Chlorimipramine: A novel anticancer agent with a mitochondrial target

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Abstract

Mitochondria have been suggested to be a potential intracellular target for cancer chemotherapy. In this report, we demonstrate the ability of the tricyclic antidepressant chlorimipramine to kill human glioma cells in vitro by a molecular mechanism resulting in an increase in caspase 3 activity following inhibition of glioma oxygen consumption. Studies with isolated rat mitochondria showed that chlorimipramine specifically inhibited mitochondrial complex III activity, which causes decreased mitochondrial membrane potential as well as mitochondrial swelling and vacuolation. The use of chlorimipramine in human as an effective, non-toxic cancer therapeutic having a strong selectivity between cancer cells and normal cells on the basis of their mitochondrial function is discussed.

Keywords: Cancer; Mitochondria; Apoptosis; Glioma; Antidepressant; Chlorimipramine; Clomipramine

Chlorimipramine (CIMP) is a tricyclic antidepressant that has been in clinical use for over 30 years [1]. However, previous studies showed that CIMP can block cellular oxygen consumption in yeast and human fibroblasts [2–4]. Previous work has also been carried out investigating the effect of tricyclic drugs on various types of cancer cells. A number of studies show chlorimipramine to have positive effects against human leukaemia cells [5], human renal cancer cells [6], and solid murine tumours [7]. In addition, tricyclic antidepressants have been shown to cause apoptotic cell death in normal human lymphocytes [8] and in non-Hodkin’s lymphoma cells [9]. However, both the effects of CIMP on human glioma cells and its precise molecular mechanisms of action involved in causing cell death are unknown. Therefore, in this study, we have investigated the effects of CIMP on (A) human glioma cells isolated from brain tissue obtained from surgical biopsies and (B) purified mitochondria isolated from a variety of rat tissues.

Materials and methods

Chlorimipramine is now being assessed for its anti-cancer activity in the clinic.

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Glioma cell culture. Glioma cell lines used were derived from primary cultures of human biopsy material taken at craniotomy in the neurosurgical theatres of King’s College London and Atkinson Morley’s Hospitals. All biopsied glioma tissues used to derive the cell lines used in the studies described here were examined in parallel by experienced neuropathologists and classified according to standard neuropathological criteria [10–12] as: astrocytoma (grade II) cell line IPDDC-A2; anaplastic astrocytoma. (grade III) cell lines IPSB-18 and NP7S5-96; or glioblastoma multiforme (grade IV) cell lines IPTF-98 and IPBB-98. Scrambled biopsies obtained from consenting patients under local Ethics Committee permission (obtained at termination of pregnancy from consenting patients under local Ethics Committee permission) were mechanically disaggregated using sterile scalps to form crude suspensions, from which primary monolayer cultures were established. Cells were routinely propagated as monolayers in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% heat-inactivated foetal calf serum (FCS) and 100 IU penicillin, 100 μg streptomycin, and 0.25 μg amphotericin/ml. Tissue culture medium was maintained at physiological pH by equilibration with 5% CO2/95% air in a humidified incubator at 37 °C. Cell monolayers were washed in fresh warm Hank’s balanced salts solution (HBSS) and harvested by digestion with 0.25% trypsin EDTA solution (0.5 g trypsin, 0.2 g EDTA/L HBSS) for 5–10 min at 37 °C. The trypsin was subsequently inhibited by diluting with medium containing 10% foetal calf serum and the cells were pelleted by centrifugation at 200 g for 5 min in a bench-top centrifuge. Cell viability was measured using a haemocytometer, using the trypan blue dye exclusion method, and all preparations were greater than 95% viable when used.

Drug exposure. The cells and mitochondria used in the studies described here were exposed to a range of concentrations of CIMP. This is because it is difficult to accurately estimate the concentration of drugs such as CIMP that cells and intracellular organelles are exposed to in vivo. Chlorimipramine, for example, is highly lipophilic and the brain/plasma ratio of CIMP itself and its major metabolite, desmethylichlorimipramine 12.5 and 7.4, respectively, 2–3 h following the last dose of a 5 dose oral dosing regime of 20 mg/Kg [13].

CIMP was dissolved in 70% ethanol to give a stock solution of 57 mM. The appropriate quantity of stock solution was then added to the culture medium to achieve the required concentration. Drug exposure in the MTT assay was for 1 or 2 h at concentrations ranging from 0.9 to 228 μM. In oxygen electrode studies CIMP concentrations ranged between 0.14 and 1.4 mM. Mitochondrial studies used 20–25 μM, CIMP, as these are concentrations that known mitochondrial inhibitors such as the 1-methyl-4-phenylpyridinium ion (MPP+) have been shown to have significant effects [14].

MTT assay. The MTT assay as optimised for glioma cells by Nikkhah et al. [15,16] was used. Cells were harvested by trypsinisation and plated into 96-well microtitre plates at a density of 1 × 104 cells/well in 200 μl DMEM. Cells were left overnight to adhere, after which the medium was replaced with fresh medium containing a range of concentrations of the test compound and incubated for 1–2 h at 37 °C. Drug solutions were subsequently removed and the wells were washed in PBS to remove any phenol red, which could affect the spectrophotometric readings of the formazan. Each well was then incubated with 100 μl MTT solution (1 mg/ml) for 2 h whereupon it was removed and 200 μl dimethyl sulphoxide (DMSO) was added to each well to dissolve any formazan crystals that had formed. The absorbance of the DMSO/formazan solution was then measured at 570 nm on a Dynatech MR 700 microplate reader. Two rows of wells were left untreated with either drug or MTT prior to addition of DMSO; these served as controls for background absorbance.

Cellular oxygen consumption measurements. Oxygen consumption of the cell line IPTF-98 was measured polarographically at 37 °C (in a thermostatically controlled water jacket) with a Clark-type oxygen electrode, an amplifier, and a personal computer using the Oxygraph System Software (all from Hansatech Instruments, UK). Cells were harvested from culture and resuspended in 1 ml culture medium containing 10 mM glutamine as the substrate. 0.5 ml of the cell suspension was then added to the electrode chamber and the rate of oxygen consumption was measured. After 3 min, one 10 μl aliquot of various concentrations of CIMP (to give a final concentration of 140, 280, 560 or 1400 μM) was made to the samples in the oxygen electrode, using a Hamilton syringe. The cells were incubated with the drug for 20 min. The rate of oxygen consumption was measured at four different time points both before and after the addition of the drug. Control experiments were also performed with cells in the absence of drug. Oxygen consumption rates were calculated following the addition of 114 μM protein content. Concentrations of CIMP used for these experiments are higher than that for the MTT assay, as the oxygen electrode is a closed system and is unable to run for the length of time used in the viability studies due to oxygen levels decreasing to zero in the incubation chamber. Oxygen consumption was measured at four different time points throughout the whole oxygen electrode experiment. Two minutes after addition of the cells to the oxygen electrode incubation chamber (in the absence of drug). The drug was then added and oxygen consumption was measured at 5, 10, and 15 min after addition of the drug.

Caspase 3 activity measurements. Caspase 3 activity was measured by its ability to cleave Acetyl-Asp-Glu-Val-Asp-Aminomethylcoumarin (Ac-DEVD-AMC) [17]. Cleavage was monitored by measuring the fluorescence of the cleaved–AMC. Cells were exposed to either 114 μM CIMP or 1 μM staurosporine as a positive control for induction of apoptosis [18] for 0, 1, 2, 4, 8, 16, and 24 h at 37 °C before being harvested using a rubber policeman. Both medium and scraped cells were centrifuged at 200 g for 5 min and the cell pellet was resuspended in PBS. Cells were then lysed by freeze–thawing in liquid nitrogen three times prior to being centrifuged at 12,000 g for 30 min at 4 °C to produce a cytosolic fraction. The cell lysates were then added to a 96-well plate in duplicate containing a reaction mixture consisting of 100 mM Hepes, 100 mM NaCl, 10% sucrose, 0.1% Chaps, 1 mM EDTA, and 10 mM DTT, pH 7.4, in a total volume of 200 μl. Into one set of samples, (0.2 mM f.c.) z-VAD-fmk (pan-caspase inhibitor) (R&D Systems, Abingdon, Oxon, England) was added and incubated for 1 h at 37 °C. One hundred microlitres of reaction mixture was then transferred from both control and inhibited wells to cuvettes containing H2O2 giving a 1:20 dilution. Production of AMC was measured at room temperature in a fluorimeter (Perkin–Elmer LS50-B) using an excitation wavelength of 375 nm and emission wavelength of 450 nm. Background readings where no sample was added to the reaction mixture were also taken. Untreated controls were run in parallel.

Isolation of purified mitochondria. To isolate glioma cell mitochondria in sufficient numbers (in order to use the techniques described below) was beyond the resources available to us. Therefore in order to investigate the effects of CIMP on mitochondrial function, mitochondria were isolated from the heart, liver, kidney, and brain tissue of adult male Wistar rats using minor modifications of established methods incorporating homogenisation and differential centrifugation [14,19–21].

Mitochondrial membrane potential. Mitochondrial membrane potential was measured fluorometrically [22] in the presence of rhodamine 123 (0.2 μM f.c.). Rhodamine 123 is taken up by energised mitochondria and released when there is a decrease in membrane potential. The mitochondrial uncoupler carbonyl cyanide m-fluorophenylhydrazone (FCCP) was used as a positive control to dissipate the mitochondrial membrane potential resulting in maximum fluorescence increase and confirming the presence or absence of any membrane potential remaining following the addition of CIMP.

Measurement of mitochondrial complex activities. Mitochondria from heart, liver, kidney, and brain (0.5 mg/ml) were incubated in respiration buffer (100 mM KCl, 75 mM mannitol, 25 mM sucrose, 100 mM Hepes, 100 mM NaCl, 10% sucrose, 0.1% Chaps, 1 mM EDTA, and 10 mM DTT, pH 7.4, in a total volume of 200 μl). One hundred microlitres of reaction mixture was then transferred from both control and inhibited wells to cuvettes containing H2O2 giving a 1:20 dilution. Production of AMC was measured at room temperature in a fluorimeter (Perkin–Elmer LS50-B) using an excitation wavelength of 375 nm and emission wavelength of 450 nm. Background readings where no sample was added to the reaction mixture were also taken. Untreated controls were run in parallel.
10 mM Tris, 0.1 mM EDTA, and 10 mM phosphate-Tris (pH 7.4) at 37 °C in the presence or absence of 25 μM CIMP, for 5 min. Samples were frozen in liquid nitrogen for storage. Immediately before assay, samples were freeze–thawed three times in liquid nitrogen and vortex mixed to ensure mitochondrial lysis.

**Mitochondrial complex I assay.** Mitochondrial complex I [EC 1.6.5.3] activities were measured spectrophotometrically (using a Molecular Devices SpectraMax Plus) as the rotenone sensitive rate of NADH oxidation (at 340 nm and 30 °C) in a 96-well polystyrene plate [23]. The reaction mixture contained: 25 mM potassium phosphate, pH 7.2, 0.2 mM NADH, 10 mM MgCl2, 1 mM KCN, 2.5 μM fat-free BSA, and approximately 20 μg of mitochondrial protein in a final volume of 0.25 ml.

**Mitochondrial complex II–III assay.** Mitochondrial complex II–III (EC 1.3.5.1 + EC 1.10.2.2) activities were measured spectrophotometrically in a 96-well plate reader (a Molecular Devices SpectraMax Plus) as the rate of cytochrome c reduction (at 550 nm and 30 °C) using succinate as the substrate [23]. The reaction mixture contained: 25 mM potassium phosphate, pH 7.4, 0.3 mM potassium EDTA, 1 mM KCN, 100 μM cytochrome c, and approximately 20 μg of mitochondrial protein in a final volume of 0.25 ml.

**Mitochondrial complex II assay.** Mitochondrial complex II activity (EC 1.3.5.1) was measured spectrophotometrically by observing the 2-thionylfluoroacetone (TTF-A) sensitive rate of reduction of 6,6-di-chlorophenolindophenol (DCPIP) by ubiquinol at 600 nm and 30 °C. The reaction mixture contained: 100 mM potassium phosphate, pH 7.4, 10 mM EDTA, 5 mM MgCl2, 1 μM KCN, 100 μM cytochrome c, and approximately 20 μg of mitochondrial protein in a final volume of 0.25 ml.

**Mitochondrial complex IV assay.** Mitochondrial complex IV (EC 1.9.3.1) activities were measured spectrophotometrically (using a Molecular Devices SpectraMax Plus) as the rate of cytochrome c oxidation (at 550 nm and 30 °C) as the antimycin a sensitive rate of reduction of cytochrome c using ubiquinol as a substrate [23]. The assay buffer contained: 50 mM potassium phosphate, pH 7.4, 10 mM EDTA, 5 mM MgCl2, 2 mM KCN, 1 μM rotenone, and 1.25 mg/ml mitochondrial protein in a final volume of 0.25 ml. The reaction was initiated by adding 100 μM ubiquinol. The first order rate constant (k) was calculated from the difference between the natural logarithms of the absorbance at t = 0 and at three time points 1, 2, and 3 min after adding the mitochondrial sample. The mean of these calculated values was then taken to be (k) and the activity was expressed in k/min/mg protein.

**Protein assay.** The protein contents of the cell suspensions and mitochondrial samples used were determined using the Lowry D.C. Protein assay kit (Bio-Rad, Hemel Hempstead, Hertfordshire, UK) using bovine serum albumin as a concentration standard.

**Electron microscopy of mitochondria.** Isolated heart mitochondria (0.25 mg/ml) were incubated in a plastic 3 ml fluorimeter cuvette in respiration buffer containing 125 mM KCl, 2 mM KH2PO4, 1 mM MgCl2, 1 μM EGTA, 20 mM Tris (pH 7.2 at 37 °C), 5 mM glutamate, 5 mM malate, and 250 μg/ml BSA (fatty acid free). The experiments were performed in a stirred water-jacketed cuvette holder at 37 °C. In each experiment, mitochondria were incubated with CIMP (25 μM) or control (no CIMP) for 10 min then pipetted into Eppendorf tubes, and centrifuged at 13,000×g for 5 min. The supernatant was removed and the mitochondrial pellet obtained was then fixed overnight in 3% buffered glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) at 4 °C. Tissue samples were post-fixed in 2% osmium tetroxide, stained with uranyl acetate in 50% ethanol, dehydrated through an ethanol buffered glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2 at 37 °C), and embedded in araldite. Ultra-thin sections were stained with uranyl acetate and lead citrate, and examined using a Philips M301 electron microscope [22,26]. Statistical analysis was performed using Student’s t test. Significance was attributed when p < 0.05.

**Results**

**Chlorimipramine causes changes in glioma cell morphology**

The microscopical examination of glioma and glial cells showed a time-dependent change in glioma cell.
morphology (rounding up of the cells) after 1 h exposure to CIMP, with many cells detaching from the tissue culture plate following 2 h exposure to CIMP (Fig. 1). Foetal astrocytes exposed to CIMP showed no changes in morphology and did not become detached from the tissue culture plate.

**Chlorimipramine decreases glioma cell viability**

The MTT assay demonstrated a concentration-dependent decrease in IPBB-98, IPDDC-98 and NP785-96 cell viability as measured by formazan absorbance at 570 nm following 1 h exposure to CIMP (Fig. 2). This decrease in cell viability reached statistical significance at a concentration of 114 µM CIMP.

**Chlorimipramine inhibits glioma cell oxygen consumption**

Oxygen consumption of IPTP-98 cells treated with CIMP was inhibited both in a concentration-dependent and time-dependent manner (Fig. 3). By 5 min, 1.4 mM CIMP produced a significant (95%) decrease in O₂ consumption compared to controls. By 10 min, a concentration of 0.57 mM caused a significant decrease in oxygen consumption; 0.28 mM CIMP caused a significant decrease in O₂ consumption by 15 min.

**Chlorimipramine increases glioma cell caspase 3 activity**

In the IPBB-98 cell line staurosporine treatment induced significant caspase 3 activity after 2 h (Fig. 4), which rose to a peak after 4 h and then gradually...
decreased although it was still significantly increased after 24 h. Caspase 3 activity was higher in the CIMP treated cells compared to those treated with staurosporine. The CIMP-induced activity followed a similar time course, with maximum activity being between 4 and 8 h. As with the staurosporine treated cells, caspase 3 activity was significantly elevated after 2, 4, 8, 16, and 24 h of treatment compared to untreated controls.

**Chlorimipramine decreases mitochondrial membrane potential**

CIMP causes a concentration-dependent decrease in membrane potential in isolated rat heart mitochondria (Fig. 5), producing a 50% increase in fluorescence after three additions of 29 μM CIMP (final concentration of 87 μM). The addition of the uncoupler FCCP caused a large increase in fluorescence, demonstrating that although CIMP had decreased the membrane potential the mitochondria still have some membrane potential.

**Chlorimipramine specifically inhibits mitochondrial complex III activity**

Fig. 6 shows mitochondrial complex activities following incubation of isolated rat heart mitochondria with CIMP. CIMP caused a small, but statistically significant, decrease in heart and kidney complex I activity, but not in liver mitochondria, whereas rotenone completely inhibited complex I activity in mitochondria from heart, liver, and kidney. CIMP completely inhibited complex II/III activity, as did the complex III inhibitor, antimycin a. CIMP had no significant effect on complex II activity, unlike the classical complex II inhibitor, TTFA, which totally inhibited complex II activity in mitochondria from all tissue types used. CIMP caused a large statistically significant decrease in complex III activity in heart, kidney, liver, and brain mitochondria, but not to the same extent as the complex III inhibitor, antimycin a, which totally inhibited complex III activity in mitochondria from all tissue types. CIMP had no significant effect on complex IV activity unlike the complex IV inhibitor, KCN, which totally inhibited complex IV activity in mitochondria from all tissue types.

**Chlorimipramine alters mitochondrial ultrastructure**

Fig. 7A shows an electron micrograph (10,000× magnification) of control (untreated) heart mitochondria. These untreated mitochondria are relatively homogenous with intact cristae and double membrane integrity. Fig. 7B shows an electron micrograph
Fig. 5. (A) Effects of CIMP and rotenone on complex I activity in isolated mitochondria from heart, liver, and kidney of rat. Data are expressed as means ± SEM of three separate experiments. Significance between control and CIMP ($p < 0.05$) is denoted by *. (B) Effects of CIMP and TTFA on complex II activity in isolated mitochondria from heart, liver, and kidney of rat. Data are expressed as means ± SEM of three separate experiments. Significance ($p < 0.05$) is denoted by *. (C) Effects of CIMP and antimycin a on complex II/III activity in isolated mitochondria from heart, liver, and kidney of rat. Data are expressed as means ± SEM of three separate experiments. Significance ($p < 0.05$) is denoted by *. (D) Effects of CIMP and antimycin a on complex III activity in isolated mitochondria from heart, liver, kidney, and brain of rat. Data are expressed as means ± SEM of three separate experiments. Significance ($p < 0.05$) is denoted by *. (E) Effects of CIMP and KCN on complex IV activity in isolated mitochondria from heart, liver, and kidney of rat. Data are expressed as means ± SEM of three separate experiments.
Fig. 6. Measurement of mitochondrial membrane potential using rhodamine 123. On addition of the mitochondria to the fluorimeter cuvette, fluorescence intensity decreases as rhodamine 123 partitions into the mitochondrial inner membrane. On the first addition of CIMP (f.c. 29 μM), the Rhodamine 123 fluorescence increases slightly, showing a decrease in mitochondrial membrane potential. The fluorescence intensity is increased even further on the second addition of CIMP (f.c. 58 μM) and then stabilises. The third, and final, addition of CIMP (f.c. 87 μM) gave a rapid increase in fluorescence intensity, suggesting a rapid decrease in mitochondrial membrane potential, which then levels off over time. Addition of the uncoupler FCCP completely depolarises the mitochondria resulting in a large increase in fluorescence.

Fig. 7. (A) Electron micrograph (10,000× magnification) showing control heart mitochondria. The morphology of these untreated mitochondria appears as expected, with intact mitochondrial cristae and double membrane integrity. (B) Electron micrograph (10,000× magnification) showing isolated heart mitochondria that have been incubated with chlorimipramine (25 μM) for 10 min. A large percentage of mitochondria show swelling and membrane rupture (highlighted by white rectangles). Some mitochondria also have vacuoles and damaged cristae (highlighted by red rectangles). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)
(10,000× magnification) of isolated rat heart mitochondria that have been incubated with chlorimipramine (25 μM) for 10 min. Many mitochondria show swelling and membrane rupture (highlighted by white rectangles). Some mitochondria also have vacuoles and damaged cristae (highlighted by red rectangles).

Discussion

Approximately 95% of a cell’s oxygen utilisation is by the mitochondrial complex IV [27]. Thus, the decrease in oxygen uptake measured in glioma cells in the presence of CIMP suggests a blockade of mitochondrial electron transport. Using the specific mitochondrial enzyme assays on mitochondria isolated from brain, heart, liver, and kidney it is clear that CIMP inhibits both mitochondrial complex I and complex III activities. However, 1 μM antimycin a caused total inhibition of complex III activity, whereas 20 μM CIMP did not produce complete inhibition of complex III activity. This suggests that antimycin a is a more tightly binding inhibitor of complex III than CIMP, and is consistent with antimycin a having a very high affinity for its binding site in complex III [28]. Another explanation is that CIMP either binds to a different site in complex III or competes with the exogenous ubiquinol used in the complex III assay. Interestingly in the complex II/III assay 20 μM CIMP had a similar inhibitory effect to 1 μM antimycin a. This could possibly be explained by the addition of exogenous ubiquinol in the complex III assay, which could compete with CIMP for the binding site on complex III. In the complex II/III assay only the endogenous levels of ubiquinone/ubiquinol exist which would not be high enough to compete with the 20 μM CIMP present. The fact that CIMP inhibits complex III activity is likely to result in an electrochemically reduced respiratory chain, and a significant increase in production of superoxide, as complex III is the major site of superoxide production within mitochondria [29]. This increase in reactive oxygen species production seen following blockade of electron transport through complex III is likely to lead to secondary damage to mitochondrial complex I [30], which is reflected in the small but significant decrease in complex I activity seen in our experiments.

Loss of cell viability as measured by MTT staining, will, depending on the concentration of CIMP that the mitochondria are exposed to could push the cell into apoptosis, which has generally been thought to be an energy-dependent process, requiring the de novo synthesis of enzymes such as caspases [31].

However, in gliomas there appear to be caspases already present at significant levels [32], and therefore the ATP requirement for apoptotic cell death in these cells may be lower than for normal (non-malignant) cells. If higher concentrations of CIMP are delivered to the complex III protein in glioma mitochondria, then a necrotic form of cell death could be induced which could be either caspase-dependent or caspase-independent [33].

It is likely that in whatever cancer cell type CIMP is used, if sufficient drug molecules come into contact with the cancer cell’s mitochondria, then this will result in binding to complex III, increased superoxide and hence hydrogen peroxide production [14], and decreased membrane potential, either with or without mitochondrial permeability transition pore formation [22]. In addition, there is likely to be an associated release of pro-apoptotic molecules such as cytochrome c, pro-caspase 9, caspase 3, and endonuclease G resulting in protein and DNA degradation, and cell death of apoptotic and/or necrotic phenotype [34].

ATP levels appear to play a central role in the process whereby a cell is “directed” into either apoptotic or necrotic pathways of cell death. Chemical energy is required for many of the pathways leading to apoptosis, and if ATP levels fall too low necrosis may ensue [35]. In glioma cells, as in many other types of cancer cells, glycolytic respiration is the dominant mechanism of ATP production. This would suggest that if cancer cells undergo a toxic insult that disrupts mitochondrial function thus depleting ATP, the amount of ATP produced by the glycolytic pathway may be sufficient for apoptosis to proceed.

Mitochondrial dysfunction in cancer cells has been widely reported since the initial observation of increased glycolytic rates and low respiratory rates of neoplastic cells by Warburg [36]. Since that time, both morphological and biochemical studies have shown numerous aberrations within mitochondria of neoplastic tissue. This is also the case in brain tumours, where dysfunctional mitochondria with depressed respiratory capacity in both malignant and benign brain tumours [37]; deformed mitochondria showing signs of degeneration [38]; and decreases in metabolic enzyme activities [39], have all been reported previously. With the recent rebirth of interest in mitochondria, attention has been drawn to the mitochondrial genome. For some time mitochondrial DNA has been known to show a number of abnormalities within neoplastic cells [40,41]. Such abnormalities are also present in brain tumours [42]. Respiratory complexes I, III, IV, and V all contain subunits encoded for by mitochondrial DNA [43]. Therefore, mutations in mitochondrial DNA are likely to result in impaired mitochondrial complex I, III, IV, and/or V activity, and impaired respiratory control within tumours.

These alterations in the mitochondrial genome in brain tumour tissue are also reflected in protein levels and enzymatic activities. For example, Lowry et al. [39] found activities of mitochondrial enzymes to be generally lower in tumour tissue compared to normal brain
tissue. Similar results were obtained by Meixensberger et al. [44] who showed that the activities of respiratory chain complexes II, III, and IV in astrocytomas were lower than in normal cortical tissue. This implies that at lower concentrations of CIMP, only mitochondria of tumour cells are likely to be affected, with normal brain cells being able to resist partial inhibition of their complex III activity, as this would produce no functional change in mitochondrial membrane potential, due to their having greater amounts of fully functional complex III protein, and therefore much higher activities of complex III. This is consistent with metabolic control theory, which would suggest a higher threshold for inhibition in normal tissue (as opposed to cancer tissue) before integrated mitochondrial functions such as the maintenance of membrane potential and mitochondrial superoxide production are significantly affected [30].

In conclusion, in this study we have demonstrated that CIMP can cause cell death in human glioma cells in a caspase 3-dependent manner without affecting primary human glia. In addition, we have shown that the mechanisms of CIMP-induced glioma cell death appear to be mitochondrionally mediated, by CIMP binding specifically to complex III of the respiratory chain. The mitochondrial genome has no introns and no none-coding sequences, and is known to have mutations and deletions in several cancer types. This may provide a functional basis at the level of mitochondrial protein expression for anti-mitochondrial therapies in cancer. Therefore, the development of novel compounds that induce cell death in cancer cells via a mitochondrial mechanism [45] (be it apoptotic or necrotic, caspase dependent, caspase independent, cathepsin dependent [46], or dependent on other as yet unknown molecular mechanisms) may be a highly useful clinical tool in cancer chemotherapy.

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References


