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Radical production by hydrogen peroxide/bicarbonate and copper uptake in mammalian cells: Modulation by Cu(II) complexes

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ABSTRACT

The presence of the bicarbonate/carbon dioxide pair is known to accelerate the transition metal ion-catalysed oxidation of various biotargets. It has been shown that stable Cu(II) complexes formed with imine ligands that allow redox cycling between Cu(I) and Cu(II) display diverse apoptotic effects on cell cultures. It is also reported that Cu(II)–tetraglycine can form a stable Cu(III) complex. In the present study, radical generation from H_2O_2 and H_2O_2/HCO_3^- in the presence of these two different classes of Cu(II) complexes was evaluated by monitoring the oxidation of dihydrorhodamine 123 and NADH and by the quantitative determination of thiobarbituric acid reactive substances (TBARs method). Cu(II)–imine complexes produced low levels of reactive species whereas Cu(II)–Gly-derived complexes, as well as the free Cu(II) ion, produced oxygen-derived radicals in significantly larger amounts. The effects of these two classes of complexes on mammalian tumour cell viability were equally distinct, in that Cu(II)–imine complexes caused apoptosis, entered in cell and remained almost unaffected in high levels whilst, at the same concentrations, Cu(II)–Gly peptide complexes and Cu(II) sulphate stimulated cell proliferation, with the cell managing copper efficiently. Taken together, these results highlight the different biological effects of Cu(II) complexes, some of which have been recently studied as anti-tumour drugs and radical system generators, and also update the effects of reactive oxygen species generation on cell cycle control.

1. Introduction

Copper is present as an essential trace element within all respiring tissues [1–3]. Under certain pathological conditions, however, copper homeostasis may become unbalanced allowing the build-up of toxic levels of the metal. The toxicity of copper has been attributed, in part, to its ability to catalyse oxidative tissue damage through oxidation/reduction reactions involving Cu(I) and Cu(II) cycling. In the presence of partially reduced oxygen species, for example hydrogen peroxide and the superoxide anion ($O_2^{\bullet -}$), redox cycling can result in the formation of the highly reactive and damaging hydroxyl radical ($^{\bullet}OH$) *via* the copper(II)/(I) cycle generating superoxide and hydroxyl radical (Eqs. (1)–(3)) [4–6].

$$Cu(II) + H_2O_2 \rightarrow Cu(I) + O_2^{\bullet -} + 2H+ \quad (1)$$

$$2O_2^{\bullet -} + 2H+ \rightarrow H_2O_2 + O_2 \quad (2)$$

$$Cu(I) + H_2O_2 \rightarrow Cu(II) + {}^{\bullet}OH + {}^{-}OH \quad (3)$$

The second order rate constant (k_2) for Fenton reaction (Eq. (3)) with Cu(I) is $4.7 \times 10^3\ M^{-1} s^{-1}$, using copper(I)–acqua as ligand [7]. In the absence of reduction agents and in the presence of Cu(II) complexes and hydrogen peroxide, competitive reactions as superoxide dismutation ($k_2 \sim 10^9\ M^{-1} s^{-1}$) [7] can also occur depending on hydrogen peroxide concentrations. For Cu,Zn–superoxide dismutase (SOD), the rate of reduction by H_2O_2 (reaction 1) was measured in $k_2 = 50\ M^{-1} s^{-1}$ [5,6].

It is well-known that the bicarbonate/carbon dioxide pair, the presence of which is important in maintaining physiological pH in extracellular body fluids, can accelerate the transition metal ion-catalysed oxidation of various biotargets. Despite of its relevance, however, most of the mechanisms that have been proposed to account for this important effect remain controversial [8–21]. On the other hand, it is accepted that the bicarbonate/carbon dioxide pair can increase peroxynitrite-mediated one-electron oxidation and nitration via formation of the carbonate radical and nitrogen dioxide [22,23]. In this context, the unequivocal demonstration by EPR that the reaction between peroxynitrite and carbon dioxide produces $CO_3^{\bullet -}$ [24] is strong evidence for the involvement of this radical in bicarbonate/carbon dioxide pair-stimulated peroxidations. Although less oxidizing than the $^{\bullet}OH$ ($E^o = 2.3$ V, pH 7.0) [5–7], the carbonate radical is a strong one-electron oxidant ($E^o = 1.8$ V, pH 7.0) [5–7] which, in contrast to the former, does not add to biomolecules. Since the carbonate radical is more specific than the hydroxyl radical, it may increase oxidation/nitration of particular biotargets [11,22,25].

In addition to the above, several lines of evidence support the hypothesis that the carbonate radical is the major diffusible oxidant

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resulting from the peroxidase activity of copper/zinc-superoxide dismutase [26,27]. However, although this enzyme has received considerable research attention in recent years by virtue of its potential relationship with familial amyotrophic lateral sclerosis, it is still unclear whether the immediate precursor of the carbonate radical is bicarbonate [19,26], carbon dioxide [14,30] or peroxymonocarbonate (HCO_4^-) [27–29]. Strong evidence for the involvement of peroxymonocarbonate in the formation of $CO_3^{\bullet-}$ derives from kinetic studies of bovine serum albumin (BSA-cysSH) and glutathione (GSH) peroxidation in the presence of bicarbonate [25], and the demonstration that the formation and reduction of peroxymonocarbonate is facilitated by the many metal centres of xanthine oxidase [31].

Copper-catalysed, hydrogen peroxide/bicarbonate-induced oxidative damage to proteins is also believed to be associated with the production of the carbonate radical [11]. Although initial studies employed Cu(II) chloride as a model of the copper complex, other investigations have revealed that the ligand environment around the Cu(II) ion is extremely important in determining the oxidative damage to biomolecules caused by the endogenous metal complexed with aqua-ligand, organic ligands or protein [32–34].

Various studies have demonstrated the ability of the free Cu(II) ion and Cu(II) complexes to induce apoptosis in mammalian tumour cells, whilst some of these complexes have been indicated for use in cancer therapy [35–38]. Although it has been proposed that the ability of such complexes to induce apoptosis in tumour cells *in vitro* derives from their facility to generate free radicals, the relationship between apoptotic activity and the reactive species produced is not clear [35–39].

The aim of the present study was to determine the effects of imine ligands and low molecular weight Gly-derived ligands on the capacity of the respective Cu(II) complexes to catalyse the generation of reactive oxygen species (ROS) by hydrogen peroxide in the presence of the bicarbonate/carbon dioxide pair. Additionally, the two classes of complexes were compared with respect to their effects on the copper uptake and growth of human neuroblastoma cells.

2. Experimental methods

2.1. Chemicals

Reagents of analytical grade or better were purchased from Sigma, Aldrich, Merck or Fisher Scientific. Solutions were prepared with distilled water that had been purified using a Millipore Milli-Q system, and buffers were pre-treated with Chellex-100 to remove contaminating metal ions. The concentration of hydrogen peroxide was determined spectrophotometrically ($\varepsilon_{240\ nm} = 43.6\ M^{-1}\ cm^{-1}$) [40].

Condensation of the amine ligands 1,3-diaminepropane (*pn*), ethylenediamine (*en*), 2-aminoethyl pyridine (*epy*) or 8-aminoquinoline (*amiquin*) with isatin (*isa*), followed by metallation with Cu(II) perchlorate, yielded the Cu(II)–isatin–diimine complexes [Cu(*isa-pn*)]$(ClO_4)_2$, [Cu(*isa-en*)(H_2O)]$ClO_4 \cdot 2H_2O$, [Cu(*isa-epy*)$_2$]$(ClO_4)_2 \cdot 2H_2O$ and [Cu(*isa-amiquin*)(H_2O)]ClO_4 as previously reported [41–43]. The structures of the complexes (Fig. 1) were confirmed by elemental analysis and comparison of their UV–visible (UV–VIS) and EPR spectra with literature data. Cu(II) complexes with the ligands tetraglycine ($[Cu^{II}(H_{-2}G4)]^-$), triglycine ($[Cu^{II}(H_{-2}G3)]^-$) and glycylglycylhistidine ($[Cu^{II}(H_{-2}GGH)]^-$) were prepared by mixing an aqueous solution of Cu(II) chloride with 1.25 equivalents of the peptide solution. The structures of the complexes were confirmed by comparison of their UV–VIS and EPR spectra with published data for these compounds [44–47]. Both classes of complexes showed to be structurally stable in aqueous solutions at all conditions used in experiments.

2.2. Dihydrorhodamine 123 (DHR) assay

Reaction mixtures (final volume = 1.00 mL) containing bicarbonate (25 mM), ascorbate (maintained in stock buffer solution pH = 4.0, 100 μM), hydrogen peroxide (3 mM) and DHR (50 μM) in 10 mM phosphate buffer (pH 7.4) were incubated in the presence or absence of Cu(II) sulphate or Cu(II)–imine complexes (50 μM) in order to assay the generation of oxygen-derived radicals with the capacity to bring about the one-electron oxidation of DHR generating $DHR^{\bullet+}$ (measured spectrophotometrically at 500 nm; $\varepsilon = 7.88 \times 10^4\ M^{-1}\ cm^{-1}$) [11].

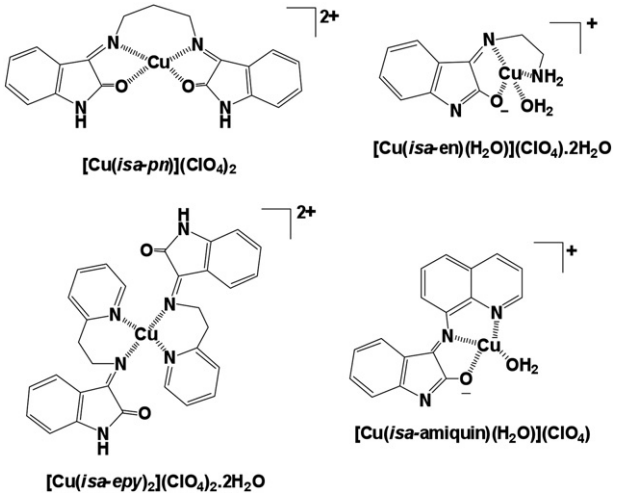

Fig. 1. Structures of Cu(II)–imine complexes employed in this study [41–43].

2.3. NADH assay

Reaction mixtures (final volume = 1.00 mL) containing bicarbonate (25 mM), ascorbate (maintained in stock buffer solution pH = 4.0, 100 μM), hydrogen peroxide (3 mM) and NADH (50 μM) in 10 mM phosphate buffer (pH 7.4) were incubated in the presence or absence of Cu(II) sulphate or Cu(II)–imine complexes (50 μM) in order to assay the generation of oxygen-derived radicals with the capacity to bring about the one-electron oxidation of NADH generating $NADH^{\bullet+}$ (measured spectrophotometrically at 340 nm; $\varepsilon = 0.62 \times 10^4\ M^{-1}\ cm^{-1}$) [16].

2.4. Degradative oxidation of carbohydrate

The 2-thiobarbituric acid reactive species (TBARs) method was used to assay the oxidation of 2-deoxy-D-ribose by monitoring the formation of a red chromophore similar to that formed with malonaldehyde [33,48]. Reaction mixtures (final volume 1 mL) containing 2-deoxy-D-ribose (2.5 mM), sodium bicarbonate (25 mM), hydrogen peroxide (3 mM), and Cu(II) sulphate or Cu(II) complexed with imines or Gly-derived ligands (50 μM) in 50 mM phosphate buffer (pH 7.4) were incubated at 37 °C for 1 h. A 500 μL aliquot of 1% (w/v) 2-thiobarbituric acid was then added, the solution was heated to 100 °C for 15 min, and allowed to cool, and the absorbance was read at 532 nm ($\varepsilon = 1.36 \times 10^5\ M^{-1}\ cm^{-1}$).

2.5. Cell growth studies

Human neuroblastoma cells SH-SY5Y were purchased from the American Type Cell Culture (ATCC) and incubated in Dulbecco's MEM-F12 medium supplemented with 10% foetal calf serum (FCS) at 37 °C in an atmosphere of 5% CO_2 in air. In order to treat cells with Cu(II) complexes with Gly-derived ligands, fresh solutions containing 12 mM Cu (GlyGlyGly), Cu(GlyGlyGlyGly) or Cu(GlyGlyHis) were used to prepare MEM-F12/FCS medium supplemented with 50 μM of complex. This concentration was chosen for all experiments since it allowed reasonable cell growth at all incubation times investigated. Experimental cells were plated at a density of $4 \times 10^4/cm^2$ and incubated at 37 °C in an atmosphere of 5% CO_2 in air. Following incubation, cells were trypsinised and adherent

cells combined, washed with phosphate buffered saline [PBS; containing potassium chloride (2.7 mM) and sodium chloride (137 mM) in 10 mM phosphate buffer (pH 7.4)], stained with Trypan blue and counted under the optical microscope using a Newbauer's chamber.

2.6. Atomic absorption experiments

Human neuroblastoma cells SH-SY5Y was incubated in Dulbecco's MEM-F12 medium supplemented with 10% foetal calf serum (FCS) at 37 °C in an atmosphere of 5% CO_2 in air. In order to treat cells with Cu(II) complexes with Gly-derived and imine-derivative ligands, fresh solutions containing 12 mM Cu(II) complexed with imines or Gly-derived ligands were used to prepare DMEM-F12/FCS medium supplemented with 50 μM of complex. Experimental cells were plated at a density of $4\times10^4/cm^2$ and incubated at 37 °C in an atmosphere of 5% CO_2 in air, in distinct triplicate experiments. Following incubation, cells were trypsinised and adherent cells combined, and washed 5 times with phosphate buffered saline [PBS; containing potassium chloride (2.7 mM) and sodium chloride (137 mM) in 10 mM phosphate buffer (pH 7.4)] containing EDTA 1.0 mM, to remove residual Cu(II) from medium. To perform atomic absorption experiments high purity water provided by a Milli-Q water purification system (Millipore, Bedford, MA, USA), nitric acid (Merck) and analytical solutions containing 1000 mg L^{-1} of Cu ($CuCl_2$) (Titrisol®, Merck) were used. Calibrations curves were obtained by using reference solutions containing 0.5–5 mg L^{-1} of Cu^{2+} in 0.1% vol/vol HNO_3. Direct analysis of cells was performed by weighing masses around 0.25 mg directly onto the graphite boat-type platform. A ZEEnit® 60 atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) equipped with a manual solid sampling accessory, pyrolytic graphite tube atomizer and boat-type platform and hollow cathode lamp (wavelength = 216.5 nm, bandpass = 0.8 nm and lamp current = 4.0 mA) was used. A stainless steel microspatula was used to transfer the samples to the pyrolytic boat-type platform. Microbalance Auto Balance AD-4 (Perkin-Elmer, Norwalk, USA) with a precision of 0.001 mg was used to weight samples. The heating program used for the direct determination of Cu in cells was adapted from a previous program developed by our group (step: temperature/°C, ramp/°C s^{-1}, hold/s): (drying: 180, 50, and 10), (pyrolysis: 1200, 100, and 15), (atomization: 2500, 2500, and 5) and (cleaning: 2600, 1200, and 3) [49].

2.7. Statistical analyses

All experiments were repeated at least five times (except where otherwise stated) and data expressed as mean values and standard

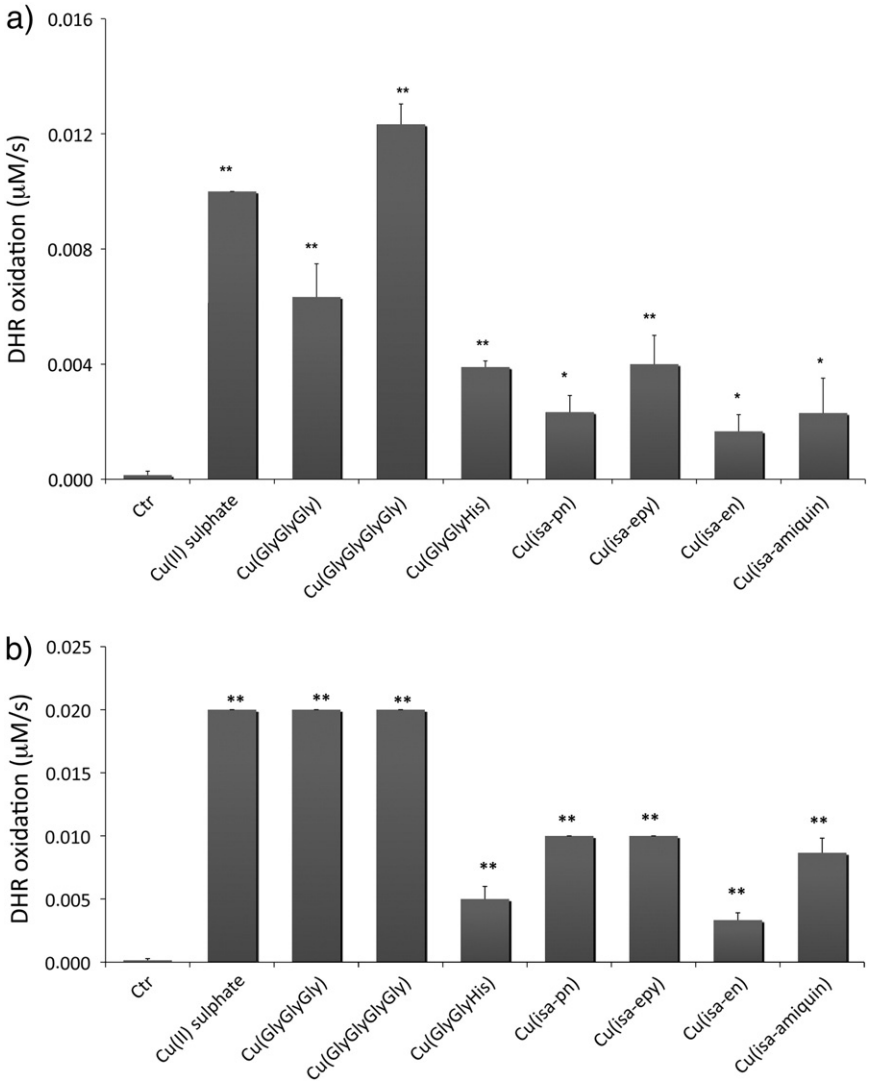

Fig. 2. Assay of dihydrorhodamine 1,2,3 (DHR) oxidation generating the $DHR^{\bullet+}$ monitored at 500 nm ($\varepsilon = 7.88\times10^4\ M^{-1}cm^{-1}$). The reaction mixture (final volume = 1.00 mL) contained hydrogen peroxide (3 mM), ascorbate (100 μM) and DHR (50 μM) in 10 mM phosphate buffer (pH 7.4) together with Cu(II) sulphate or the indicated Cu(II)–imine or Cu–Gly derivative complex (50 μM); a) without bicarbonate; b) with bicarbonate (25 mM) in the incubation. In both panels: Ctr = control assay without Cu(II) additive; data are reported as mean values ± SD ($n = 5$); the significance of the difference between the treatment mean and that of the control is indicated by $^*p<0.05$ and $^{**}p<0.001$.

deviation. Differences between means were assessed by ANOVA with Bonferroni's correction, and those with p values <0.05 were considered significant.

3. Results and discussion

The aim of the study was to gain an insight into the mechanism by which the bicarbonate/carbon dioxide pair influences the generation of reactive species from hydrogen peroxide in the presence of different Cu(II) ions and complexes thereof. For this purpose, we have investigated the effect on oxygen-derived radical formation of Cu(II) complexed with four different stable imine ligands [41–43], cycling the metal between the 2+ and 1+ redox states, and with three low molecular weight peptide ligands known to form stable Cu (III) complexes in solution [44–47].

Assay of the rates of the copper-catalysed H_2O_2/HCO_3^- or H_2O_2-induced oxidation of DHR and NADH *in vitro* revealed that the generation of oxygen-derived radicals was much higher in the presence of Cu(II) sulphate than when Cu(II) imine complexes were present (Figs. 2 and 3). This unexpected finding indicates that imine complexes generate lower levels of reactive oxygen species (ROS) than the free Cu (II) ion and Cu(II) peptide ligands, except Cu(GlyGlyHis). Such a result challenges the use of these complexes in cancer cell therapy to induce apoptosis in mammalian tumour cells *in vitro* on the basis of their facility to generate free radical and reactive species [35–39]. Comparative results were similar in the presence or absence of bicarbonate; however, with bicarbonate/carbon dioxide in the reaction medium, more oxidation of both DHR and NADH was verified. This is probably due the carbonate radical production from hydroxyl radical and bicarbonate with a second order rate constant of $8.5\times10^6\,M^{-1}s^{-1}$ [22] and posterior probe oxidation by both carbonate and hydroxyl radical, as they are not specific [50]. In the case of DHR, hydroxyl radicals are the most reactive but least efficient in generating fluorescent products, probably because of lower selectivity of attack than carbonate radical [50]. In the case of NADH oxidation, the observed higher oxidation when bicarbonate is present probably reside in the fact that hydroxyl radical can either add or oxidize targets, whereas carbonate radical only oxidize the biomolecule, a direct observation derived from their different redox potential and chemical reactivity [22].

In order to confirm the results obtained, the TBARs method was used to assess the rate of oxidation of 2-deoxy-D-ribose mediated by Cu(II) sulphate and Cu(II) complexes with imines or Gly-derived ligands. As can be observed from Fig. 4, the relatively low level of generation of oxidizing radicals by Cu(II)–imine complexes was confirmed. On the other hand, in the presence of Cu(II) complexed with Gly-derived ligands the rate of oxidation of 2-deoxy-D-ribose was higher than that established for the free Cu(II) ion. It appears, therefore, that Cu(II)–Gly-derived complexes possess a different mechanism of action in their augmentation of biomolecular oxidation by the H_2O_2/HCO_3^- system. The second order rate constant for reactions with hydroxyl radical with 2-deoxy-D-ribose is $4.1\times10^9\,M^{-1}s^{-1}$ at pH = 7.0 [5], with indicates that it is much faster than carbonate radical reaction with this substrate, as the hydroxyl radical reacts with HCO_3^- in a $8.5\times10^6\,M^{-1}s^{-1}$ second order rate constant. At this time it is possible that at experimental conditions used in the

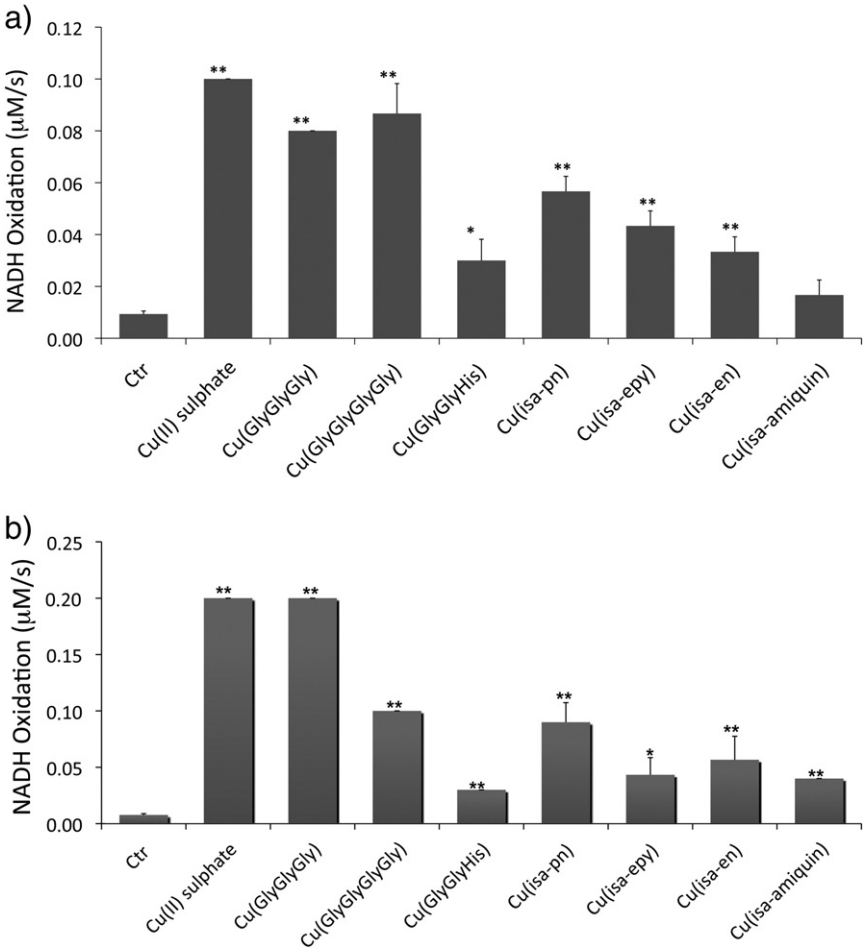

Fig. 3. Assay of NADH oxidation generating the $NADH^{\bullet+}$ monitored at 340 nm ($\varepsilon = 0.62\times10^4\,M^{-1}cm^{-1}$). The reaction mixture (final volume = 1.00 mL) contained hydrogen peroxide (3 mM) and NADH (50 μM) in 10 mM phosphate buffer (pH 7.4) together with Cu(II) sulphate or the indicated Cu(II)–imine or Cu–Gly derivative complex (50 μM); a) without bicarbonate; b) with bicarbonate (25 mM) in the incubation. In both panels: Ctr = control assay without Cu(II) additive; data are reported as mean values ± SD ($n = 5$); the significance of the difference between the treatment mean and that of the control is indicated by $^*p<0.05$ and $^{**}p<0.001$.

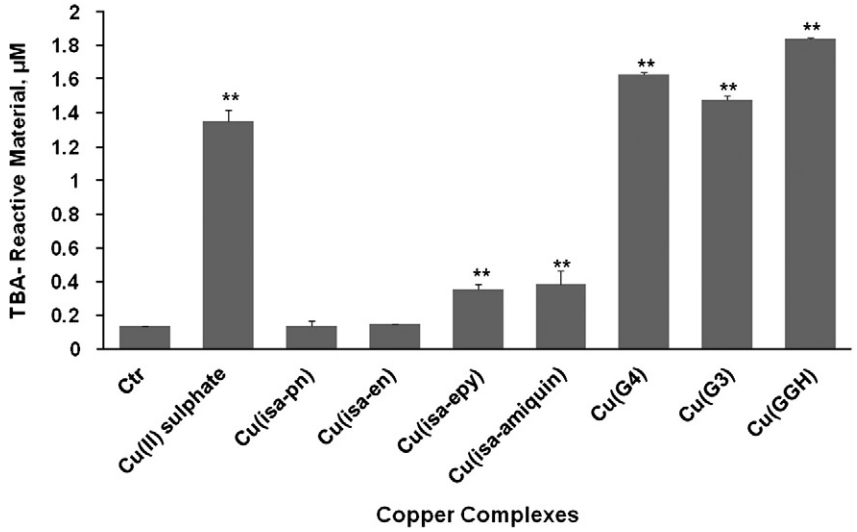

Fig. 4. Oxidative damage to 2-deoxy-D-ribose (2.5 mM) promoted by Cu(II) complexes (50 µM) after incubation at 37 °C for 1 h in phosphate buffer (50 mM; pH 7.4) containing hydrogen peroxide (3 mM), ascorbate (100 µM) and bicarbonate (25 mM). The degradation of the ribose ring to malonaldehyde-like products was monitored at 532 nm ($\varepsilon = 1.36 \times 10^5\ M^{-1}cm^{-1}$) after reaction with 2-thiobarbituric acid. Ctr = control assay without Cu(II) additive; data are reported as mean values ± SD ($n = 4$); the significance of the difference between the treatment mean and that of the control is indicated by $^*p<0.05$ and $^{**}p<0.001$.

experiment, we were able to measure the hydroxyl radical production from the copper complexes and oxidants.

Since the apoptotic and anti-proliferative activities of Cu(II) imine complexes have already been demonstrated in respect of mammalian neuroblastoma cells SH-SY5Y [39,41], we were interested to determine whether Cu(II)–Gly-derived complexes exhibited similar activities and also to evaluate the contribution of ROS generation to such effects. Previous results at similar experimental conditions [41] showed that Cu (isa-pn) decrease the SH-SY5Y cell viability in 20%, Cu(isa-amiquin) in 15% and Cu(isa-epy) in 35% at 24 h of treatment and copper complex concentration of 50 µM. The viabilities of SH-SY5Y cells in the presence of Cu(GlyGlyGly), Cu(GlyGlyGlyGly) or Cu(GlyGlyHis) were investigated *in vitro*, and the results (Fig. 5) revealed a stimulatory effect of these complexes on the tumour cells. It seems, therefore, that the Cu(II) complexes that generated higher ROS *in vitro* did not cause an apoptotic cell response but instead initiated a proliferation of growth, which was particularly intense during the first 12 h of incubation. Free Cu(II) ion, as exemplified by the results obtained with 50 µM Cu(II) sulphate as medium supplement, also showed stimulation of SH-SY5Y proliferation at all incubation times. In contrast, an earlier study involving SH-SY5Y cells demonstrated that the presence of Cu(II) sulphate at concentrations greater than 150 µM damaged mitochondria and induced cell death [51], an effect that was attributed to ROS production by free Cu(II) ion. One of these complexes, Cu(isa-epy) showed a capacity of act as a delocalized lipophilic cation in mitochondria [52].

To distinguish the capability of both classes of Cu(II) complexes to enter the cells and the kinetics of their accumulation, acting as a free radical generator inside the cell, we followed copper uptake by atomic absorption analyses (Fig. 6). Results shows that treatments with Cu(II)–imine-derivative ligands generally resulted in a rapid increase of intracellular copper content. This result was particularly significant, especially when compared with that obtained with copper sulphate, used as control of cellular incorporation of the metal ion. Cu(isa-epy) seems to be more efficiently incorporated within the cells with respect to others Cu–imine ligands and others Cu(II)–glycine-derivative ligands. Interestingly, Cu(isa-epy) confirmed to be the most dangerous to cell growing, showing a direct effect on cell death by apoptosis induced by mitochondrial damage [39,52]. The Cu(II)–glycine-derivative ligands did not penetrate into cells, except Cu(GlyGlyHis), that showed to be more similar with Cu–imine-derivative complexes in ROS generation

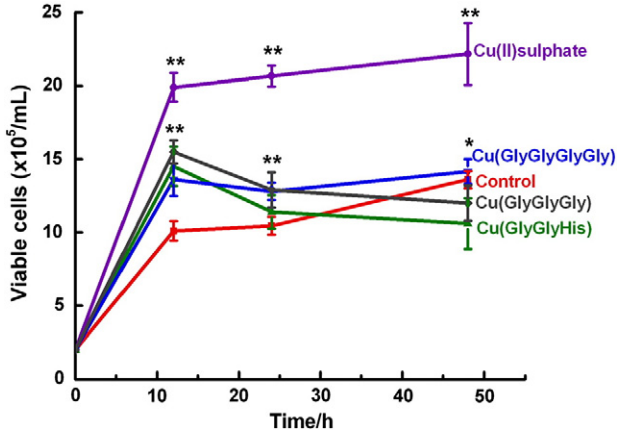

Fig. 5. Effects of the Cu(II)–Gly-derived complexes Cu(GlyGlyGly), Cu(GlyGlyGlyGly) and Cu(GlyGlyHis) on the viability of SH-SY5Y cells. Cells were treated with the complex at a concentration of 50 µM for 12, 24 and 48 h and viable cells were counted after staining with Trypan blue. Ctr = control assay without Cu(II) additive; data are reported as mean values ± SD ($n = 6$); the significance of the difference between the treatment mean and that of the control is indicated by $^*p<0.05$ and $^{**}p<0.001$.

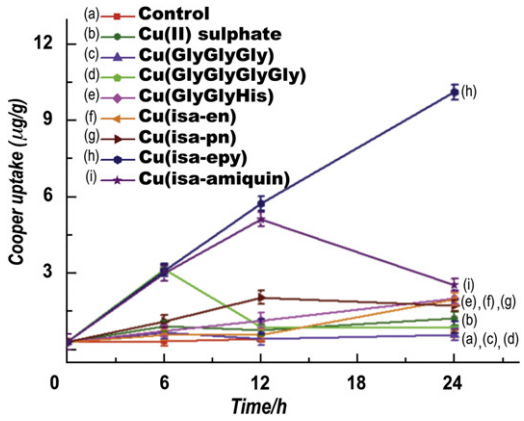

Fig. 6. Copper uptake (µg/g cell) measured by absorption atomic spectrometry on SH-SY5Y cells. Cells were treated with the complex at a concentration of 50 µM for 6, 12 and 24 h, extensively washed by PBS/EDTA and PBS and digested with HNO_3. Ctr = control assay without Cu(II) additive; data are reported as mean values ± SD ($n = 3$); the significance of the difference between the treatment mean and that of the control was $p<0.05$ in all experiments.

studies (Figs. 2 and 3). These results demonstrated a direct relationship between copper uptake and the cell viability, with Cu–imine-derivative ligands being permeating and more efficient in inducing cell death than Cu-glycine ones.

To the best of our knowledge, it is currently believed that ROS generation by Cu(II) redox cycling gives rise to cell death by apoptosis [34,36], and that this effect has been proposed as a possible anticancer strategy. However, a relationship between the levels of ROS generated, copper uptake and the observed apoptotic effects has not been clearly established. The present study has revealed that there is a narrow threshold for which ROS generation caused by cell uptake of copper(II) complexes can activate cell proliferation rather than cell death defined by copper cell metabolism.

4. Conclusions

Low levels of free radical generation were observed during reactions of H_2O_2 with Cu(II)–imine complexes in the presence of the HCO_3^-/CO_2 pair, but these complexes were able to enter in cell and carry out an efficient copper uptake, with no excretion of Cu(II) ion. In contrast, Cu (II) complexes with Gly-derived ligands catalysed a significantly larger production of reactive species in a H_2O_2/HCO_3^- oxidation system, but with no capability to remain inside the cell during copper metabolism, remaining similar to Cu(II) sulphate salt. Viability analysis with the mammalian cancer cell line SH-SY5Y revealed that free Cu(II) ion and Cu (II) complexes with Gly-derived ligands stimulated cell growth and proliferation rather than apoptosis, a direct observed effect of copper uptake from these different complexes. Cu(II)–imine complexes act as a free copper ion inside the cell as they are absorbed by cell membrane and remain inside the cell for the time of the treatment. On the contrary Cu(II)–Gly derivative complexes cannot be absorbed by cell membrane and consequently are not available to produce ROS inside the cell.

The results provide a better understanding of the biological role of the Cu(II) ion and ligand complexes in cancer cell therapy. Cu(II)–imine and Cu(II)–Gly-derived complexes clearly exhibit different mechanisms of action in their augmentation of biomolecular oxidation by the H_2O_2/HCO_3^- system. Furthermore, it is proposed that copper uptake by cells can also have an effect on apoptosis in mammalian cancer cell.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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