

review

The role of tricyclic drugs in selective triggering of mitochondrially-mediated apoptosis in neoplastic glia: a therapeutic option in malignant glioma?

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We have previously demonstrated that the tricyclic antidepressant, Clomipramine, exerts a concentration-dependant, tumour cell specific, pro-apoptotic effect on human glioma cells *in vitro* and that this effect is not mirrored in non-neoplastic human astrocytes. Moreover, the drug acts by triggering mitochondrially-mediated apoptosis by way of complex 3 of the respiratory chain. Here, through reduced reactive oxygen species and neoplastic cell specific, altered membrane potential, cytochrome c is released, thereby activating a caspase pathway to apoptosis. In addition, while we and others have shown that further antidepressants, including those of the selective serotonin reuptake inhibitor (SSRI) group, also mediate cancer cell apoptosis in both glioma and lymphoma, clomipramine appears to be most effective in this context. More recently, other groups have reported that clomipramine causes apoptosis, preceded by a rapid increase in p-c-Jun levels, cytochrome c release from mitochondria and increased caspase-3-like activity. In addition to clomipramine we have investigated the possible pro-apoptotic activity of a range of further tricyclic drugs. Only two such agents (amitriptyline and doxepin) showed a similar, or better, effect when compared with clomipramine. Since both orally administered clomipramine and amitriptyline are metabolised to desmethyl clomipramine (norclomipramine) and nortriptyline respectively it is necessary for testing at a tumour cell level to be carried out with both the parent tricyclic and the metabolic product. In addition, reversal of multidrug resistance in a number of solid cancers following treatment with both clomipramine and amitriptyline has been reported. This additional role for tricyclics may be of some significance in the treatment of primary and secondary brain tumours. Since a substantial number of patients with malignant glioma have already received and are receiving clomipramine, both anecdotally and within a clinical trial, we have carried out CYP (P450) gene expression studies and determined blood plasma levels of clomipramine and norclomipramine, in order to ascertain whether differences in individual patient metabolism influence clinical outcome. While the pro-apoptotic effect of norclomipramine appears to be inferior to that of the parent tricyclic, amitriptyline and nortriptyline share a similar propensity for eliciting apoptosis in neoplastic but not non-neoplastic astrocytes. The potential value of these agents as adjuvants in the management of patients with malignant glioma is apparent.

Key words: brain neoplasms – drug therapy; glioma; clomipramine; antidepressive agents, tricyclic; apoptosis

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Introduction

Tricyclic drugs and neoplastic cells

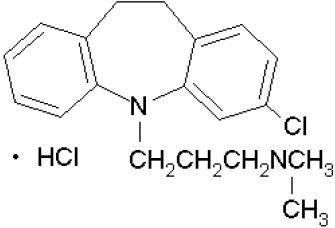
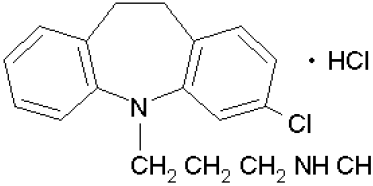
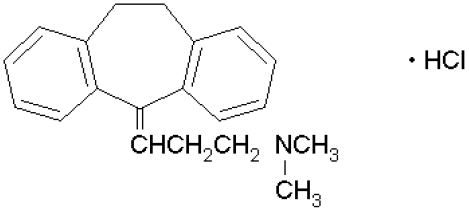
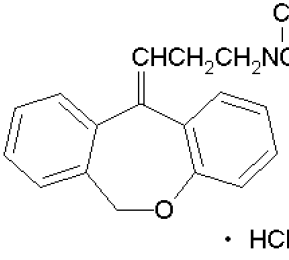
Tricyclic drugs, whose name is derived from their characteristic three ring nucleus (Table 1), were first thought to be useful as antihistamines with sedative properties and later as anti-psychotics. They include an important group of tricyclic antidepressants (TCAs) which have been in clinical use over 40 years. In the 1970's, it was found that TCAs showed selective inhibition of mitochondrial activity in yeast cells.¹ It was surmised that the wide range of actions shown by the TCAs *in vivo* was due to interactions with membranes and membrane bound enzymes, in particular the mitochondrial membrane¹, resulting in inhibition of cellular respiration and limitation of adenosine triphosphate (ATP) production. Further experiments showed that cancer cells were much more susceptible to the inhibitory effects of TCAs than non-transformed cells.² After treatment with TCAs, it was observed that the respiration rate of transformed cells was significantly less than their normal counterparts in oxygen electrode studies.² It was concluded that anti-mitochondrial drugs, such as TCAs, depress mitochondrial activity in cancer cells, thereby leading to cell death, whereas non-transformed cells were able to recover after treatment.² This mode of action of the TCAs was found to be a common feature amongst members of the group but there appears to be no clear relationship between chemical structure and pharmacological

action.³ However, the chlorine containing drugs are said to be more toxic than others to the functions of the mitochondrial membrane.⁴

Impairments of mitochondrial function may lead to ATP depletion and necrotic cell death.⁵ More recently, however, mitochondria have been implicated in both the regulation of apoptotic cell death and cancer formation.³ It has been reported that mitochondrial respiration is decreased in neoplastic tissue, along with a lowering of the cellular content of mitochondria. These findings indicate that tumour cells rely upon glycolysis as an energy source and this enables them to survive under hypoxic conditions.⁶ There are at least three established mechanisms through which mitochondria can trigger apoptosis although these events may be inter-related.⁷ Apoptosis may be triggered by disruption of electron transport, oxidative phosphorylation and ATP transport, release of proteins that trigger activation of caspases and alteration of cellular redox potential.⁷ A number of agents appear to target the mitochondria and promote the release of cytochrome c and other pro-apoptotic proteins, which can trigger caspase activation resulting in cell death.⁵ Caspases are cysteine proteases and exist in a latent state in 'healthy' cells.⁸ In response to damage or a malfunction of vital metabolic processes, cells generate signals that lead to activation of caspases, which result in apoptotic cell death.⁸ Figure 1 shows some of the signalling pathways involved in tricyclic-initiated, mitochondrially-mediated neoplastic cell apoptosis.

Defects in apoptosis signaling pathways are, however, common in cancer cells. Moreover, tumour development, progression and resistance to radiotherapy and chemotherapy are all the direct result of defects in the regulation of apoptosis in glioma⁹, due to raised apoptotic thresholds. Human mitochondrial DNA (mtDNA) consists of a small circular genome of 165kb that enco-

Table 1. Chemical structure of the tricyclic antidepressants used in laboratory studies

Name	Chemical	structure
Chemical structure of clomipramine hydrochloride		
Chemical structure of norclomipramine hydrochloride (N-desmethylclomipramine)		
Chemical structure of amitriptyline hydrochloride		
Chemical structure of doxepin hydrochloride		

des a complex array of proteins including 13 respiratory chain sub-units. Expression of the entire genome is required to maintain proper function of the mitochondria. The identification of the specific proteins responsible for the regulation of apoptosis may be expected to lead to the development of cancer therapies directed at altering the levels of expression of pro-apoptotic proteins and enhancing the effects of current radiotherapy and chemotherapy. The *bcl-2* proto-oncogene represses a number of cellular apoptotic pathways and is

known to be expressed in increasing amounts in glial tumours with increasing degree of malignancy.¹⁰ Transfection of glioma cells with antisense *bcl-2* has been reported to result in an increase in apoptotic cell death. This indicates that *bcl-2* plays a role in tumour progression of gliomas by acting as an oncogene and inhibition of the *bcl-2* gene could have a therapeutic effect.¹⁰

It has been determined that chemotherapeutic drug-induced apoptosis of human malignant glioma cells involves the death receptor-independent activation of caspa-

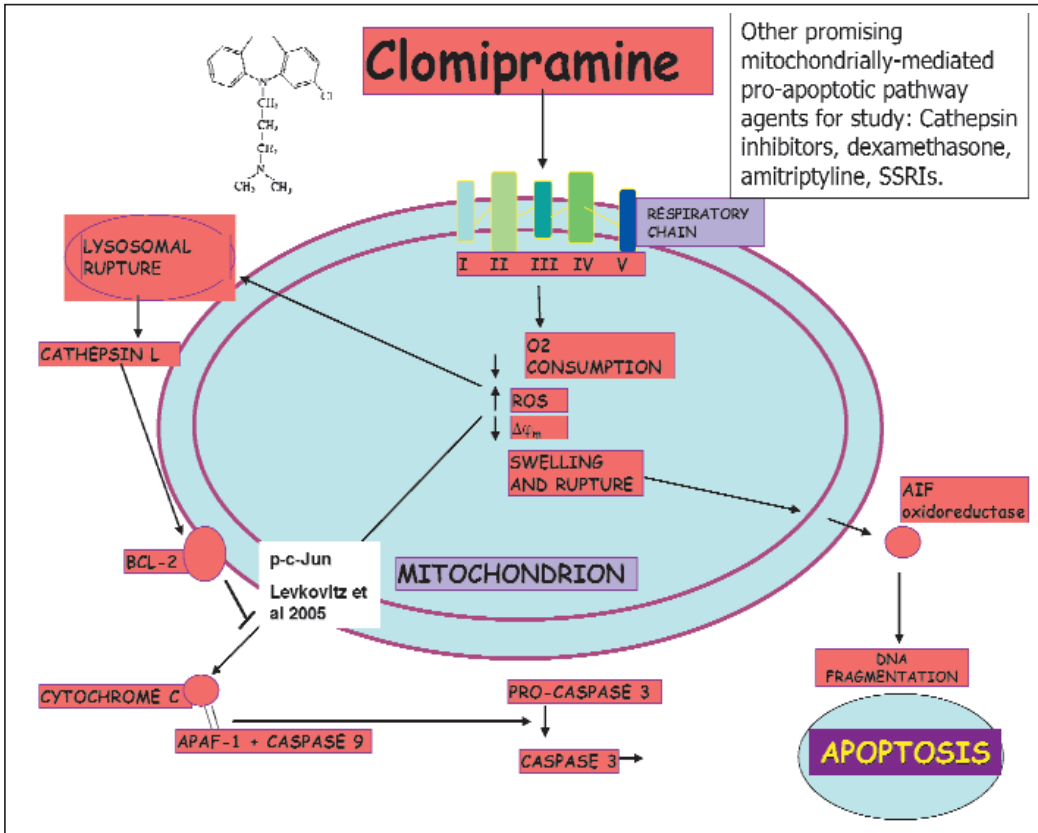


Figure 1. Flow pathway of clomipramine pro-apoptotic effect.

ses other than 3 and 8.¹¹ Caspases 1, 2, 3, 7, 8 and 9 are constitutively expressed in most human malignant glioma cell lines and drug-induced apoptosis involves delayed activation of caspases 2, 7 and 9 and is blocked by a broad spectrum caspase inhibitor.¹¹ It has also been established that the cytotoxic effects of many chemotherapeutic agents are mediated via apoptotic pathways; therefore developing drugs that target the mitochondria may provide a new strategy to induce apoptosis in tumour cells.¹²

It has been shown that the TCAs imipramine and clomipramine, and the selective serotonin reuptake inhibitor (SSRI) citalopram, induce apoptosis in cancer cells and that this process is associated with an ear-

ly increase in the production of reactive oxygen species (ROS) and subsequent loss of mitochondrial membrane potential.¹³ The literature suggests that TCAs can induce apoptosis in acute myeloid leukemic cells¹⁴ and lymphomas¹⁵ as well as gliomas.^{3,16,17} The mechanism of action of clomipramine involves the inhibition of complex III of the respiratory chain, resulting in elevated levels of ROS, cytochrome c release and caspase-activated apoptosis.¹⁶ Indeed the data presented in a study carried out by Daley *et al.* indicated that clomipramine might be useful in the treatment of patients with primary brain tumours.¹⁶ In fact it is estimated that there are now over 300 'anecdotal' cases of patients with a range of different primary brain tumours

who are taking, or have taken, clomipramine in the UK. With respect to these cases, there have been numerous reports of survival benefit and increased quality of life. Currently, there is a clinical study in progress in which patients newly diagnosed with either an anaplastic astrocytoma or glioblastoma multiforme receive an initial dose of 25mg clomipramine, escalating to 150mg in steps of 25mg at 3-day intervals.³

In addition, reversal of multidrug resistance in a number of solid cancers following treatment with both clomipramine^{18,19} and amitriptyline²⁰ has been reported. This additional role for tricyclics may, albeit at differing concentrations, provide an additional novel approach to the treatment of primary and secondary brain tumours.

In order to address some of the issues related to a possible further role of TCAs in the therapy of glioma we are carrying out the following studies:

Clinical

- Determination of blood plasma levels of clomipramine and its metabolite, norclomipramine, in patients with brain tumour taking the drug.
- Assessment of CYP (P450) gene expression in glioma patients taking clomipramine.
- Monitoring of outcome of »anecdotal« glioma patients treated with clomipramine through the Samantha Dickson Research Trust (www.sdrt.co.uk).
- Clinical trial at King's College Hospital, London in newly diagnosed patients with histologically verified anaplastic astrocytoma and glioblastoma multiforme.

Laboratory

- Assessment of viability in a dose response series of in vitro experiments in low passage, biopsy derived glioma cultures, high passage, established glioma cell lines and non-neoplastic human astrocytes to clomipramine.

- Assessment of oxygen utilisation of the above cells after treatment with clomipramine.
- Assessment of apoptosis of the above cells after treatment with clomipramine.
- Repeating the above studies with norclomipramine, amitriptyline, nortriptyline and various combinations of amitriptyline and clomipramine.
- Establishing the possible influence of different concentrations of dexamethasone on clomipramine-induced apoptosis.

Methods used in ongoing laboratory studies

Blood samples/clomipramine distribution

Blood plasma samples taken at regular intervals, from both anecdotal and trial patients taking clomipramine, are analysed using standard high-performance liquid chromatography (HPLC), to detect both clomipramine and its metabolite norclomipramine. A methodology is currently being developed for the measurement of dexamethasone via HPLC, and amitriptyline/nortriptyline can potentially be added to the range of tricyclics that we are able to offer testing for, should it be required. The data taken from the analysis of blood plasma will be used to track the metabolic progress of individual patients, and over a period of months could be used to 'tailor' the individual dose according to side effects. The preliminary studies that have been carried out are based upon the therapeutic window for patients using clomipramine as an antidepressant, however when enough data is gathered it will be possible to determine the target range for use in malignant glioma. In combination with the plasma testing of patients, it will be possible for a series of basic liver function tests (aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gammaglutamyltransferase (GGT)) to be performed 'in-house'.

Blood samples taken from patients included in the above studies are also analysed for the presence/absence of markers related to the metabolism of clomipramine. DNA extracted from Whatman FTA cards is analysed by PCR using primers for the CYP genes 2D6 and 2C19. By determining the gene expression of the individual patient it will be possible to classify them as 'good' or 'poor' metabolisers of clomipramine and this information will be of use when clinical decisions are taken concerning the optimal daily dose.

Treatment of cells

Neoplastic and non-neoplastic glial cells are used for treatment with the following agents: amitriptyline, nortriptyline, clomipramine, norclomipramine, dexamethasone & sodium valproate (valproic acid). These experiments will show if there is any synergy, additive effect or antagonism between agents in combination.

Cell viability

Cell viability is used, in conjunction with clonogenicity assays to determine the efficacy of the drugs in vitro. Studies are also performed using normal human astrocytes (Cambrex Biosciences) to demonstrate that the tricyclic drugs affected only neoplastic cells in the brain. The MTT, Neutral Red and Alamar Blue cytotoxicity assays are used to initially determine the IC₅₀ for each of the agents, and then subsequent studies are performed using pertinent concentrations of the tricyclics. Using a Beckman Coulter Vi-Cell XR trypan blue analyzer cells exposed to test agents for varying lengths of time can be analysed to determine percentage cell death, via uptake of trypan blue. The instrument also provides information about viability of different sub-populations based upon cell size after drug

exposure and is used to prepare growth curves and population doubling times via its »Bioprocess« software programme.

Oxygen electrode assay

Oxygen electrode studies using Hansatech multiple Oxytherm/Oxygraph O₂ electrode apparatus are performed to establish any decrease in oxygen uptake on tumour cell exposure to the test agents. Reduction in oxygen utilisation gives an indication of the affects of test agents on mitochondrial function and is a useful indicator of events culminating in mitochondrially-mediated apoptotic cell death.

Apoptosis assays

Annexin V/Propidium iodide flow cytometry: is used to determine the mechanism of cell death subsequent to exposure to the test agent by way of a BD FACScalibur flow cytometer. The annexin V fluorochrome binds to the 'flipped' phosphatidyl serine residues of the inner leaflet of the cell membrane, after the apoptotic signalling cascade has been activated. The assay can differentiate between early and late apoptotic cells as well as necrotic cells. The protocol had to be optimised when using it to study the tricyclics in combination with dexamethasone as the propidium iodide, which is taken up by the 'leaky' cell membrane of necrotic cells, is also taken up due to the effect that dexamethasone exerts on the pores of the cell membrane via the glucocorticoid receptors.

Live cell imaging: with monolayers of tumour cells is carried out over periods of up to 72 hours of drug exposure using a Zeiss Axiovert 200M incorporating a temperature/humidity/CO₂ controlled chamber together with Improvision Openlab, Velocity and Acquisition software. Cell proliferation and apoptotic events are recorded as time-lapse DVDs.

Change in Oxygen Utilisation of Cell Line IPSB-18 Following Addition of Various Concentrations of Amitriptyline

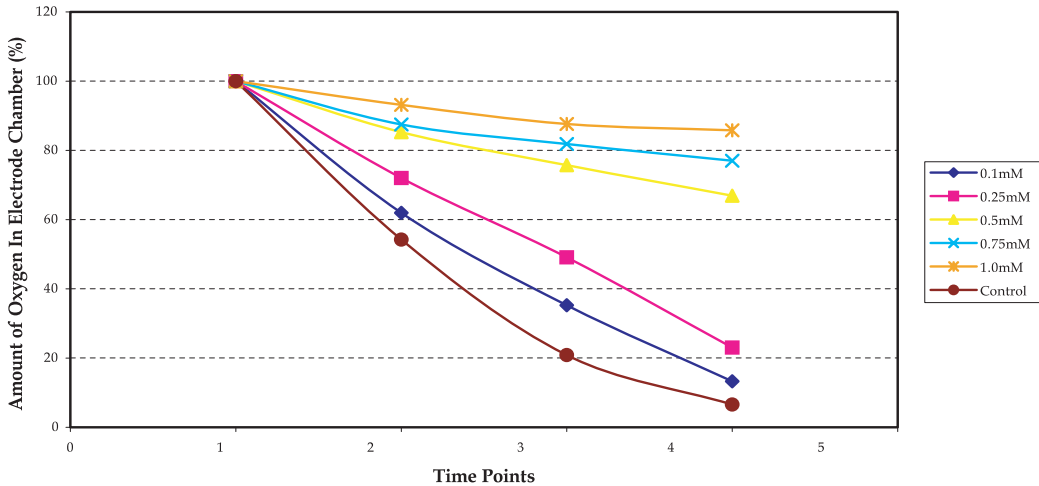


Figure 2. Graph representing the oxygen consumption of an anaplastic astrocytoma after treatment with different concentrations of Clomipramine.

Caspase 3 Activity: Caspase 3 activity is measured by its ability to cleave Ac-DEVD-AMC, whereby it produces a fluorescent AMC subunit. Cytosol extracted from cells exposed to the test agents, are read on a Mithras LB950 plate reader (Berthold Technologies) and compared to controls in which a pan-caspase inhibitor is added.

Results

Additional tricyclics and neoplastic cell apoptosis

In addition to clomipramine we have, in pilot experiments, investigated the possible pro-apoptotic activity of a range of further tricyclic drugs. Only two such agents (amitriptyline and doxepin) showed a similar (doxepin), or better (amitriptyline), effect when compared with Clomipramine. Amitriptyline has previously been reported to reduce proliferation in cancer cell lines.²¹ Preliminary studies carried out using amitriptyline and nortriptyline show that it exerts a cytotoxic effect on the established

anaplastic astrocytoma line (IPSB-18 p39) and the glioblastoma-derived culture (CLOM 15 p23). We have also found that Amitriptyline induces a dose-dependent reduction in oxygen utilisation in human glioma cells (Figure 2) as well as apoptosis as seen in Annexin V/PI flow cytometry. Moreover, when early passage cultures of human glioma were treated sequentially with clomipramine & amitriptyline apoptosis was initiated. Only a small proportion of cells recovered from this treatment

The possible role of dexamethasone in modulation of tricyclic drug-mediated brain tumour cell apoptosis

In the UK the great majority of patients suffering from malignant glioma receive the glucocorticoid steroid, dexamethasone, to reduce raised intracranial pressure.²² This steroid has been reported to be both anti- and pro-apoptotic, in its own right, according to concentration, in various cancer cells.²³ In addition, it has been shown to protect established glioblastoma-derived cultu-

res from temozolomide-induced apoptosis by influence on caspase-3 activity and Bax: Bcl-2 ratio.^{24,25} In our laboratories when studying concomitant dexamethasone/clomipramine treatment of glioma cells and dexamethasone pre-treatment prior to clomipramine treatment, we were able to demonstrate both inhibition and potentiation of clomipramine-mediated apoptosis.²⁶ These studies, however, merit greater investigation using different combinations and doses primary and early passage glioma-derived cultures as well as established cell lines.

Clinical studies with TCAs in brain tumour

Since a substantial number of patients with malignant glioma have already received and are receiving clomipramine, both anecdotally and within a clinical trial at King's College Hospital, London we are carrying out two experiments. One to determine the CYP (P450) genetic profile of individuals and the other to determine blood plasma levels of clomipramine and norclomipramine, in order to determine whether differences in individual patient metabolism influences clinical outcome. CYP (450) are hydroxylases situated on the P450 loci and are responsible for the breakdown of antidepressant in particular CYP2C19 and CYP2D6, which are highly polymorphic.

The first of these was to determine the CYP (P450) (27, 28) genotypic profile of individuals, in particular the CYP2D6 and CYP2C19 and the other was to test blood plasma levels of clomipramine and norclomipramine in order to determine whether differences in individual patient metabolism, measured by HPLC analysis, influences clinical outcome. We now wish, in collaboration with our clinical colleagues, to extend these studies in order to obtain statistically meaningful data with which to inform clinical practice.

Discussion and future studies

The pro-apoptotic role of clomipramine in neoplastic cells

Clomipramine acts by triggering mitochondrially-mediated apoptosis by way of complex 3 of the respiratory chain. Here, through reduced reactive oxygen species and neoplastic cell specific, altered membrane potential, cytochrome c is released thereby activating a caspase pathway to apoptosis (Figure 1).¹⁶ Indeed, Xia *et al.*^{13,14} previously reported that clomipramine induced increases in reactive oxygen species, lead to mitochondrial membrane potential alterations and increased caspase-3 activity in human acute leukaemia HL-60 cells which preceded apoptosis. Similarly, the tricyclic analog, desipramine, has also been shown to induce mitochondrially-mediated apoptosis in C6 glioma cells via increased caspase-3 gene expression and intracellular calcium homeostasis changes.²⁹ In addition, while we and others have shown that further antidepressants, including those of the selective serotonin reuptake inhibitor (SSRI) group, also mediate cancer cell apoptosis in both glioma and lymphoma, clomipramine appears to be most effective in this context.¹⁵ Very recently, Levkovitz *et al.*³⁰ independently reported that clomipramine, in a comparative study between SSRIs and clomipramine in C6 rat glioma and human neuroblastoma cells, caused apoptosis preceded by a rapid increase in p-c-Jun levels, cytochrome c release from mitochondria and caspase-3-like activity. Significantly lower sensitivity to the drug's pro-apoptotic activity was demonstrated in primary mouse brain and neuronal cultures. The authors therefore concluded – as we had previously – that the high sensitivity of cancer cells to the drug suggested that clomipramine may have potential in the treatment of brain tumours. We have also demonstrated the role

of cathepsin L in interfering with activity of pro-apoptotic agents such as clomipramine by use of cathepsin inhibitors and anti-sense technology.³¹

Amitriptyline has previously been reported to reduce proliferation in cancer cell lines²¹ and to decrease glioma cell viability.³² In our preliminary experiments we have found that amitriptyline induces a dose-dependent reduction in oxygen utilisation in human glioma cells as well as apoptosis as seen in Annexin V/PI flow cytometry. Moreover, when early passage cultures of human glioma were treated sequentially with clomipramine & amitriptyline apoptosis was initiated. Only a small proportion of cells recovered from this treatment. In addition, reversal of multi drug resistance in a number of solid cancers following treatment with both clomipramine^{19,33} and amitriptyline²⁰ has been reported. This additional role for tricyclics may, albeit at differing concentrations, be of some significance in the treatment of primary and secondary brain tumours.

Cancer stem cells

CD133 is a 120kDa five-transmembrane cell surface protein, originally described as a haematopoietic stem cell marker.^{34,35} More recently, however, it was shown to mark normal human neural stem cells.³⁶ Subsequently, Singh *et al.*³⁷ demonstrated CD133 positivity, by both immunohistochemistry and flow cytometry, on two common forms of paediatric brain tumour; the high grade malignancy medulloblastoma and the low grade pilocytic astrocytoma. Moreover, brain tumour stem cells can be magnetic immuno-bead and fluorescence activated cell sorted by use of dissociated cell suspensions using CD133 antibodies. The subsequent CD133 positive selected sub-population of tumour cells also express nestin but fail to express markers associated with dif-

ferentiated cells of neural lineage.³⁸ Although these CD133/nestin positive stem cells represent a minority fraction of the overall tumour cell complement, they are able to generate clonal tumour neurospheres in suspension culture. They also show increased self-renewal capacity and can be induced to differentiate into cells phenotypically similar to those seen in the original patient histology. The same group then developed an *in vivo*, serially-transplantable, xenograft model in NOD-SCID (non-obese diabetic, severe combined immunodeficient) mouse brains by injecting as few as 100 CD133-positive brain tumour stem cells. The histological appearance of the resulting tumours resembled that of the original resected tumour. Conversely, injection of as many as 10⁵ CD133-negative cells failed to produce tumours.³⁹ We have noted that while human glioma biopsies normally grow well in standard DMEM growth conditions, cells from four clomipramine treated patients cells taken at second biopsy grow poorly in DMEM culture media. We hypothesise that cancer stem cells, as denoted by CD133 (plus CD44/CD24/nestin/Musashi-1) expression may increase in number & are resistant to clomipramine.⁴⁰ We, therefore, propose to culture these second (recurrent case) biopsies, as well as new cases of glioma in stem cell defined medium to see if yield of CD133 +ve cells has increased. Primary/*ex-vivo* cultures will be prepared from human glioma obtained from King's College Hospital (KCH) London (LREC 00-173) and Hurstwood Park Neurological Centre, Haywards Heath, Sussex (LREC applied for). Epilepsy surgical brain resection tissue from KCH will be used to provide additional non-neoplastic astrocyte cultures (LREC 02-056). Biopsied glioblastoma primary cultures taken from both newly diagnosed patients and those previously treated with clomipramine and isolated *in vitro*⁴¹ in stem cell defined fee-

der cell conditions.⁴² The monoclonal AC133 antibody (Miltenyi Biotech) will be used to identify CD133 positive stem cells and early progenitor cells. Immunocytochemistry, using fluorescence/TIRF microscopy, and flow cytometry will be used to identify and quantify CD133 antigen expression. CD133 positive cells will then be separated either by MACS/CD133 immunobeads (Miltenyi Biotech) or by sterile FACS and grown in bulk culture for subsequent testing with various drug combinations. Although we expect a low yield of CD133-positive cells we feel this would be a timely study.

Valproic acid

Many brain tumour patients also suffer from seizures and are, consequently, prescribed anti-convulsants. One particular anti-convulsant, the histone deacetylase inhibitor, sodium valproate (valproic acid), has recently attracted attention for its potential anti-cancer properties. Histone deacetylation is critical for regulation of gene expression which may affect chromatin structure and chromatin interaction with regulatory factors. In this context valproic acid has been shown to rapidly hyperacetylate histones H3 and H4 in breast cancer cells and depleted the structural maintenance of chromatin proteins, DNA methyltransferase and heterochromatin proteins with a consequent enhanced sensitivity of DNA to DNA-damaging agents, both *in vitro* and in xenograft models.⁴³ In addition, valproic acid has been reported to enhance radiosensitivity of human brain tumour cell lines and xenografts.⁴⁴ Combination therapy of histone deacetylase inhibitors and radiotherapy has also resulted in increased neuroblastoma cell necrosis and apoptosis compared with either single modality treatment. Interestingly, Beecken *et al.*⁴⁵ have shown that it also positively modulates ne-

ural cell adhesion molecule (NCAM) polysialylation, thereby blocking adhesion of several neuroectodermal tumour-derived cell lines to HUVEC (human umbilical vein endothelial cells) while downregulation of CD44 expression has been reported on human and rat glioma cells *in vitro*.⁴⁶ These findings may be suggestive of reduced invasion but increased tumour cell differentiation and apoptosis have also been reported in human brain tumour xenograft models.⁴⁶ Indeed, enhanced differentiated gene expression, growth inhibition, cell cycle arrest, induction of apoptosis and downregulation of the pro-survival genes bcl-2 and bcl-xl has also been reported in thyroid cancer cells.^{47,48} We are, therefore, eager to explore the potential of valproic acid in combination with tricyclics.

Current literature available on dexamethasone and its actions on glioma cells is conflicting. It has been reported that glucocorticoids have a functional role at the level of the mitochondria.⁴⁹ It has also been shown that glucocorticoids are neurotoxic and appear to play a role in neuronal cell loss following neuropathological insults.⁵⁰ Dexamethasone has been shown to enhance necrotic cell death of glioma cells induced by serum deprivation.⁵⁰ The steroid also reversibly and significantly inhibits growth of C6 glioma cells both at early and late passage.⁵¹ Despite evidence suggesting dexamethasone exerts a necrotic type of cell death, some studies have indicated that its mechanism of action is via apoptosis and interference with apoptotic pathways. In leukaemia cells dexamethasone-induced apoptosis has been demonstrated through the mitochondria-dependent pathway.⁵² Glucocorticoids are known to influence the ability of cells to undergo apoptosis, directly inducing apoptosis in thymocytes while inhibiting it in hepatoma and carcinoma cells.²³ It has been suggested that dexamethasone inhibits the induction of

apoptosis in astrocytoma cells, probably via up-regulation of *Bcl-x_L*, which could prevent cytochrome c release from mitochondria and subsequent caspase activation.²³ Dexamethasone was also shown to confer protection against the induction of apoptosis by anti-cancer agents.²³ This indicates that dexamethasone could potentially interfere with the efficacy of chemotherapeutic agents. The laboratory and clinical studies described are aimed at identifying a possible role for tricyclics in combination with standard therapies for glioma patients. It is hoped that such a combinatorial, and possibly customised, approach may enhance both quality of life and survival time for patients suffering from malignant brain tumour.

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Pomen tricikličnih zdravil pri selektivnem proženju mitohondrijske apoptoze pri neoplastični gliji. Nova možnost zdravljenja malignih gliomov?

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Izhodišča. Naše raziskave so že pokazale, da ima triciklični antidepresiv klomipramin *in vitro* specifičen pro-apoptotičen učinek na humanih malignih gliomskih celicah. Učinek je odvisen od koncentracije klomipramina in ga ni opaziti na normalnih humanih astrocitih. Zdravilo sproža apoptozo tako, da deluje na mitohondrije, kjer učinkuje na dihalno verigo. Čeprav so ob naših tudi druge raziskave pokazale, da imajo različni antidepresivi (vključno s selektivnimi zaviralci ponovnega prevzema serotonina – SSRI) vpliv na apoptozo pri limfomih in gliomih, je klomipramin najbolj učinkovit. Ugotavljali smo tudi pro-apoptotično aktivnost drugih tricikličnih zdravil in odkrili, da imata le dve takšni zdravili (amitriptilin in doksepin) enako ali boljšo učinkovitost kot klomipramin. *Per os* zaužiti zdravili klomipramin in amitriptilin se metabolizirata v desmetil klomipramin (norklomipramin) in nortriptilin. Menimo, da bi bilo potrebno testirati tumorske celice na omenjeni zdravili in na njuna metabolita. Učinkovitost obeh zdravil je namreč lahko močno zmanjšana zaradi odpornosti na zdravila (multidrug resistance), ki pa je pri obeh zdravilih različna. Ugotovili smo tudi, da ima metabolit klomipramina norklomipramin slabši pro-apoptotični učinek, medtem ko ima metabolit amitriptina nortriptilin enak učinek kot amitriptin.

Zaključki. Menimo, da bo potrebno v kliničnih raziskavah ugotoviti učinkovitost tricikličnih antidepresivov pri malignem gliomu, najprej kot dopolnilno zdravljenje.