primary glioma cell cultures grown in the presence of serum and obtained five GR-positive and four GR-negative cultures. They showed that dexamethasone in concentrations ranging from 40 nM to 5 μM stimulates the proliferation of four of the five GR-positive cultures, while dexamethasone at the same concentrations does not influence the growth of the GR-negative cultures. Using dexamethasone at 125 μM, they observed a significant inhibition of all the cultures, independently of their GR status. In summary, it appears that the presence of GR is necessary [68, 106] but not sufficient [66] to explain the proliferative effects of dexamethasone below 10 μM. On the other hand, the inhibitory effects of dexamethasone above 125 μM do not seem mediated by GR, but rather by aspecific mechanisms, such as membrane alterations [66].

Two studies have specifically addressed the influence of serum complementation or deprivation on the response of cell culture to glucocorticoids. Using dexamethasone at 1 nM on a primary culture of anaplastic astrocytoma, McLean et al. [57] obtained an inhibition of astrocytoma cell proliferation in the presence of 10% foetal bovine serum (FBS). By contrast, glucocorticoids restored the proliferation of astrocytoma cells which had been markedly reduced by serum deprivation. In a more recent paper, Morita [59] made the opposite observation and reported that in serum-free cultures dexamethasone at 1μM inhibits the proliferation of C6 rat cells, but has no effect in the presence of 10% FBS. In this model, dexamethasone is thought to exacerbate the cytotoxic effect of serum deprivation which depends on the activation of GR [59].

Several authors have studied in vitro how dexamethasone interacts with drugs used for chemotherapy. In 1977, using rat C6 glioma cells, Grasso et al. [33] showed that the growth inhibition induced by the antineoplastic agent BCNU was accentuated when cells were pre-treated by dexamethasone 1 μM but not when both drugs were given in combination. In 1997, Weller et al. [98] studied the in vitro interactions between dexamethasone and several anti-neoplastic agents (ACNU, VM-26, vincristine, cytarabine, methotrexate and adriamycin) in a T98G and a LN-229 serum-free model. Dexamethasone was added 24 h prior to anti-neoplastic agents and maintained for 72 h. The authors showed that dexamethasone (maximal effect at 100 nM) attenuates cytotoxic and growth inhibitory effects of all chemotherapy drugs. Nevertheless, the withdrawal of dexamethasone after 7 days of pre-treatment resulted in significant restoration of sensitivity to most drugs tested. Using T98G and C6 glioma cells, Wolff et al. [100, 101] showed that dexamethasone at 1μM induces partial resistance to cisplatinium at 50 μM for 72 h, by a mechanism involving the GR. More recently, dexamethasone has been shown to protect glioblastoma U87MG [18] and T98G cell lines [87] against temozolomide-induced apoptosis. In addition, dexamethasone may interfere with suicide gene therapy as in the Herpes Simplex Virus thymidine kinase gene (HSV-tk) model [73]. In conclusion, in vitro studies suggest that dexamethasone antagonizes the cytotoxic and anti-proliferative effects of several anti-neoplastic agents. This observation highlights the importance of timing in combined anti-tumour chemotherapy.

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Table 1 *Glioma cell proliferation: in vitro studies*

Author	Established cell line or primary cell culture	Inoculation cell density[a]	Dose and duration of glucocorticoid treatment	Serum	Cell growth assays	Proliferative response to glucocorticoids
Freshney [26]	Primary culture of human AA	15-50 cells/cm^2	β-met 25 μM; 3 weeks	20% FBS	[3H]thymidine and cell counts	< 50 cells per colony: S; > 50 cells per colony : I
Langeveld [46]	Hs 683, D384, U251, WF and 3 primary culture of glioma	1,500-25,000 cells/cm^2	Dex 1 nM-1 μM; 144 h	10% FCS	Cell counts and MTT assay	S, particularly for low density (D384, Dex 1 μM)
Pinski [68]	U-87 MG	2,500 cells/cm^2	Dex 0.01 nM-10 μM; 96 h	5% dextran-coated charcoal FBS	Cell counts	S (Dex 10 nM-10 μM) significantly reduced by RU 486 1 μM and 10 μM
Zibera [106]	HU-197	4,200 cells/cm^2	Dex 1.6 nM-1 μM; 120 h	15% FCS	Cell counts	S (Dex 40 nM-1 μM); effect of Dex 40 nM is completely inhibited by DXB 16 μM
Paoletti [66]	Ten primary cultures of gliomas	1,500-3,000 cells/cm^2	Dex 40 nM-125 μM; 96 h	10% FCS	Cell counts	Dex 40 nM-5 μM: S of GR positive cultures, no effect on GR negative cultures; Dex 125 μM: I of GR-positive and GR-negative cultures
McLean [57]	Seven primary cultures of human AA	Not specified	Dex 1 nM-10 μM; 24 h	10% FBS and without serum	[3H]thymidine and cell counts	With serum: I (Dex 250 nM); without serum: S (Dex 2.5 nM)
Mealey [58]	Eight primary cultures of GBM	Not specified	Dex 625 nM-250 μM; 144 h	10% FCS	Cell counts	I more pronounced for higher doses (Dex 625 nM-250 μM)
Morita [59]	C6	50,000 cells/cm^2	Dex 1 μM; 24, 48 and 72 h	10% FBS and without serum	Determination of cell viability (neutral red)	Without serum : I; with serum: no effect
Grasso [32]	C6	20,000-30,000 cells/cm^2	Dex 0.1 nM-10 μM; 24, 48, 96 and 144 h	10% FCS	Cell counts	I more pronounced for higher doses

[a] Surface used for conversion: 96-well, 0.32 cm^2; 24-well, 2 cm^2; 6-well, 9.6 cm^2

MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, *AA* anaplastic astrocytoma, *GBM* glioblastoma, *Dex:* dexamethasone, *β-met:* betamethasone, *S* stimulation of cell proliferation, *I* inhibition of cell proliferation

Tumour growth: in vivo experiments

Several in vivo studies have shown that tumour xenografts tend to regress in mice treated by glucocorticoids, improving survival [36, 90, 102, 104]. Histology of the xenografts suggests that glucocorticoids may inhibit angiogenesis. Wright et al. [104] used a mixed rat glioma cell line induced by intravenous injections of *n*-nitrosomethylurea and injected a cell suspension in the flank of 25 albino Wistar rats. They administered methylprednisolone acetate at 100 mg/kg twice weekly from the day of transplantation. None of the 13 rats receiving dexamethasone developed the neoplasm, while all 12 of the controls produced tumour. Tamargo et al. [90] generated subcutaneous gliomas in Fischer 344 rats by implantation of a 2-4 mm^3 piece of 9L gliosarcoma and studied the effect of local treatment by cortisone acetate on tumour growth. After 14 days, they observed a significant inhibition of tumour growth in animals treated by cortisone acetate. In parallel, they studied the effect of cortisone given locally on angiogenesis in VX2 carcinomas implanted in 60 rabbit corneas. After 21 days, they obtained a significant inhibition of angiogenesis by cortisone acetate, suggesting a vascular component in the tumour growth inhibition induced by glucocorticoids. Guerin [36] and Wolff [102] injected 9L and C6 rat glioma cells into the left putamen of syngenic rats. Two days after implantation, they administered dexamethasone at 3 mg/kg/day and obtained a significant inhibition of tumour growth associated with a reduction of vascular density and an enhancement of survival. Using a rat intra-cerebral 9L gliosarcoma model, several authors [60] demonstrated that intra-peritoneal injections of dexamethasone (1.5 mg/kg repeated twice to 3 mg/kg repeated five times) on day 10-15 after inoculation significantly decreases tumour volume and diminishes, in vessels larger than 25 μm, the tumour vessel dilatation associated with neoplastic angiogenesis [4].

Finally, Pinski et al. [68] investigated the influence of RU486, a GR antagonist, on the growth of U-87 MG cells in nude mice. They showed that daily injection of RU486 at 0.1 or 0.5 mg/animal significantly inhibited tumour growth at 4 weeks. However, this result should be interpreted with caution since RU486 has been show to present GR agonist effects in some circumstances (see above).

In mice, dexamethasone pre-treatment enhances the anti-tumour effects and reduces the hemato-toxicity of several chemotherapeutic agents, as demonstrated by two in vivo studies [71, 97]. In C3H/HeJ mice treated with carboplatin (600 mg/m^2), Rinehart et al. [72] showed that dexamethasone pre-treatment (3 or 7 days, at 0.5 mg/day) significantly reduced mortality and induced less severe granulocytes and platelet nadirs. In nude mice with subcutaneous U-87 MG glioma xenografts, Wang et al. [97] demonstrated that dexamethasone pre-treatment (0.1 mg/day for 5 days) significantly increased the therapeutic effects of combined therapy with gemcitabine and carboplatin.

Tumour-associated oedema: in vivo models

In 1967, Klatzo [45] clarified the pathophysiology of brain oedema and distinguished the "cytotoxic" and the "vasogenic" types of oedema. "Cytotoxic" oedema results from a disturbance of cell metabolism, due, for example, to ischaemic or anoxic conditions. "Vasogenic" oedema develops in case of blood-brain-barrier damage, such as in brain tumour progression.

Animal studies have helped to clarify the pathophysiology of tumour-associated oedema [39] and have shown that it has both "vasogenic" and "cytotoxic" components.

The vasogenic component is due to an increased micro-vascular permeability of the blood-brain and blood-tumour barrier leading to the extravasation of whole plasma, even at distance from the tumour. For this reason, the term "tumour-associated" oedema is now preferred to that of "peritumoural oedema". Several mechanisms contribute to the vasogenic oedema [19]. Firstly, malignant gliomas produce molecular factors that increase the permeability of the capillary bed in the surrounding brain tissue and that are likely to be the source of tumour-associated oedema [63]. Among them, vascular permeability factor (VPF) is of particular interest, its in vivo activity being inhibited by dexamethasone through a pathway implicating de novo synthesis of a polypeptide, maybe ANXA1 [15, 16]. Secondly, newly formed micro-vessels within the tumour present characteristic features that contrast with the normal architecture of the blood-brain barrier: widened intercellular junctions, discontinuous tight junctions, membranous fenestrations, non-contiguous basement membranes, active micropinocytosis and paucity of mitochondria (M.W. Brightman and T.S. Reese, unpublished data [9, 64]). Finally, immunological factors and inflammatory processes have also been implicated, such as prostaglandins, arachidonic acid [93] and leukotrienes [79], although there is no consensus on role of the latter [92].

The cytotoxic component is induced by the generation of oedematogenous metabolites by tumour cells, including lactate, produced by aerobic glycolysis [70]. Their osmotic activity explains the protein-free cytotoxic component of the brain oedema.

Experimental models of gliomas confirmed that tumour-associated oedema responds favourably to local or systemic corticotherapy (Table 2) [91]. Matsuoka and Hossmann [54] produced brain gliomas in nine cats by implantation of RG2 cells and injected, after 2-3 weeks, a single dose of 10 mg dexamethasone intramuscularly. In the treated group, they observed lower peritumoural white matter water content, electrolyte shifts and vascular

resistance than in the control group. Furthermore, cerebral blood flow was conserved while it was decreased in untreated group. Using a rat intra-cerebral 9L glioma model, Guerin et al. [36] studied the effect of glucocorticoids on vascular permeability and vascular density. Two days after tumour implantation, dexamethasone at 3 mg/kg was administered daily by intra-peritoneal injections. Glucocorticoid therapy reduced vascular permeability as demonstrated by the lower content of Evans blue in treated tumours. It also significantly reduced vascular density as shown by immunostaining for laminin. Using the same model, others [4] assessed anti-angiogenic effects of dexamethasone by magnetic resonance imaging (MRI), cerebral blood volume maps and microscopic evaluation, and demonstrated that glucocorticoid diminishes, in vessels larger than 25 μm, the tumour vessel dilatation associated with neoplastic angiogenesis. Authors suggest that this effect could be linked either to glucocorticoid-induced down-regulation of VEGF or to glucocorticoid-induced vasoconstriction. Working with C6 glioma xenografts in rodents and doses of dexamethasone in the clinical range (0.22 mg/kg/day), Swaroop et al. showed that glucocorticoids decrease tumour capillary permeability without modifying the local cerebral blood flow. They suggested that these effects are mediated by a reduction of the inducible nitric oxide synthetase (iNOS) expression within and around the tumour [78, 88, 89]. In rabbits, Lindvall-Axelsson and Owman [50] showed that betamethasone administered daily for 5 days significantly reduces cerebral spinal fluid production. Finally, Chang et al. [13] studied the effect of ANXA1 on the resolution of peritumoural oedema. They produced intra-cerebral tumours by implantation of C6 cells in Wistar rats. They showed that the cortical and tumoural water content was decreased by dexamethasone but not by ANXA1, suggesting that glucocorticoids do not exert their anti-oedema properties through induction of ANXA1.

Table 2 *Effects of glucocorticoids on tumour-associated oedema: in vivo models*

Reference	Glucocorticoid effect	In vivo model
Guerin [36]	Decrease of tumour capillary permeability	Rat intra-cerebral 9L glioma model
Guerin [36]	Reduction of vascular density	Rat intra-cerebral 9L glioma model
Badruddoja [4]	Anti-angiogenic effect (down-regulation of VEGF or vasoconstriction)	Rat intra-cerebral 9L glioma model
Lindvall-Axelsson and Owman [50]	Reduction of cerebral spinal fluid production (CSF)	CSF production determination by ventriculo-cisternal perfusion with radioactive inulin in rabbits

Glucocorticoids in human gliomas: clinical and neuro-imaging studies

The development of brain imaging studies—CT-scan, positron emission tomography (PET) and MRI—have led to a better understanding of the effects of glucocorticoids on tumour-associated oedema (Table 3).

CT-scan studies are based on the analysis of midline shift, ventricular compression, oedema, contrast enhancement intensity and area, before and after glucocorticoid administration. These parameters are improved after glucocorticoid treatment in most patients. Maximum effect occurs within 2 weeks. The contrast enhancement within tumours is reduced in patients treated with glucocorticoids suggesting a partial restoration of blood-brain barrier integrity [12].

Positron emission tomography makes it possible to non-invasively measure cerebral blood flow, cerebral blood volume, blood to tumour transport rate constant and metabolic processes, such as oxygen utilization. This technique demonstrated that glucocorticoids exert a direct effect on cerebral vessels and cause vasoconstriction as suggested by the reduction of regional cerebral blood flow (rCBF) and regional cerebral blood volume (rCB V) and enhancement of oxygen extraction without variation in oxygen utilization [48]. Furthermore, Jarden et al. [42] have demonstrated that glucocorticoids reduce tumour capillary permeability within 6 h after a single pharmacological dose.

Magnetic resonance imaging is a good tool for studying brain oedema, because the relaxation time 71 (longitudinal relaxation time) [53] and *T*2 (transverse relaxation time) are positively correlated to the water content of the tissue studied [1]. Derived techniques, such as the use of Gd-DTPA, ^{99}Tc-DTPA [3], quantitative dynamic susceptibility contrast perfusion MRI (DSC-MRI) [47] or diffusion tensor MRI (DT-MRI) [52, 81] provide an even more accurate means of studying brain oedema. Bell et al., using a mean T1 determination, found that glucocorticoids had no significant effects on water content and concluded that the clinical improvement observed in all of their patients could not be explained by any effect of glucocorticoids on the extent of brain oedema [8]. Nevertheless, the highest value in the histogram of T1 ("super-oedema") represents a more sensitive monitoring of changes in tumour-associated oedema because of its high heterogeneity. Using this technique, Andersen et al. [1] showed a significant glucocorticoid effect on brain oedema, and postulated that glucocorticoids act on the leaky blood-brain barrier within the tumour. The reduction of capillary trans-

endothelial diffusion of Gd-DTPA in gliomas observed after glucocorticoid treatment tends to confirm this hypothesis [2]. DT-MRI and DSC-MRI studies demonstrated that glucocorticoids act by locally reducing the extra-cellular water fraction in tumour-associated brain oedema [81] and subsequently increasing perfusion in the oedematous brain [6]. Quantitative MRI can therefore measure steroid treatment response or failure in patients with malignant gliomas [82].

In short, early brain imaging studies [8] did not find any detectable decrease of tumour-associated oedema following glucocorticoids, but attributed the clinical benefit of glucocorticoids to vasoconstriction [7]. However, the development of more accurate techniques (PET, "super-oedema" determination, Gd-DTPA and DT-MRI) clearly demonstrated that glucocorticoids reduce tumour-associated oedema by a direct effect on tumour capillary permeability [1, 2, 42] and related this action to the rapid clinical improvement following glucocorticoid therapy.

Table 3 *Effects of glucocorticoids on tumour-associated oedema: neuro-imaging studies*

Reference	Glucocorticoid effect	Neuro-imaging study
Cairncross [12]	Decrease of tumour capillary permeability	CT-scan, MRI and PET
Andersen [1] Andersen and Jensen [2] Jarden [42] Brooks [10]		
Sinha [81]	Reduction of tumour-associated oedema water content by a local effect on the extra-cellular water fraction	DT-MRI, DSC-MRI
Bastin [6]		
Leenders [48]	Vasoconstriction	PET

Recommendation and guidelines for corticotherapy in patients with gliomas

Although glucocorticoids are widely used in the treatment of patients with intracranial gliomas, no systematic review or meta-analysis has ever been conducted. The few published randomized trials deal with metastatic brain tumours [38, 95, 103]. In consequence, the indications, choice of glucocorticoids, dose and duration of treatment are still empirical and vary according to institutional practices.

In 2003, Sarin and Murthy [75] proposed a practical guideline for the use of glucocorticoids as medical decompressive therapy for primary and metastatic intracranial tumours. They recommend corticotherapy for patients who have intracranial tumours with symptoms of raised intracranial pressure or rapidly progressive focal neurological deficits. Corticotherapy is not recommended if the patient has seizures, or disturbances of higher mental functions, or for prophylactic use during cranial irradiation. The daily dose (6, 12 or 24 mg dexamethasone, given in two fractionated doses) is based on the severity of headache or vomiting and the presence of focal deficit or altered consciousness. Doses up to 100 mg of dexamethasone or 2,000 mg of methylprednisolone have been proposed in the absence of improvement [49, 69]. Nevertheless, in view of major secondary effects, Sarin and Murthy [75] recommend a maximal dose of 24 mg dexamethasone outside clinical trial settings. The treatment should be stopped in the absence of improvement after 48 h. In case of improvement or stabilization, dexamethasone should be tapered every 48 h. Glucocorticoids are generally given from diagnosis until radiotherapy is completed, a period of about 8-12 weeks [40]. High-dose steroid use and radiotherapy are sufficient to induce severe immuno-suppression in patients with primary brain cancer and prophylactic antibiotherapy has been advocated for patients with low CD4 counts [40].

Evaluation of the Human Corticotropin-Releasing Factor (hCRF) as an alternative to dexamethasone is currently in progress in a phase III study ("A Phase III Randomized, Double-Blind Study Comparing Human Corticotropin-Releasing Factor to Dexamethasone for Control of Symptoms Associated with Peritumoral Brain Edema in Patients with Primary Malignant Glioma"). The purpose of this study is to examine the safety and effectiveness of hCRF compared to dexamethasone in patients with primary malignant glioma who require increased dexamethasone doses to control symptoms of tumour-associated oedema (http:// www.clinicaltrials.gov, identifier NCT00226668, sponsored by Neurobiological Technologies).

To the best of our knowledge, only one trial has studied the use of glucocorticoids alone as a potential chemotherapeutic agent. In this randomized controlled trial, Green et al. [34] compared the survival of patients with supratentorial malignant gliomas treated with carmustine (BCNU) or procarbazine or high-dose methylprednisolone alone or BCNU plus high-dose methylprednisolone, in addition to radiotherapy. Patients treated with BCNU or procarbazine had a significantly longer survival than patients receiving high-dose methylprednisolone. Furthermore, the combination of BCNU plus high-dose methylprednisolone tended to be

less effective than BCNU alone in patients with poor prognosis.

Other trials included glucocorticoids as part of a larger chemotherapeutic regimen. Pendergrass et al. [67] studied the "eight-drugs-in-one-day" (eight-in-one) regimen—comprising high-dose methylprednisolone, vincristine, hydroxyurea, procarbazine, CCNU, cisplatin, cytosine arabinoside and either cyclophosphamide or dacarbazine—in the treatment of 107 children with recurrent or incompletely resected brain tumours. Methylprednisolone was included for anti-emetic, anti-oedema and possible anti-tumour effects. After two cycles of chemotherapy and within a 4- to 6-week interval, 3 of the 10 patients with recurrent grade III and IV gliomas had an objective response including two complete responses. Seven of 17 patients with newly diagnosed grade III and IV gliomas had an objective response including three complete responses. In 1976, the Children's Cancer Study Group initiated a randomized clinical trial, CCG-943, to study the effectiveness of chemotherapy—prednisone, CCNU and vincristine—in the treatment of 58 children with high-grade astrocytoma [84]. Prednisone was included to minimize undesirable neurological reactions to drug therapy and for the possibility that its effects on cell membrane permeability might enhance the action of the other chemotherapeutic agents. The addition of chemotherapy to surgery and radiotherapy significantly prolonged survival and event-free survival of children with supratentorial or cerebellar high-grade astrocytoma. In a phase III trial, Finlay et al. [24] compared the eight-in-one regimen with prednisone, CCNU and vincristine in the treatment of children with high grade astrocytomas and showed that the eight-in-one chemotherapy provides no benefit to the three-drug regimen used in the CCG-943 study.

Conclusions

Glucocorticoids have been used for more than 40 years in the treatment of intracranial gliomas. However, little is known about the precise mechanisms by which they affect tumour growth, reduce tumour-associated oedema and improve patients' clinical status. Interpretation of experimental data is difficult due to the high variability of tested parameters and experimental conditions. Furthermore, there have been surprisingly few clinical studies on the use of glucocorticoids in this context. However, in view of their widespread use in patients with gliomas and their multiple interactions with other treatments, elucidating the exact mechanisms of glucocorticoid action in gliomas should be a research priority.

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