

# Stoichiometry and conditional stability constants of Cu(II) or Zn(II) clioquinol complexes; implications for Alzheimer's and Huntington's disease therapy

Evandro Ferrada<sup>a,\*</sup>, Verónica Arancibia<sup>b</sup>, Bárbara Loeb<sup>b</sup>, Ester Norambuena<sup>c</sup>,  
Claudio Olea-Azar<sup>d</sup>, J. Pablo Huidobro-Toro<sup>a</sup>

<sup>a</sup> Centro de Regulación Celular y Patología, Prof. J.V. Luco e Instituto Milenio de Biología Fundamental y Aplicada, MIFAB, Departamento Fisiología, Facultad de Ciencias Biológicas y, Santiago, Chile

<sup>b</sup> Facultad de Química, Pontificia Universidad Católica de Chile, Casilla 114 D, Santiago, Chile

<sup>c</sup> Depto de Química, Facultad de Ciencias Básicas, UMCE, Santiago, Chile

<sup>d</sup> Depto de Química Inorgánica y Analítica, Facultad Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile

---

## Abstract

Successful trials with 5-chloro-7-iodo-8-hydroxyquinoline (clioquinol, CQ) for Alzheimer's disease treatment prompted renewed interest in assessing whether its therapeutic action is related to the coordination of neurotoxic trace metals, such as Cu(II) and Zn(II). We now report conditional stability constants ( $K_C$ ) for CQ Cu(II) and Zn(II) complexes measured in a biological buffer containing Ca(II) and Mg(II) ions. UV-vis spectroscopy and polarography evidenced a 1:2 stoichiometry of Cu(II) and Zn(II) CQ complexes; the  $K_C$ s calculated were: Cu(CQ)<sub>2</sub>  $1.2 \times 10^{10}$ , and Zn(CQ)<sub>2</sub>  $7.0 \times 10^8 \text{ M}^{-2}$ ; the CQ affinity for Cu(II) is at least an order of magnitude higher than for Zn(II). To test the possible functional relevance of the Cu(II) CQ complexes in the brain, we bioassayed free Cu(II) concentration by the metal-induced inhibition of ATP-gated currents of the P2X<sub>4</sub> receptor, a predominant brain P2X receptor. CQ reduced concentration-dependently the Cu(II) inhibition of the ATP-gated currents. In view that the stability constant of CQ for Zn(II) is similar to that of Aβ-amyloid for Zn(II), and the fact that CQ may form complexes with Cu(II), even in the presence of competing ions, the present results highlight that the formation of Cu(II) CQ complexes in the brain may act by diminishing free Cu(II) concentrations modifying thereby brain excitability, or favoring the degradation of β-amyloid plaques or huntingtin, rather than through a specific effect of CQ itself.

**Keywords:** Clioquinol; Oxine; Cu(II); Zn(II); Clioquinol complexes; Neurodegenerative diseases; Alzheimer's and Huntington's diseases

---

## 1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by deposits of extracellular amyloid plaques in the brain cortex. A main constituent of these plaques is β-amyloid, a 42-amino acid peptide found complexed with trace

metals specially Zn(II), Cu(II) and Fe(III). *In vitro* assays showed that Zn(II) and Cu(II) precipitate β-amyloid into insoluble aggregates which are dissolved by metal chelators (Masters et al., 1985; Bush, 2002). This observation prompted the use of trace metal ligands to revert plaque formation, favoring the dissolution of the amyloid deposits. The report that 5-chloro-7-iodo-8-hydroxyquinoline (clioquinol, CQ) attenuated AD symptoms in human clinical trials (Ritchie et al., 2003) incited renewed interest in its pharmacodynamics, linking its possible beneficial therapeutical action to the chelation of Cu(II)/Zn(II) in the brain. Recent reports indicate that CQ also mitigates Huntington's disease neuropathological symptoms in a mouse model of this disorder (Nguyen et al., 2005); CQ treatment decreased the accumulation of huntingtin aggregates,

---

*Abbreviations:* CQ, clioquinol; Ox, 8-hydroxyquinoline; ESR, electron spin resonance; IR, infra red spectroscopy; Cu(II), cupric ion; Zn(II), zinc ion; AD, Alzheimer disease

\* Corresponding author at: Department of Physiology, Neurohumoral Regulation Unit, Faculty of Biological Sciences, P. Catholic University of Chile, P.O. Box 114-D, Santiago 1, Chile. Tel.: +56 2 6862579.

E-mail address: eaferrad@puc.cl (E. Ferrada).

suggesting commonalities in the etiology of both neurological pathologies. These results suggest a broader clinical potential of CQ in the treatment of neurodegenerative diseases.

Until the late sixties, CQ was widely used in the clinic to treat human intestinal amebiasis; this drug was not exempt of adverse effects. It became evident that prolonged treatments with CQ, a halogenated derivative of 8-hydroxyquinoline (oxine, Ox); resulted in optic atrophy particularly in the Japanese population; the CQ–cobalt interaction reduced cobalamin (vitamin B<sub>12</sub>) bioavailability (Goodman and Gilman, 1970). These toxic side effects, together with the introduction of novel antibiotics, largely discontinued its human use. The reevaluation of CQ for the treatment of neurological disorders warrants extended studies on its physicochemical properties and mechanism of action in the CNS.

In spite of successful human trials with CQ for the treatment of AD, the coordination chemistry of this ligand with Cu(II) or Zn(II) and its interaction with  $\beta$ -amyloid in the brain remains unknown. For a better understanding of its mechanism of action, we were incited to study the formation of CQ–metal complexes in a media with an ionic composition similar to the brain extracellular environment. Recently, Di Vaira et al. (2004), reported the X-ray structural characterization of Cu(II) and Zn(II) CQ complexes, synthesized in tetrahydrofuran and other organic solvents. The synthesis and thermodynamic constants of Cu(II) and Zn(II) Ox complexes in organic/water mixtures are known (Johnston and Freiser, 1952; Lane et al., 1960); however, considering the possible application of CQ in the treatment of neurological disorders, we aimed at determining the conditional stability constants ( $K_C$ ) of CQ trace metal complexes in a buffer mimicking the brain extracellular media, competing with divalent cations, such as Ca(II) or Mg(II), common constituents of the brain extracellular fluids. To assess the possible significance of the Cu(II) CQ complexes to synaptic excitability, we examined whether CQ decreased the allosteric inhibition elicited by Cu(II) in the ATP-gated currents of the P2X<sub>4</sub> receptor, the most abundant brain nucleotide receptor (Soto et al., 1996). As a bioassay we used *Xenopus laevis* oocytes injected with the rat P2X<sub>4</sub> cDNA, as previously reported by Coddou et al. (2002). The conditional stability constants ( $K_C$ ) values were determined in the presence of both trace metals, a condition particularly relevant to extrapolate present results to AD therapeutics. Moreover, to interpret the influence of the halogen substitutions in the formation of these metal CQ complexes, we also synthesized and determined the conditional equilibrium constants for Cu(II) and Zn(II) Ox complexes. The present results show for the first time  $K_C$  values for CQ trace metal complexes derived in the presence of both trace metals and other divalent metals, and that CQ blocked concentration-dependently the Cu(II) inhibition of the ATP-gated currents elicited by a brain purinoceptor. We discuss the implications of the present findings at the light of recent publications on the therapeutics of CQ for neurodegenerative diseases, and conclude that CQ increases the availability of intracellular Cu(II).

## 2. Materials and methods

CQ, Ox, HEPES, Cu(II) and Zn(II) chlorides, adenosine 5'-triphosphate (ATP), as the disodium salt, antibiotics and collagenase were purchased from Sigma (St. Louis, MO, USA). All the salts and chemicals used to prepare the Barth's buffer, were analytical grade and purchased from Merck (Darmstadt, Germany). The Barth's buffer composition includes (mM): NaCl, 88; KCl, 1; HEPES, 10; MgSO<sub>4</sub>, 0.82; NaHCO<sub>3</sub>, 2.4; CaCl<sub>2</sub>, 0.91 and Ca(NO<sub>3</sub>)<sub>2</sub>, 0.33 adjusted to pH 7.4.

### 2.1. Preparation of metal CQ complexes

Cu(II) and Zn(II) complexes of CQ or Ox were generated at room temperature (23–25 °C) separately in Barth's buffer by mixing ligand and Cu(II) or Zn(II) in two-fold ligand excess. Due to the low ligand solubility in Barth's, millimolar CQ or Ox solutions were prepared in nitric acid and diluted 1000-fold in Barth's solution. Solid complexes were prepared likewise and crystallized from the Barth's buffer. Microanalysis was used to analyze the stoichiometry of the Cu(II) and Zn(II) CQ or Ox complexes. Titration of ligands with Cu(II) or Zn(II) were analyzed in solution studying the UV–vis spectra; the shift of  $\pi$ – $\pi^*$  and  $n$ – $\pi^*$  transitions indicated metal CQ coordination (Fig. 1).

### 2.2. Procedure to determine the conditional stability constants ( $K_C$ ) for CQ trace metal complexes in Barth's solution

Since this work aimed at determining the  $K_C$  in a media similar to the extracellular brain ionic composition, we performed our studies in a buffer containing divalent cations, such as Ca(II), Mg(II) and trace metals.  $K_C$ 's differ from thermodynamic constants in that the former considers the affinity of a ligand for a particular ion in the presence of a complex mixture of solutes; while the latter is determined in water (standard conditions and ionic strength = 0, at 25 °C); occasionally organic solvents are used. The stoichiometry for each trace metal complex was derived from the absorbance

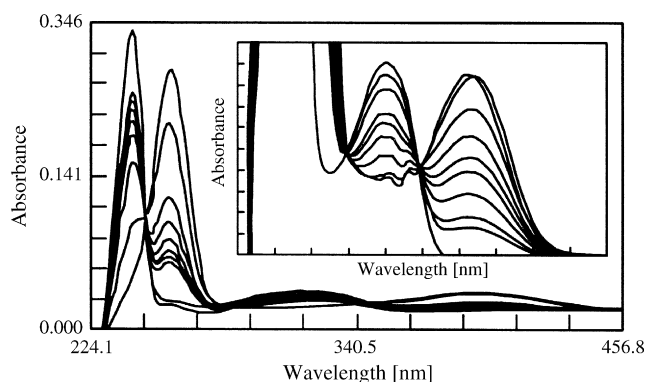


Fig. 1. UV-vis titration of Cu(II) CQ complexes in Barth's solution. The 0.1 mM CQ solution was titrated with CuCl<sub>2</sub>. The spectrum shows 250 and 362 nm signals for the respective transition sets. This last band, correspond to the Cu(II) CQ complex formation. *Inset*: Amplifies isobestic point titrated at 0.1 mM CQ.

versus the metal concentration titration curve; from the inflection point of these curves we inferred approximate  $K_C$ 's. For more precise  $K_C$ 's values, we next used pseudopolarography (Skoog and Leary, 1994; Croot et al., 1999) and determined the  $E^{1/2}$  for each complex. The magnitude of the  $\Delta E$  is directly proportional to  $K_C$ , according to the expression:

$$E_{\text{Cu(II)CQ}}^{1/2} - E_{\text{Cu(II)}}^{1/2} = \frac{0.059}{n \log K_C}$$

Voltammograms of Cu(II) CQ and Zn(II) CQ complexes were scanned between  $-1.3$  and  $-0.3$  V versus Ag/AgCl, at several electrolysis potentials.  $E^{1/2}$  for the Cu(II) or the Zn(II) CQ complexes were obtained from the pseudopolarogram plot (peak current versus electrolysis potential curve, see inset of Fig. 2), allowing the calculation of  $K_C$ 's.

### 2.3. Electrophysiological bioassay using *Xenopus oocytes* microinjected with $P2X_4$ receptors

A segment of the *X. laevis* ovary was surgically removed under anaesthesia; oocytes were manually defolliculated and next incubated with collagenase as detailed by Acuña-Castillo et al. (2000). Oocytes were injected intranuclearly with 3–5 ng cDNA coding for the rat  $P2X_4$  receptor; to record ATP-gated currents, oocytes were clamped at  $-70$  mV using the two-electrode voltage-clamp configuration with an OC-725C clamper (Warner Instruments Corp., Hamden, CT, USA). To allow optimal receptor expression, oocytes were incubated for 36–48 h at  $15$  °C in Barth's solution supplemented with 10 IU/L penicillin plus 10 mg streptomycin and 2 mM pyruvate. ATP-gated currents were recorded following a  $10$   $\mu\text{M}$  ATP

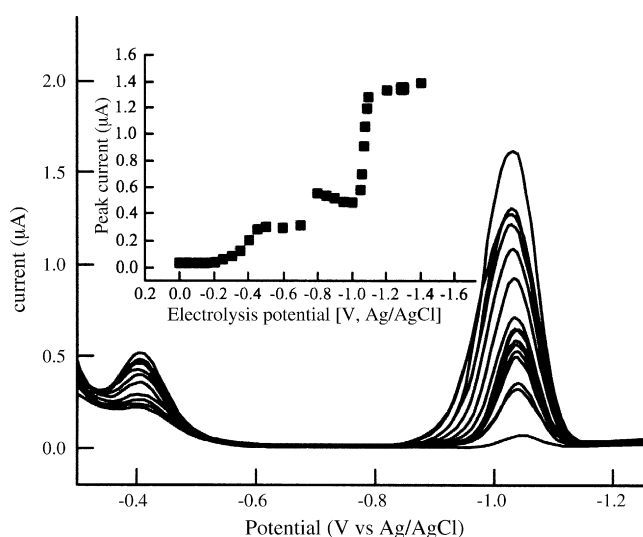


Fig. 2. Determination of conditional stability constants in Barth's solution. The voltammogram allowed derivation of the peak currents ( $-0.4$  V for Cu(II) CQ and  $-1.1$  V for Zn(II) CQ complexes) which were plotted as a function of electrolysis potential (pseudopolarogram, shown in the inset). From the two curve inflections of the pseudopolarogram plot, the  $E^{1/2}$ 's were derived for Cu(II) and Zn(II) CQ complexes; the first corresponds to the Cu(II) CQ while the second corresponds to the Zn(II) CQ complex. Cu(II) and Zn(II) concentrations in the solution were  $1.0$   $\mu\text{M}$ , and CQ  $0.2$  mM (pH 7.4,  $25$  °C). Voltage step:  $5$  mV, amplitude:  $25$  mV; frequency:  $25$  Hz; sweep rate:  $126$   $\text{mV s}^{-1}$ .

application dissolved in Barth's solution for 10 s. ATP and metal chloride salts dissolved in Barth's solution were perfused using a peristaltic pump operating at a constant flow of  $2$  mL/min. ATP ( $10$   $\mu\text{M}$ ) challenges were repeated regularly at 15-min intervals, minimizing receptor desensitization.

## 3. Results

### 3.1. Conditional stability constants for Cu(II) and Zn(II) CQ complexes

The presence of an isobestic point revealed the formation of a unique Cu(II) CQ complex (Fig. 1); titration analysis was consistent with a 1:2 metal ligand ratio. A similar pattern was observed for the Ox complexes, except for the anticipated minor differences in  $\lambda_{\text{max}}$  (Cheatum et al., 2001). Consonant with these results, microanalysis showed a 1:2 metal ligand coordination ratio for both the Cu(II) or Zn(II) CQ solid complexes; IR spectra of these compounds gave further support for the ratio, in agreement with Di Vaira et al. (2004). In contrast, the Cu(II) Ox complex was 1:1, while the ratio with Zn(II) was 1:2, emphasizing the influence of the halogens in the complex formation. The inferred  $K_C$ 's values were confirmed by pseudopolarography and are summarized in Table 1.

The geometry of the complexes generated in a biological buffer were characterized by a likely square planar or tetragonal Cu(II) CQ coordination geometry. ESR spectra complex showed  $g_{\parallel} = 2.167$  G,  $g_{\perp} = 2.067$  and  $A_{\parallel} = 77.88$  G values. The hyperfine ESR spectrum pattern agreed with the 1:2 metal ligand stoichiometry of CQ complexes. Additionally, Spartan calculations of the CQ complexes, confirmed a planar-tetragonal conformation characterized by a  $53^{\circ}$  angle between planes formed by the two ligands. Altogether our findings are consistent with the crystallography of the Zn(II) CQ complex reported by Di Vaira et al. (2004) obtained in tetrahydrofuran. In contrast, the Zn(II) CQ complex revealed a planar conformation in agreement with reports in the solid state (Di Vaira et al., 2004; López et al., 1998). Although these calculations are oversimplified, the results suggest that the geometry of the CQ and Ox complexes are different, revealing the influence of the halogen inductive effect.

### 3.2. CQ blocked the Cu(II)-evoked inhibition in $P2X_4$ receptor expressed in *X. laevis* oocytes

CQ blocked concentration-dependently the  $10$   $\mu\text{M}$  ATP-gated currents elicited in this receptor bioassay. Application of

Table 1  
Conditional equilibrium constants for trace metals with CQ and Ox in Barth's media derived by pseudopolarography

Ligand	Metal	Ratio (M:L)	$K_C$ ( $\times 10^8 \text{ M}^{-2}$ )
CQ	Copper	1:2	120
	Zinc	1:2	7
Ox	Copper	1:2	800
	Zinc	1:2	20

10  $\mu\text{M}$  Cu(II) for 1-min inhibited  $81.3 \pm 6.4\%$  the 10  $\mu\text{M}$  ATP-evoked currents; the magnitude of the ATP-evoked current gradually augmented when the metal was co-incubated with larger CQ concentrations (30–100  $\mu\text{M}$ , Fig. 3). CQ alone did not interfere with the ATP-gated currents.

#### 4. Discussion

$K_C$ 's are key for quantifying and therefore understanding reactions that may be relevant to biology and therapeutics of drugs and their interactions with endogenous substances.  $K_C$  depend on pH, solvent, and the concentrations of competing cations which in this case included divalent ions and trace metals, such as Cu(II) and Zn(II), normal constituents of biological fluids. Several methods for  $K_C$  determinations are routinely used, including spectrophotometric titration and voltammetry; both methods were used in the present study.

$K_C$ 's inferred from the spectroscopic and polarographic methods were almost coincident, indicating the consistency of these methodologies. The  $K_C$ 's for Cu(II) or Zn(II) CQ complexes derived in Barth's buffer are the first reported values relevant to the therapeutic use of CQ. We are aware that the  $K_C$  determinations performed in a solvent without competing ions, the values would likely have been larger, since the determinations for the formation of CQ complexes with Ca(II) or Mg(II) are orders of magnitude less (Angel et al., 2002).

The P2X<sub>4</sub> receptor is the most abundant brain P2X receptor (Soto et al., 1996); it is inhibited by free Cu(II), as reported by Acuña-Castillo et al. (2000) and Coddou et al. (2002). The bioassay used to examine synaptic activity was also conducted in Barth's media where we observed that CQ decreased the Cu(II) inhibition of this ATP-gated channel. The most likely interpretation for this finding indicates that CQ diminishes the free Cu(II) concentration available to interact with the P2X<sub>4</sub> receptor; consequently, the ATP-gated currents elicited by the

activation of this receptor increase, since Cu(II) is an allosteric inhibitor modulator of the P2X<sub>4</sub> receptor (Acuña-Castillo et al., 2000). Two factors may account for the fact that the bioassay results do not conform to the 1:2 metal ligand ratio as expected from the stoichiometry of the Cu(II) CQ complex. On the one hand, the P2X<sub>4</sub> receptor has its own affinity for Cu(II) at the allosteric modulator site of the receptor; therefore, free Cu(II) competes between CQ and the receptor. The finding that even at 100  $\mu\text{M}$  CQ the effect of Cu(II) did not completely overcome the Cu(II)-induced inhibition, suggests that the affinity of CQ for Cu(II) is less than that of the P2X<sub>4</sub> receptor for the metal. Second, it is improbable that each oocyte attains the same level of receptor concentration, which varies depending on the level of cDNA expression per oocyte. Altogether the present findings highlight common features between the eventual formation of CQ trace metal complexes in the brain and the predicted biological properties. The present results fully support and strengthen the view that CQ forms metal coordination complexes, in agreement with the proposal that the beneficial clinical effect of CQ in AD treatment might be due to the reduction  $\beta$ -amyloid deposits in the brain since CQ removes trace metals from the amyloid plaques (Bush, 2003). We conclude that the beneficial effect of CQ is likely related to the formation of CQ Cu(II) complexes rather than a specific action of CQ by itself, since other endogenous or exogenous compounds that chelate Cu(II) or Zn(II) have similar properties in the P2X<sub>4</sub> receptor bioassay. Noteworthy, the endogenous dipeptide carnosine and a segment of human prion protein also decreased the Cu(II)-induced inhibition of the P2X<sub>4</sub> receptor activity in the oocyte bioassay, an action interpreted to be due to the free Cu(II) concentration as demonstrated by Coddou et al. (2002) and Lorca et al. (2003). Moreover, in the same study, Coddou et al. (2002) and Lorca et al. (2003) observed that these peptides were selective for Cu(II) and not for Zn(II), consistent with their differential affinities for these trace metals. However, we cannot discard that CQ may evidence possible side effects unrelated to its trace metals chelator properties, but indirectly related to its action on brain excitability or other properties, as yet not clarified.

Chemists have known for over 50 years the thermodynamic equilibrium constants for the formation of Cu(II) and Zn(II) Ox complexes; however, these values were derived in 50% dioxane–water solution (Johnston and Freiser, 1952). The present determinations in Barth's buffer as well as that of Johnston and Freiser (1952), coincide to indicate that the constants are more than one order of magnitude larger for Cu(II) than for Zn(II). Due to the instability of Cu(I) it is highly unlikely that this metal specie participates in these reactions. Consonant with the higher affinity of Ox for Cu(II), the constants for a series of structural analogs related to 4-hydroxybenzimidazole are 1–2 orders of magnitude larger for Cu(II) than Zn(II) (Lane et al., 1960). The CQ  $K_C$ 's also show a higher affinity for Cu(II) than Zn(II), however the difference in the magnitude of these constants (Ox versus CQ), is two-fold less (Table 1); a finding that may have implications for the therapeutic use of CQ. The 17-fold higher affinity of CQ for Cu(II) than for Zn(II), strengthens the relevance of CQ in

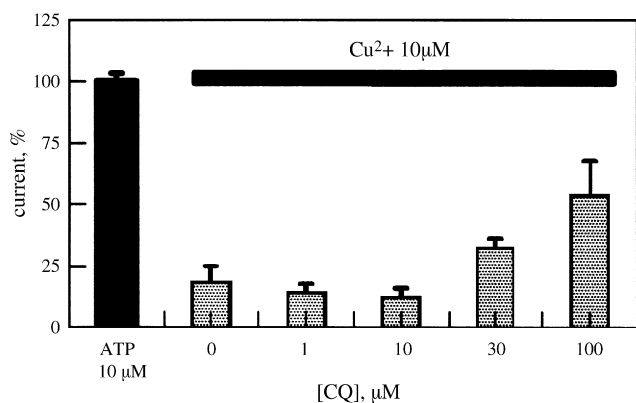


Fig. 3. CQ reduced concentration-dependently the ATP-gated current in oocytes expressing the brain P2X<sub>4</sub> receptors. *X. laevis* oocytes expressing rat P2X<sub>4</sub> receptors consistently showed that 10  $\mu\text{M}$  ATP elicited inward currents that were reversibly inhibited by a 1-min incubation with 10  $\mu\text{M}$  Cu(II). Co-incubation of Cu(II) with increasing concentrations of CQ reduced proportionally the Cu(II)-evoked inhibition. Solid column represent the currents gated by 10  $\mu\text{M}$  alone; grey columns represent the current gated by 10  $\mu\text{M}$  ATP in the presence of varying concentrations of CQ. Columns indicate mean values; bars indicate the standard error of the mean.

human therapeutics. The estimated concentration of Cu(II) or Zn(II) in the  $\beta$ -amyloid plaques may reach *circa* 0.4 and 1 mM, respectively, values that favor the formation of CQ metal complexes in the brain. Although CQ showed a preferential affinity for Cu(II) and its concentration in the  $\beta$ -amyloid plaques is lower than Zn(II), we cannot ignore that CQ may also interact with Zn(II), in view of its preponderant brain concentration. Unfortunately, at present we do not have estimates of the CQ concentration that reaches the brain and are found in the plaques of patients with AD. The use of CQ in clinical trials is now supplemented with vitamin B<sub>12</sub>, avoiding the recognized neuropathy associated to CQ toxicity (Takeshi, 2001).

While the CQ  $K_C$  for Zn(II) are in the same range as that of  $\beta$ -amyloid for Zn(II); the affinity of  $\beta$ -amyloid for Cu(II) is in the atto molar range (Bush, 2003), a finding that may be relevant to the pharmacodynamics of CQ. Moreover, considering that Cu(II) is more neurotoxic than Zn(II), two mechanisms may be proposed in relation to the clinical effect of CQ: (1) the CQ Zn(II) complex may account for partial dissolution of  $\beta$ -amyloid Zn(II) complexes; (2) CQ may facilitate metal uptake by neurons and/or promote diffusion of the Cu(II) CQ complex into neurons, a finding observed by Treiber et al. (2004); CQ's lipophilicity may support this proposal. At the light of the finding that Cu(II) may either increase the synthesis of proteins, such as huntingtin (Nguyen et al., 2005), or prion protein (Varela-Nallar et al., 2005), or attenuate the processing of  $\beta$ -amyloid precursor protein reducing  $\beta$ -amyloid formation (Treiber et al., 2004), the total increase of neuronal Cu(II) concentration may ultimately change gene expression. In agreement with this proposal, White et al. (2006) reported that an increase in intracellular Cu(II) resulted in a selective up regulation of metalloprotease activity, which in the extracellular matrix could rapidly degrade the  $\beta$ -amyloid peptide. In view that intracellular Cu(II) is tightly bound to chaperones, the elucidation of the mechanism of Cu(II) transcription elements warrants further research in the etiology of neurodegenerative pathologies.

In sum, the  $K_C$ 's determinations that we now report, strengthen the most likely formation of CQ Cu(II) over CQ Zn(II) complexes in the brain, highlighting the basis for the beneficial action of CQ in the treatment of AD. The formation of Cu(II) CQ complex ultimately alters free Cu(II) concentrations, increasing the intracellular availability of Cu(II) which favors the degradation of  $\beta$ -amyloid plaques or huntingtin, rather than by a specific effect of CQ itself. Moreover, the present  $K_C$ 's values are in agreement with current hypothesis related to the CQ-induced changes in intracellular Cu(II) availability in neurons.

## Acknowledgments

We appreciate the assistance of P. Ramdohr with the art graphics and R. Lorca with the oocyte bioassay. The International Copper Association (ICA), and Center for Cell Regulation and Pathology (grant 13980001) funded part of this

research. The Millennium Institute for Fundamental and Applied Biology (MIFAB) contributed to funding the Center.

## References

- Acuña-Castillo C, Morales B, Huidobro-Toro JP. Zinc and copper modulate differentially the P2X<sub>4</sub> receptor. *J Neurochem* 2000;74:1529.
- Angel I, Bar A, Horovitz T, Taler G, Krakovsky M, Retnisky D, et al. Metal ion chelation in neurodegenerative disorders. *Drug Dev Res* 2002;56:300.
- Bush AI. Metal complexing agents as therapies for Alzheimer's disease. *Neurobiol Aging* 2002;23:1031.
- Bush AI. The metallobiology of Alzheimer's disease. *Trends Neurosci* 2003;4:207.
- Coddou C, Villalobos C, González J, Acuña-Castillo C, Loeb B, Huidobro-Toro JP. Formation of carnosine-Cu(II) complexes prevents and reverts the inhibitory action of copper in P2X<sub>4</sub> and P2X<sub>7</sub> receptors. *J Neurochem* 2002;80:626.
- Croot PL, Moffett JW, Luther GW. Polarographic determination of half-wave potentials for copper organic complexes in sea water. *Mar Chem* 1999;67:219.
- Cheatum CM, Heckscher MM, Crim FF. Excited-state dynamics in 8-hydroxyquinoline dimers. *Chem Phys Lett* 2001;349:37.
- Di Vaira C, Bazzicalupi C, Orioli P, Messori L, Bruni B, Zatta P. Clioquinol a drug for Alzheimer's disease specifically interfering with brain metal metabolism: structural characterization of its zinc(II) and copper(II) complexes. *Inorg Chem* 2004;43:3795.
- Johnston WD, Freiser H. Structure and behavior of organic analytical reagents. III. Stability of chelates of 8-hydroxyquinoline and analogous reagents. *J Am Chem Soc* 1952;74:5239.
- Lane TJ, Sam CS, Kandathil AJ. Chelate stabilities of certain oxine-type compounds. *J Am Chem Soc* 1960;82:4462.
- Lorca RA, Chacon M, Barria MI, Inestrosa NC, Huidobro-Toro JP. The human prion octarepeat fragment prevents and reverses the inhibitory action of copper in the P2X<sub>4</sub> receptor without modifying the zinc action. *J Neurochem* 2003;85:709.
- López R, Boys D, Loeb B, Zuloaga F. Synthesis, crystal structure, molecular orbital calculations and electronic properties of 2,3-di(2-pyridyl)naphtho[2,3-f]quinoxaline-7,12-quinone (Aqdpq). *J Chem Soc Perkin Trans* 1998;2:877-83.
- Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down Syndrome. *Proc Natl Acad Sci USA* 1985;82:4245.
- Nguyen T, Hamby A, Massa S. Clioquinol down-regulates mutant huntingtin expression in vitro and mitigates pathology in a Huntington's disease mouse model. *Proc Natl Acad Sci USA* 2005;102:11840.
- Ritchie CW, Bush AI, Mackinnon A, Macfarlane S, Mastwyk M, MacGregor L, et al. Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting A $\beta$  amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial. *Arch Neurol* 2003;60:1685.
- Skog D, Leary J. Instrumental analysis. McGraw-Hill; 1994.
- Soto F, García-Guzmán M, Gómez-Hernández JM, Hollmann M, Karschin C, Stühmer W. P2X<sub>4</sub>: an ATP-activated ionotropic receptor cloned from rat brain. *Proc Natl Acad Sci USA* 1996;93:3684.
- Takeshi T. Clioquinol's return: cautions from Japan. *Science* 2001;292:2251.
- Treiber C, Simons A, Strauss M, Hafner M, Cappai R, Bayer TA, et al. Clioquinol mediates copper uptake and counteracts copper efflux activities of the amyloid precursor protein of Alzheimer's disease. *J Biol Chem* 2004;279:51958.
- Varela-Nallar L, Toledo EM, Larrondo LF, Cabrol AL, Martins VR, Inestrosa NC. Induction of cellular prion protein gene expression by copper in neurons. *Am J Physiol Cell Physiol* 2005;290:C271.
- White AR, Du T, Laughton KM, Volitakis I, Sharples RA, Xilinas ME, et al. Degradation of the Alzheimer's disease amyloid beta peptide by metal-dependent up-regulation of metalloprotease activity. *J Biol Chem* 2006;281:17670.