JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 1681–1686

Received March 21, 2002
Accepted August 14, 2002
DOI 10.1211/002235702261
ISSN 0022-3573

Cimetidine: antioxidant and metal-binding properties

Zaynab Lambat, Janice L. Limson and Santy Daya

Abstract

Cimetidine is one of the most potent H_2 receptor antagonists for inhibiting excessive histamine-induced acid secretion and is currently used worldwide to treat peptic ulcers. In this study, levels of free radicals were assessed and the ability of cimetidine to act as an antioxidant was determined using nitroblue-tetrazolium assay and lipid peroxidation assays. Free radical generation in the brain is promoted by the presence of iron, as occurs in the Fenton reaction. The results show that cimetidine reduces the generation of superoxide anion formed in the nitroblue-tetrazolium assay. In addition, cimetidine (1 m_M) is able to reduce the iron-induced rise in lipid peroxidation in rat brain homogenates. Electrochemistry, UV/Vis spectroscopy and HPLC experiments show metal–ligand interactions between cimetidine and transition metals. The results imply that cimetidine provides a neuroprotective effect by binding to iron and copper, thus making them unavailable for free radical production.

Introduction

The mechanism by which biological systems generate free radicals has been studied extensively in the pathophysiology of many neurological disorders including Alzheimer's and Parkinson's diseases. The brain is particularly sensitive to free radical destruction because of high levels of polyunsaturated lipids that comprise the lipid membrane, and moreover, the high oxygen utilisation by the brain (Halliwell 1992). Although aerobic lifestyles are advantageous in many ways, the cellular utilization of oxygen for many biochemical reactions results in the formation of highly destructive free radical products.

Free radical generation in the brain is further assisted by the presence of large amounts of iron required by the Fenton reaction (Braughler & Hall 1989; Halliwell 1992; Daya 1999). The role of iron in free radical and lipid peroxidation reactions has been studied extensively and its role in this regard is widely accepted (Daya 1999). Under physiological conditions, iron is only slightly soluble, tending to form precipitates with anions such as the hydroxyl radical. However, a variety of agents greatly increase the solubility of iron. For example, the addition of ethylene diamine tetra-acetate (EDTA) to a free-radical generating system in the presence of iron markedly potentiates cytotoxicity, hydroxyl radical formation and, consequently, lipid peroxidation (Fahn & Cohen 1992).

There has been recent evidence that cimetidine forms complexes with copper which exhibit a superoxide dismutase activity and thereby reduce free radical formation (Greenaway et al 1980; Kimura et al 1986). Hence, it was further determined whether the reputed binding of cimetidine to metals reduces free radical production.

Although the superoxide anion is a free radical, it is not a particularly damaging species. It is mostly reductive in nature and its main significance is probably as a source of hydrogen peroxide and as a reductant of transition metal ions (Cheeseman & Slater 1993).

Under normal conditions, the antioxidant defence system within the body can easily handle free radicals that are produced. During times of increased oxygen flux, free radical production may exceed that of removal and ultimately result in lipid peroxidation. The hydroxyl radical, specifically, is the most damaging of all free radicals

Faculty of Pharmacy, Rhodes University, Box 94 Grahamstown, 6140, South Africa

Zaynab Lambat, Janice L. Limson, Santy Daya

Correspondence: S. Daya, Faculty of Pharmacy, Rhodes University, Grahamstown, Box 94, 6140 South Africa. E-mail: s.daya@ru.ac.za

Acknowledgement and funding: This work was funded by grants from the South African Medical Research Council and the National Research Foundation. Janice Limson thanks the Medical Research Council (South Africa) for post-doctoral fellowship. Professor Tebello Nyokong is thanked for use of voltammetric equipment.

and ultimately causes the most damage (Halliwell & Gutteridge 1985). The extensive damage to membrane structure and integrity, an indicator of hydroxyl radical generation, was measured using the thiobarbituric acid test.

Additional studies were carried out to evaluate binding of Fe^{2+}, Fe^{3+} and Cu^{2+} using ultra-violet/visible spectroscopy studies (UV/Vis) and HPLC, as well as electrochemistry. Electrochemical methods have been employed with much success in the analysis of metal–ligand interactions (Limson et al 1998).

Materials and Methods

Chemicals

All reagents were of the highest quality available. Cimetidine, nitroblue-tetrazolium, nitroblue-diformazan, resorcinol, 1, 1, 3, 3-tetramethoxypropane (98%), butylated hydroxytoluene and 2-thiobarbituric acid (98%) were purchased from Sigma (St Louis, MO). Iron sulfate was purchased from BDH Laboratory Supplies (Poole, UK). Trichloroacetic acid, ascorbic acid, Fe_2SO_4, $FeCl_3$, $CuSO_4$ and EDTA were purchased from Saarchem (Krugersdorp, South Africa). Isolute J C_{18} solid-phase extraction (SPE) columns were obtained from International Sorbent Technology (Mid Glamorgan, UK).

Animals

Adult male rats of the Wistar strain, 250–300 g, were used. The rats were housed in a controlled environment with a 12-h light–dark cycle, and were given free access to standard laboratory chow and water. The Rhodes University ethics committee approved protocols for the experiments.

Homogenate preparation

Rats were killed by cervical dislocation and the brains were rapidly removed and homogenized with 0.1 M phosphate-buffered saline (PBS), pH 7.4, so as to give a final concentration of 10% w/v. The homogenate was frozen in liquid nitrogen and stored at −70°C until needed. All samples were used within 2 days of homogenate preparation.

Lipid peroxidation determination

The method used in this experiment was a modification of an earlier method (Anoopkumar-Dukie et al 2001). In a final volume of 1 mL, the iron sulfate (5 mM), EDTA (100 μM), H_2O_2 (2.8 mM), cimetidine (0.0, 0.5 and 1 mM), and ascorbate (100 μM) were added in this sequence.

The reaction mixture was incubated in an oscillating water bath for 1 h at 37°C. At the end of the incubation period, 0.5 mL butylated hydroxytoluene (0.5 mg mL^{-1} in methanol) and 1 mL trichloracetic acid (15% in water) were added to the mixture. The tubes were then placed in a hot water bath maintained at 80°C for up to 20 min to release protein-bound malondialdehyde. To avoid adsorption of malondialdehyde onto insoluble protein, the samples were cooled and centrifuged at 2000 *g* for 15 min. Following centrifugation, 1 mL of the protein-free supernatant was removed from each tube and 1 mL of 2-thiobarbituric acid (0.33% in water) was added to this fraction. The tubes were sealed and incubated in a boiling water bath at acidic pH for 30 min.

After cooling, thiobarbituric acid–malondialdehyde was separated from other possible interfering thiobarbituric acid-reactive substances using an Isolute J C_{18} SPE column that was pre-washed with 2 mL of methanol followed by 2 mL distilled water. The sample (1 mL) was loaded onto the column that was subsequently washed with 2 mL distilled water. The thiobarbituric acid–malondialdehyde complex was eluted with 1 mL methanol. The methanol was then evaporated using an N-EVAP analytical evaporator at 65°C under a gentle stream of nitrogen. The residue was dissolved in distilled water (0.5 mL) containing 0.1 mg mL^{-1} resorcinol. These samples were analysed by HPLC as described above. The malondialdehyde levels were obtained from a calibration curve generated using 1, 1, 3, 3-tetramethoxypropane in the same way described above. The ratio of the peak height of thiobarbituric acid–malondialdehyde to the peak height of resorcinol (external standard) was plotted against the concentration of malondialdehyde in the complex injected. Final results are expressed as nmol (mg tissue)$^{-1}$.

Nitroblue-tetrazolium assay

This method is generally accepted as a simple and reliable method for assaying the superoxide free radical (Ottino & Duncan 1997). A modification of an earlier assay was used in this set of experiments. The principle of the assay is based on the ability of free radicals to reduce nitroblue-tetrazolium to insoluble diformazan, which can be extracted with glacial acetic acid. Homogenate (1 mL) containing KCN (0.0, 0.5 and 1 mM) alone or in combination with cimetidine (0.0, 0.5 and 1 mM) was incubated with 0.4 mL of 0.1% nitroblue-tetrazolium in an oscillating water bath for 1 h at 37°C. Termination of the assay and extraction of reduced nitroblue-tetrazolium was carried out by centrifugation of the samples at 2000 *g* and re-suspension of the pellet with 2 mL glacial acetic acid. The absorbance of the glacial acetic acid containing the nitroblue-tetrazolium extracts were measured at 560 nm and the resulting absorbance was converted to μmol diformazan using a standard curve generated from nitroblue-diformazan. Final results were expressed as μmol diformazan (g tissue)$^{-1}$.

Electrochemistry

Cyclic and stripping voltammograms were obtained with the Bio Analytical Systems (BAS) CV-50W voltammetric analyser using a BAS C2 cell stand to maintain constant

atmosphere. A 3-mm diameter glassy carbon electrode was employed as a working electrode for voltammetric experiments. A silver/silver chloride (KCl concn, 3 M) and a platinum wire were employed as reference and auxiliary electrodes, respectively, in all electrochemical work. Before use, the glassy carbon electrode was cleaned by polishing with alumina on a Buehler pad, followed by washing in nitric acid and rinsing in water followed by the buffer solution. Between scans, the glassy carbon electrode was cleaned by immersion in a dilute acid solution and rinsed with water.

For cyclic voltammetric experiments, appropriate concentrations of the metal and cimetidine in buffer were introduced into a glass cell and degassed for 5 min with nitrogen before scanning a potential window.

For adsorptive stripping experiments, appropriate concentrations of the metal (Fe^{2+} or Fe^{3+}) and of the ligand (cimetidine) were introduced into an electrochemical cell. The electrolyte used was pH 7.3 Tris-HCl buffer for Fe^{3+} and pH 3.5 citric acid buffer for Fe^{2+}. For work with copper, pH 4.5 sodium acetate buffer was used. The solution was then de-aerated with nitrogen for 5 min, after which a flow of nitrogen was maintained over the solution throughout the measurement. Optimum deposition potential of 0.10 V or 0.15 V vs Ag/AgCl was applied for 60 s to effect the formation and adsorption of the metal and ligand species onto the glassy carbon electrode. The voltammograms were then scanned in the negative direction from the deposition potential to –0.6 V vs Ag/AgCl at the scan rate of 0.1 V s^{-1} to strip the adsorbed metal–ligand species from the electrode. During the stripping step, current response due to the reduction of the metal–ligand species was measured as a function of potential. All potential values quoted are referenced against the Ag/AgCl chloride reference electrode.

UV/Vis studies

The UV/Vis spectra were monitored with a Cary 500 UV/Vis/NIR spectrophotometer. Concentrations of each metal were kept constant (1 mg mL^{-1}) and spectra measured on subsequent additions of cimetidine (1 mg mL^{-1}) at different concentrations. Spectra were scanned over a wavelength range of 190–800 nm, immediately after addition of cimetidine solution to the metal solutions.

HPLC analysis

Samples were analysed on a modular, isocratic HPLC system. The chromatographic system used consisted of a Spectraphysics Iso Chrom LC Pump, a Linear UV/VIS 200 Detector, and a Rikadenki Recorder. Samples were introduced into the system using a Rheodyne fixed loop injector, fitted with a 20-μL loop. A mobile phase of 40 : 60, acetonitrile–water was used. The retention time (5 min) and the peak height of cimetidine (1 mM) was measured alone as well as with subsequent additions with the metals, Fe_2SO_4, $FeCl_3$ and $CuSO_4$ (1 mM).

Statistical analysis

The results were analysed using a one-way analysis of variance followed by the Student–Newman–Keuls multiple range test. The level of significance was accepted at $P < 0.05$. A $P < 0.05$ between groups was accepted as being statistically significant.

Results and Discussion

Effect of cimetidine on iron-induced lipid peroxidation

The use of iron to generate free radicals, particularly the hydroxyl radical, caused extensive damage to cell membranes and caused an increase in lipid peroxidation as compared with the control set of experiments. Exposure of whole rat brain homogenate to varying concentrations of iron sulfate (1 and 5 mM) increased lipid peroxidation compared with the control (Figure 1). Iron-induced lipid peroxidation significantly decreased in the presence of cimetidine (0.5 and 1 mM). This may occur through a mechanism by which cimetidine binds iron, making it unavailable to initiate further free-radical-induced lipid peroxidation.

Effect of cimetidine on cyanide-induced superoxide anion production

Cyanide induces neurotoxicity due to oxidative damage caused by free radicals, especially the superoxide anion. The role of cyanide in the assay for superoxide anion is to inhibit cytochrome c oxidase, the terminal enzyme of the respiratory chain present in complex IV. The inhibition

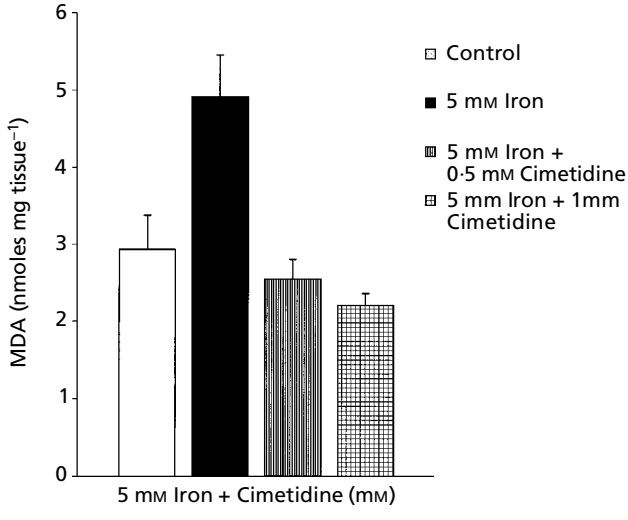

Figure 1 The effect of cimetidine on iron-induced lipid peroxidation in rat brain homogenate. Each bar represents the mean±s.e.m., n = 4 ($P < 0.05$; Student–Neuman–Keuls multiple range test).

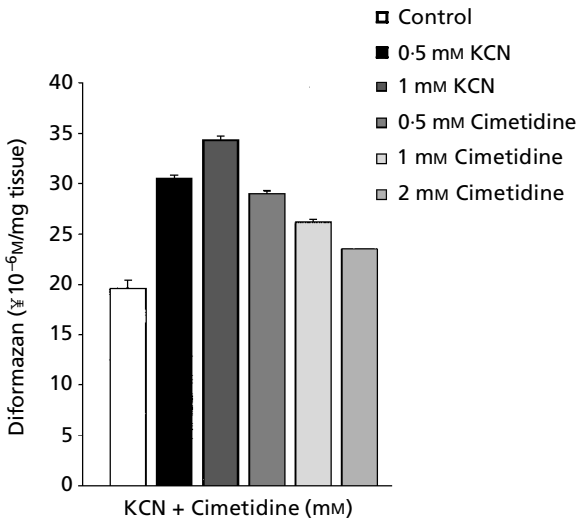

Figure 2 The effect of cimetidine on cyanide-induced superoxide anion production. Each bar represents the mean±s.e.m., n = 6 ($P < 0.05$; Student–Neuman–Keuls multiple range test).

results in a block of oxidative phosphorylation, therefore giving rise to insufficient utilisation of oxygen.

Cimetidine reduces the generation of free radicals in a concentration-dependent manner. Exposure of rat homogenate to KCN caused the generation of free radicals, which was significantly reduced on addition of cimetidine (0.5, 1.0 and 2.0 mM) (Figure 2).

UV/Vis studies

Table 1 lists the major bands observed for studies measuring the interaction of Cu^{2+} and cimetidine. The major band observed for Cu^{2+} was at 200 nm. This band shifted to 224 nm in the presence of cimetidine. Two new bands, at 338 and 378 nm, were observed on addition of cimetidine. These shifts, and the formation of new bands, strongly suggest an interaction between Cu^{2+} and cimetidine. A distinct colour change was observed from light blue to jade for the copper solution in the presence cimetidine.

Fe^{3+} showed distinct bands at a range of wavelengths – 218, 234, 251, 290, 330 and 366 nm. All of these bands shifted in the presence of cimetidine. At a ratio of 1:3, Fe^{3+}: cimetidine, a new band at 311 nm was observed. For Fe^{3+}: cimetidine in a ratio of 1:5, bands at 338 and 370 nm were observed, similar to those observed for Cu^{2+} and cimetidine, supporting a theory that cimetidine binds to both Cu^{2+} and Fe^{3+}.

The spectra obtained for the interaction between ferrous sulfate and cimetidine showed a peak for Fe^{2+} alone at 289.7 nm. In the presence of cimetidine, new bands appeared at 206, 216 and 235 nm, similar to those observed for Fe^{3+} alone. On addition of cimetidine, a colour change for Fe^{2+} was observed from pale blue/green to yellow, which darkened with time and increasing concentration of cimetidine. This colour change, as well as the similarity between the bands obtained with Fe^{3+}, suggests that the cimetidine oxidizes the Fe^{2+} to Fe^{3+}.

Table 1 UV/Vis studies showing interaction of transition metals with cimetidine.

Peak	Wavelength (nm)
Cu^{2+}	200, 212.9
Cu^{2+}+Cimetidine (1:2)	224, 332, 372
Cu^{2+}+Cimetidine (1:3)	224, 337, 378
Cu^{2+}+Cimetidine (1:5)	337, 378
Fe^{3+}	218, 234, 251, 266, 290, 330
Fe^{3+}+Cimetidine (1:2)	365
Fe^{3+}+Cimetidine (1:3)	294, 311, 367
Fe^{3+}+Cimetidine (1:5)	294, 338, 370
Fe^{2+}	289.7
Fe^{2+}+Cimetidine (1:2)	201, 235, 370.8
Fe^{2+}+Cimetidine (1:3)	206, 236, 378.5
Fe^{2+}+Cimetidine (1:5)	201, 216, 239, 378

Electrochemistry

Cimetidine is electrochemically inactive at a bare glassy carbon electrode, meaning that no redox waves are observed for this compound. However, adsorptive stripping voltammetry (ASV) and cyclic voltammetry (CV) produce reproducible oxidation and reduction waves for copper and iron species in solution. By monitoring the changes of the redox patterns for the metal species in the presence of cimetidine, the response between these metals and the ligand could be examined.

Figure 3 shows the ASV for Fe^{3+} alone at a potential of −0.47 V vs Ag/AgCl. In the presence of increasing concentrations of cimetidine, there was a strong increase in the current response for Fe^{3+} (Figure 3b and c). At the higher concentration of 2.0×10^{-5} M cimetidine, a potential shift to −0.49 V was observed. Theoretically, an increase in the observed current and a potential shift are strong indicators that the ligand (in this case cimetidine) forms a bond with the metal, facilitating its transport to the electrode and hence bringing about an increase in current response. A potential shift is a strong indication that a new species is

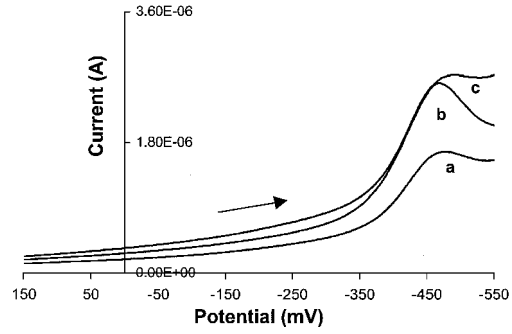

Figure 3 Anodic stripping voltammogram (ASV) for Fe^{3+} (1×10^{-5} M) alone in solution (a) or in the presence of 1×10^{-5} M cimetidine (b) or 2×10^{-5} M cimetidine (c).

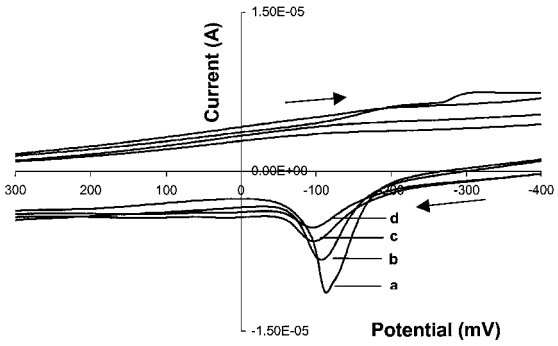

Figure 4 Cyclic voltammogram (CV) of Cu^{2+} (5×10^{-5} M) alone in solution (a) or in the presence of 2×10^{-5} M (b), 6×10^{-5} M (c) or 8×10^{-5} M cimetidine (d).

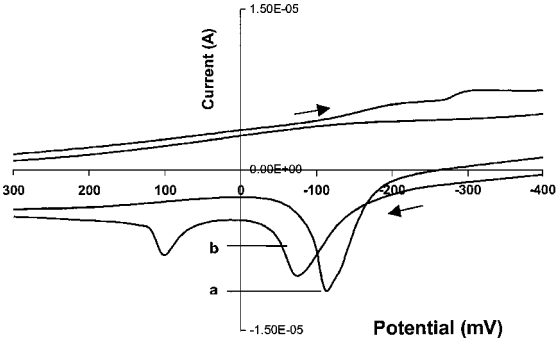

Figure 5 CV of Cu^{2+} (5×10^{-5} M) alone (a) or in the presence of 7×10^{-5} M cimetidine (b) after 3rd scan.

being reduced at the electrode, in this instance, a cimetidine–Fe^{3+} complex as opposed to just the Fe^{3+} alone.

For studies with Fe^{2+}, it was necessary to work at low pH to avoid the oxidation of Fe^{2+}. A similar response was observed for Fe^{2+} in the presence of cimetidine as for Fe^{3+} but with no potential shift observed. It is postulated that in the presence of cimetidine, the Fe^{2+} could be immediately oxidized to Fe^{3+}, as the colour change to yellow suggests. It is thus likely that, in these studies with Fe^{2+}, cimetidine forms a metal–ligand interaction with Fe^{3+} present in solution.

Reproducible adsorptive stripping voltammograms for Cu^{2+} were not obtained, and therefore cyclic voltammetry was used to gauge the interaction between Cu^{2+} and cimetidine. Figure 4(a) shows the CV for Cu^{2+} alone in solution, at a potential of -0.11 V vs Ag/AgCl. In the presence of increasing concentrations of cimetidine, the Cu^{2+} peak decreased and shifted to more positive potentials. Upon multiple scans of a solution of Cu^{2+} and cimetidine, it became apparent that a new complex was being formed. For comparison, Figure 5(a) shows the CV of Cu^{2+} alone. Figure 5(b) shows the CV after the third scan, clearly showing a peak shift for Cu^{2+} from -0.11 V to -0.076 V, while a new oxidation wave at 0.10 V is clearly visible. It is suggested that the copper peak decreases as less copper is available due to an interaction with cimetidine.

HPLC analysis

For HPLC analysis, a UV/Vis detector with wavelength set at 228 nm was used. Cimetidine was released at a retention time of 5 min. The peak height was 164 mm. In the presence of Fe^{2+}, the peak height for cimetidine decreased to 82 mm. With Fe^{3+}, a decrease in the height of the cimetidine peak to 91 mm was observed, along with a new peak with a retention time of 2 min and a height of 56 mm. Similarly, for Cu^{2+}, a decrease in the height of the cimetidine peak to 78 mm was observed, also with the appearance of a new peak with a retention time of 2 min and a height of 15 mm. The peak with the retention time of 2 min observed for both Fe^{3+} and Cu^{2+} strongly supports the theory of a cimetidine–metal complex with these metals.

Discussion

The results of this study indicate that, at a concentration of 0.5 and 1 mM, cimetidine reduces iron-induced lipid peroxidation as well as superoxide anion formation.

The superoxide anion causes damage in living cells indirectly by giving rise to potent oxidants such as the hydroxyl radical and hydrogen peroxide. A decrease in superoxide anion formation on addition of cimetidine suggests that cimetidine exhibits antioxidant properties by preventing formation of powerful oxidants. The use of iron to generate free radicals (in particular the hydroxyl radical) caused extensive damage to cell membranes, resulting in an increase in lipid peroxidation as compared with the control set of experiments. On addition of cimetidine the extent of lipid peroxidation was significantly reduced and this could be due to a mechanism by which cimetidine binds iron, making it unavailable to generate free radicals.

Studies gauging the interaction of cimetidine with Fe^{2+}, Cu^{2+} and Fe^{3+} strongly suggest that cimetidine binds certain metals. Electrochemical studies show metal–ligand interactions between cimetidine and both Cu^{2+} and Fe^{3+}. HPLC confirms this with the presence of a new band with the same retention time for cimetidine in the presence of both Cu^{2+} and Fe^{3+}.

UV/Vis studies corroborate these findings for Cu^{2+} and Fe^{3+}, with shifts in absorbance of these metals in the presence of cimetidine, along with the new bands observed for both metals at approximately 338 nm and 370 nm. Results suggest that cimetidine does not bind directly to Fe^{2+}. The similarity between the UV/Vis spectra for Fe^{2+} in the presence of cimetidine and that of Fe^{3+} alone, as well as the colour change of the Fe^{2+} solution upon addition of cimetidine, suggests that cimetidine oxidizes Fe^{2+} to Fe^{3+} and then binds to the Fe^{3+} form of the metal.

These findings raise questions concerning the manner in which cimetidine acts to reduce free radical damage and lipid peroxidation. It is suggested that cimetidine acts by binding Fe^{3+} and Cu^{2+} in a non-toxic form, thereby preventing these metals from generating free radicals. By oxidizing Fe^{2+} it is suggested that cimetidine mops up toxic free Fe^{2+} and binds it in a non-toxic form as Fe^{3+}–cimetidine complex.

In this study experiments have been carried out in-vitro and the rat brain has been used as a model to assess neurodegeneration. For cimetidine to be effective in man, clinical trials would have to be carried out to support the theory of cimetidine as treatment for neurodegenerative diseases.

The brain is particularly vulnerable to lipid peroxidation since membrane lipids in the brain are very rich in polyunsaturated fatty acids, providing an ideal medium for lipid peroxidation (Gutteridge 1994). Cimetidine, although water-soluble, has the ability to cross the blood–brain barrier and can therefore exert its effect in the CNS. Totte et al (1981) report that the sulfoxide metabolite of cimetidine, which is more lipid-soluble than cimetidine and thus more likely to penetrate the blood–brain barrier, has been found in the CNS in abnormal levels. This could provide an explanation for cimetidine's ability to penetrate the CNS.

In conclusion, since some free radical production is inevitable in neuronal cells, and lipid peroxidation is the major consequence of free radical action, antioxidant defence mechanisms have evolved to protect such cells from extensive damage (Uchida & Kawakishi 1990). From this study it can be shown that cimetidine could be used to enhance the antioxidant defence mechanism, thereby providing a role for this drug as a potential neuroprotective agent.

References

Anoopkumar-Dukie, S., Walker, R. B., Daya, S. (2001) A sensitive and reliable method for the detection of lipid peroxidation in biological tissues. *J. Pharm. Pharmacol.* **53**: 263–266

Braughler, J. M., Hall, E. D. (1989) Central nervous system trauma and stroke. *Free Rad. Biol. Med.* **6**: 289–301

Cheeseman, K. H., Slater, T. F. (1993) An introduction to free radical biochemistry. *Br. Med. Bulletin* **49**: 481–493

Daya, S. (1999) The role of melatonin as a neuroprotectant in Alzheimer's disease. *Pec. Med.* **31**: 528–537

Fahn, S., Cohen, G. (1992) The oxidant stress hypothesis in Parkinson's disease. *Ann. Neurol.* **32**: 804–812

Greenaway, F. T., Brown, L. M., Dabrowiak, J. C., Thompson, M. R., Day, V. M. (1980) Copper (II) complexes of the antiulcer drug cimetidine. *J. Am. Chem. Soc.* **102**: 7784–7786

Gutteridge, J. M. (1994) Antioxidants, nutritional supplements and life-threatening diseases. *Br. J. Biomed. Sci.* **3**: 288–295

Halliwell, B. (1992) Reactive oxygen species and the central nervous system. *J. Neurochem.* **59**: 1609–1623

Halliwell, B., Gutteridge, J. M. C. (1985) The chemistry of oxygen radicals and other oxygen-derived species. In: Halliwell, B., Gutteridge, J. M. C. (eds) *Free radicals in biology and medicine*. Oxford University Press, New York, pp 22–79

Kimura, E., Koike, T., Shimizu, Y., Kodama, M. (1986) Complexes of the histamine H_2-antagonist cimetidine with divalent and monovalent copper ions. *Inorg. Chem.* **25**: 2242–2246

Limson, J., Nyokong, T., Daya, S. (1998) The interaction of melatonin and its precursors with aluminium, cadmium, copper, iron, lead and zinc: an adsorptive voltammetric study. *J. Pineal Res.* **24**: 15–21

Ottino, P., Duncan, J. R. (1997) The effect of α-tocopherol succinate on free radical and lipid peroxidation levels in BL6 melanoma cells. *Free Rad. Biol. Med.* **22**: 1145–1151

Totte, J., Scharpe, S., Verkerk, R., Neels, H. (1981) Neurological dysfunction associated with abnormal levels of cimetidine metabolite. *Lancet* **2**: 1047

Uchida, K., Kawakishi, S. (1990) Cimetidine anti-ulcer drug as a powerful hydroxyl radical scavenger. *Agric. Biol. Chem.* **54**: 2485–2487