Copper complexing decreases the ability of amyloid beta peptide to cross the BBB and enter brain parenchyma

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1. Introduction

Alzheimer’s disease (AD) is a neurodegenerative disorder that affects the cognitive functions of the brain. It is characterized pathologically by cerebral deposits of extracellular amyloid plaques and intracellular neurofibrillary tangles in the brains of the affected individuals. Amyloid beta proteins (Aβs) are the prime components of senile plaques [5,22]. Aβ1-40 isoform is the major soluble species in biological fluids while Aβ1-42 (Aβ42) is the predominant species found in plaque deposits [1,27,30]. There are several studies which propose that AD brain is subjected to Aβ-induced oxidative stress. The neurotoxicity associated with these peptides requires the conversion of the soluble monomers to β-sheet conformation and subsequent aggregation into a structural fibrillar form. The role of metal ions like copper (Cu), iron (Fe), zinc (Zn), and aluminum (Al) have been implicated in the formation of Aβ aggregates and fibrillation [6,12,23]. A recent study reported a detailed characterization of the changes in conformational and aggregational properties of different amyloid sequences induced by Al [23]. The same study showed that Cu and Zn were much less efficient than Al in stimulating the...
spontaneous aggregation/fibrillogenesis of Aβs. However, in another study, Cu (II) was shown to cause the peptide to aggregate to a greater extent than Fe (III). Aβ possesses high-affinity binding site for Cu that mediates its protease resistance and reversible precipitation. Aβ avidly binds Cu in 1:2 ratio to form a redox active complex. A report suggested that the formation of Aβ–Cu complex enhanced the toxicity of Aβ in primary neuronal cultures that was mediated by extracellular generation of H2O2. Furthermore, it was shown that this complex acted as a cheloler allele oxidase enzyme that catalyses cholesterol to generate H2O2 [21]. Thus, the interaction between metal ions and Aβ leads to increased oxidative stress which is mainly observed in brain regions with amyloid deposition [18,20]. Moreover, complex formation with these metal ions might alter the permeability of Aβ across the BBB. Complexing with Al increases the uptake of Aβ in to the brain. Because of the significance of Aβ–Cu interactions to the pathophysiology of amyloid deposition in brain, we propose that copper might alter the permeability of Aβ across the BBB and its clearance from brain.

In this study, the effect of complexing with copper on the ability of Aβ to cross the BBB was determined by using both rat and human Aβ (42mer) peptides. Also we have analyzed whether chelation with Cu would alter Aβ-induced oxidative stress in an immortalized line of rat brain endothelial cells (RBE4). We determined the rate of clearance of Aβ–Cu from the brain and the blood and also its uptake by brain, liver, kidney, and heart.

2. Materials and methods

2.1. Chemicals

Human and Rat Aβ (Aβ42) was purchased from Biosource (Camarillo, CA). α-MEM (12561-056), Ham’s F-10 (12390-035), GlutaMAX-I (35050-061), and Geneticin (10131-035) are purchased from Gibco. The N-(1-pyrenyl)-maleimide (NPM) was purchased from Aldrich (Milwaukee, WI, USA). High-performance liquid chromatography (HPLC) grade solvents were purchased from Fisher Scientific (Fair Lawn, NJ). Copper chloride (99%), bFGF (F0291), HEPES, Bovine serum albumin, and all other chemicals were purchased from Sigma (St. Louis, MO, USA).

2.2. Radioactive labeling and purification of the peptides

Five micrograms of a peptide was dissolved in 50 μl of chloride free phosphate buffer (pH 7.4) containing two microCi of radioactive 131I. Ten micrograms of chloramine-T was added to this mixture and after 1 min the resulting radioactively labeled Aβ was purified from unreacted 131I by elution on a G-10 sephadex column.

2.3. Complexing

Radioactively labeled Aβ was used to prepare the complex, whereases, unlabeled peptide was used for the MTS assay and thiol measurement. The complex between Aβ and Cu was prepared according to the procedure described by Fuglielli et al. [21]. In brief, Cu:glycine (Gly) adduct was prepared by adding Cu and Gly in 1:6 ratio with bidistilled MilliQ water. The level of metal contamination in the solvent was assayed by atomic absorption mass spectroscopy. In all preparations, metal traces were below the detection limit. All chemicals used were of the highest purity grade. Aβ was added to Cu:Gly adduct in 1:2 ratio and allowed to react for 15 min at 20 °C. One microliter of the resulting solution was counted for 3 min in a gamma counter. The solutions for injection were prepared from this stock solution and the level of radioactivity was adjusted with Ringer’s lactated solution as required.

2.4. In vitro studies

An immortalized line of rat brain endothelial (RBE4) cells, kind gift of Roux [24], were grown in a α-MEM: Ham’s F-10 (1:1) medium supplemented with 1% (v/v) GlutaMAX-I, and BSA; 0.1% (v/v) bFGF; 0.6% Geneticin; and 0.75% HEPES. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO2. All the experiments were performed with serum-free media.

2.4.1. MTS (cell viability) assay

MTS assay (Cell Titer 96 aqueous one solution cell proliferation assay, Promega) was performed according to the procedure provided with the kit. This is a cell proliferation assay in which the administered 3-(4,5-dimethyl thiazol-2-yl)-5-(3-carboxymethoxy phenyl)-(2-(4-sulfophenyl))-2H-tetrazolium inner salt (MTS) [7] is bioreduced by viable cells to a colored formazan product that is soluble in media. In brief, cells were treated for 24 h with Aβ (rat/human; 200 nM), Aβ–Cu (200:400 nM; rat/human), or Cu only (400 nM) containing media. After the incubation period, MTS was added to each well. Two hours later, the absorbance was read at 490 nm; absorbance readings were proportional to the number of viable cells.

2.4.2. Thiol measurement

Cellular levels of thiols were determined by the method previously published as developed in our laboratory [31]. Cells were seeded on poly-α-lysine coated (0.05 mg/ml) 25 cm2 flasks (5 ml/flask). After reaching 80% confluence, flasks were washed and the cells received media containing Aβ (rat/human; 200 nM), Aβ–Cu (200:400 nM; rat/human), or Cu only (400 nM). The control groups received fresh media. After 24 h, the cells were removed from the cultures and suspended in serum borate buffer (100 mM Tris–HCl, 10 mM boric acid, 5 mM l-serine, 1 mM DETAPAC, pH 7.4). Twenty microliter of the diluted cell homogenate was added to 230 μl of 2N HCl. After reaching 80% confluence, flasks were washed and the cells received media containing Aβ (rat/human; 200 nM), Aβ–Cu (200:400 nM; rat/human), or Cu only (400 nM). The control groups received fresh media. After 24 h, the cells were removed from the cultures and suspended in serum borate buffer (100 mM Tris–HCl, 10 mM boric acid, 5 mM l-serine, 1 mM DETAPAC, pH 7.4). Twenty microliter of the diluted cell homogenate was added to 230 μl of serum borate buffer and 750 μl of NPM (1 mM in acetonitrile). The resulting solutions were incubated at room temperature for 5 min. The reaction was stopped by the addition of 5 μl of 2N HCl. The samples were then filtered through a 0.2 μm acrodisc filter and injected onto the HPLC system.

2.4.3. Efflux and Influx rates

RBE4 cells were used 5–8 days after subculture to prepare monolayers on transwell filters with a pore size of 0.4 μm. These filters were inserted into the wells of the 24-well plate and used as the in vitro BBB model. The efflux (brain-to-blood direction) and influx (blood-to-brain direction) rate of Aβ
transported across the RBE4 monolayer was reported as the permeability coefficient. The culture media was removed from the inserts with the RBE4 cells were washed twice with serum-free media. One hundred microliter of the fresh media was added to the luminal side (inside) or collecting chamber of the transwell insert. The efflux process was initiated by loading 600 μl serum-free media containing radiolabeled peptide (1 \times 10^7 \text{ cpm/ml}) on the abluminal or loading chamber (outside of the insert). The initial radioactivity in the loaded solution (600 μl) was checked. Samples (90 μl) from the collecting chamber were removed at 15, 30, 60 and 90 min and immediately replaced with an equal volume of media. Serum-free media was added to these samples to make up the total volume to 600 μl. For influx experiments, 100 μl of the radioactively labeled peptide (1 \times 10^7 \text{ cpm/ml}) was loaded in the luminal chamber and 600 μl of serum-free media in the abluminal chamber. Five hundred microliter of the sample volumes were collected at 15, 30, 60 and 90 min and immediately replaced with equal volume of media. At the end of the experiment, the contents of both the chambers were collected. All the samples were mixed with 600 μl of 30% trichloroacetic acid (TCA; final concentration 15%) and centrifuged at 5400 \times g for 15 min at 4 °C. The supernatant was discarded and the radioactivity was measured in the TCA precipitates and RBE4 cells attached to the transwell filters in a gamma counter. The permeability coefficient and clearance of TCA-precipitable radiolabeled peptide were calculated according to the method described by Dehouck et al. [9].

In brief, permeability coefficient was calculated as described in Eq. (1):

$$\frac{1}{P_{\text{app}}} = \frac{1}{P_{\text{membrane}}} + \frac{1}{P_{\text{trans}}}$$

(1)

where $P_{\text{app}}$ represents the rate of clearance at which \(^{131}\text{I}-\)labeled proteins are moving from the abluminal to luminal chamber across RBE4 monolayer and Transwell\textsuperscript{\textregistered} filter. $P_{\text{membrane}}$ denotes the slope of the clearance curve with a Transwell\textsuperscript{\textregistered} filter membrane without RBE4 cells.

The $P_{\text{trans}}$ values were divided by the surface area of the Transwell\textsuperscript{\textregistered} inserts (0.33 cm\textsuperscript{2}) to generate the permeability coefficient ($P_{\text{trans}}$, in cm/min).

Clearance is expressed as microliters (μl) of radiolabeled peptide diffusing from the abluminal to luminal (efflux) chamber and is calculated from the initial concentration of tracer in the loading chamber and final concentration in the collecting chamber as given in Eq. (2):

$$\text{Clearance (μl)} = \left| \frac{C_L}{C_C} \right| \times \frac{V_C}{t}$$

(2)

where $|C_L|$ is the initial radiolabeled peptide concentration of loading chamber (in cpm/μl), $|C_C|$ is the radiolabeled peptide concentration of collecting chamber (in cpm/μl), and $V_C$ is the volume of collecting chamber (in μl).

2.5. In vivo studies

The 2-month old male CD-1 mice from our in-house colony (VA-St. Louis) were used for the experiments. The mice were housed in a temperature-controlled (25 °C) room equipped to maintain a 12 h light–dark cycle. Standard rat chow (Purina rat chow) and water were given ad libitum.

2.5.1. Blood-to-tissue influx rates

Male CD-1 mice (2-month old) were anesthetized with an i.p. injection of 0.2 ml of urethane (40% solution). The left jugular vein and the right carotid artery were then exposed. The mice were given an injection into the left jugular vein of 0.2 ml of Ringer's lactated solution (LR) containing 500,000 cpm of the radioactive peptide. Blood was collected from the right carotid artery and the whole brain along with other tissues including liver, kidney, and heart were removed and weighed between 1 and 10 min after the i.v. injection. The whole blood was centrifuged at 5400 \times g for 10 min at 4 °C. The level of radioactivity was measured in 50 μl of the resulting serum and all the tissues with a gamma counter for 3 min. Tissue/serum ratios were calculated by the formula:

$$\frac{\text{Tissue cpm} / \text{g-tissue}}{\text{Serum cpm} / \mu l - \text{serum}}$$

The tissue/serum ratios were plotted against exposure time. The exposure time values were obtained by the following equation:

$$\text{Exposure time (min)} = \int_{t}^{\infty} \frac{C_p(t)}{C_{pt}} \, dt$$

where $C_p(t)$ is the level of radioactivity in one microliter of serum at time $t$ and $r$ is a dummy variable for time.

2.5.2. Clearance from blood

The level of radioactivity in 1 ml of serum measured in the influx study above was divided by the amount of radioactivity injected intravenously and multiplied by 100 to yield the percent of the intravenous injection present in 1 ml of serum (%Inj/ml). The log of %Inj/ml was plotted against time and the half-time clearance calculated by multiplying the inverse of the slope by 0.301.

2.5.3. Capillary depletion

Male CD-1 mice (2-month old) were anesthetized with an i.p. injection of 0.2 ml of urethane (40% solution). The left and right jugular veins were exposed and 0.2 ml of Ringer's lactated solution (LR) containing 500,000 cpm each of \(^{131}\text{I}-\text{Aβ}\) and \(^{125}\text{I}-\text{albumin}\) (the albumin used as a measure of the vascular space of the brain) and was injected into the jugular vein. At 10 min after the injection, the abdomen was opened and arterial blood was collected from the abdominal aorta. The thorax was then opened, the descending aorta clamped, both jugular veins severed, and 20 ml of LR perfused in less than 1 min through the left ventricle of the heart. After that, the mouse was decapitated and the cortex from the whole brain isolated, weighed, and placed in an ice-cold glass homogenizer. The brain was homogenized by 10 vertical strokes in 0.8 ml of physiological buffer (pH 7.4; 10 mM HEPES, 141 mM NaCl, 4 mM KCl, 2.8 mM CaCl\textsubscript{2}, 1 mM MgSO\textsubscript{4}, 1 mM Na\textsubscript{2}PO\textsubscript{4}, and 10 mM glucose). Dextran solution (1.6 ml of a 26% solution) was added to the homogenate, mixed, and
homogenized with an additional three vertical strokes. The homogenate was centrifuged at 5400 \( \times g \) for 15 min at 4 °C. The resulting supernatant (brain parenchymal fraction) and the pellet (capillary fraction) were separated. The levels of radioactivity in the capillaries and brain parenchymal fractions were counted in a gamma counter for 3 min. The results were corrected for \(^{125}\)I-albumin and expressed as the capillary/serum ratio and parenchyma/serum ratio (\( \mu l/g \)).

2.5.4. Brain-to-blood efflux
The intracerebroventricular (icv) injection method was used to study in vivo brain-to-blood passage. This method assesses BBB function at both the vascular and choroid plexus [15,16]. A hole in the skull 0.5 mm caudal and 1 mm lateral from the bregma and 3 mm deep was made in mice under urethane anesthesia. One microliter/mouse of a solution containing \( 5 \times 10^6 \) cpm of a radioactive peptide was injected through the hole. At 0, 2, 5, 10, and 20 min after the injection, the mouse was decapitated under anesthesia and the whole brain was harvested and weighed. The 0-min time point was performed on mice overdosed with 40% urethane and the icv injection was given between 10 and 30 min after death. For this group, the mouse was decapitated 10 min after icv injection. The radioactivity of the whole brain was measured in a gamma counter. The results were reported as log of brain cpm plotted against time. The slope of this line was multiplied by 0.301 to yield the half-time clearance.

2.6. Statistics
Means are presented with their standard errors. Two means were compared by Student’s t-test. Linear regression analysis was performed with the least sum of the squares method. Regression lines are reported with their \( r \) and \( p \) values. The slopes are reported with their standard deviation of the residuals and compared statistically with the software in Prism 4.0 (GraphPad, Inc., San Deigo, CA).

3. Results

3.1. Cell viability of RBE4 cells
The cell viability of RBE4 cells within each group \( (p < 0.05) \) is compared in Fig. 1. A statistically significant difference is observed between control and rat Aβ–Cu \( (p < 0.01) \). As shown in figure, Cu reduced the viability when bound to rat Aβ by 32% \( (p < 0.05) \) compared to the rat Aβ only group or the Cu only group. No statistically significant difference in the cell viability of RBE4 cells existed between other groups. Also Cu did not affect the viability compared to the control group \( (p > 0.05) \).

3.2. Thiol levels in RBE4 cells
No statistically significant differences were observed among groups for GSH and CYS levels. HCYS levels were reduced by rat Aβ–Cu, human Aβ, human Aβ–Cu, and Cu treatments \( (p < 0.01) \). Aβ did not enhance the decrease in HCYS induced by Cu alone (Fig. 2).

Fig. 1 – Cell viability. Effect of Cu on rat and human Aβ-induced cytotoxicity. Cells were exposed for 24 h to rat Aβ (200 nM), human Aβ (200 nM), rat Aβ + Cu (200 + 400 nM), human Aβ + Cu (200 + 400 nM) or Cu alone (400 nM). Cell viability was quantified by the MTS assay. Values represent means ± S.D. Statistically different values *\( p < 0.01 \) compared to control group, **\( p < 0.05 \) compared to rat Aβ + Cu were determined \( (n = 3) \).

Cu increased the in vitro efflux, but not the influx, rate of rat Aβ (Fig. 3): \( t = 3.68, df = 22, p < 0.005 \). With human Aβ, Cu had no statistically significant effect on either the efflux or influx rates.

3.3. Blood-to-tissue uptake rates of rat Aβ and Aβ–Cu
The uptake rates \( (K_i) \) of rat Aβ and Aβ–Cu for all the tissues were compared in Fig. 4. A statistically significant relation between tissue/serum ratios and exposure times existed for all tissues in both groups, indicating time-dependent sequestration of the Aβs by these tissues (Table 1). The relation between brain/serum ratios and exposure time for Aβ and Aβ–Cu demonstrates that both compounds were taken up by the BBB over time. Similarly, both compounds were taken up by the liver, kidneys, and heart at different rates, as measured by the formula for \( K_i \). Unlike for other tissues, a barely significant difference was observed in the uptake rates of the kidneys for...
3.4. Clearance of rat Aβ and Aβ–Cu from blood

A significant relation existed between log (% Inj/ml) and time for both Aβ (n = 10 time points, 3 mice/time point, \( r = 0.684, \ p < 0.0001 \)) and Aβ–Cu (n = 10 time points, 3 mice/time point, \( r = 0.727, \ p < 0.0001 \)), demonstrating that they are cleared from the blood. The apparent volume of distribution (Vd) in serum differed significantly (\( p < 0.0001 \)) between rat Aβ and Aβ–Cu while the overall disappearance rate remained unchanged. The lower Vd for Aβ–Cu shows it had a slightly larger volume of distribution. The half-time clearance from blood was 6.8 min for Aβ and 5.8 min for Aβ–Cu. No statistically significant difference existed between the slopes of the lines used to generate the half-time clearances, indicating no difference in the rate at which these compounds were cleared from the blood (Fig. 5).

3.5. Capillary depletion

Capillary depletion was performed to determine whether copper complexing altered sequestration of Aβ protein by brain capillaries after its intravenous injection. The study was performed with both rat and human Aβ and the results reported as parenchyma/serum and capillary/serum ratios (µl/g) in Fig. 6. The t-tests comparing rat or human Aβ–Cu were each significantly different from Aβ for the parenchyma/serum ratio (rat: \( p < 0.05, \ t = 3.11, \ df = 7 \); human: \( p < 0.05, \ p < 0.05 \).

Fig. 3 – Effect of Cu on the in vitro influx and efflux rates of Aβ42. (a) rat Aβ (b) human Aβ. ‘Control’ represents Aβ alone group and ‘+Cu’ represents Aβ–Cu group. Values represent means ± S.E. Statistically different values were found to be **p < 0.005 compared to corresponding control group.

Fig. 4 – Multiple regression analysis for rat Aβ and rat Aβ–Cu. The slope of the line measures \( K_i \), the uptake rates from blood into the tissues. y-axis intercept measures \( V_i \), the apparent volume of distribution. Each time point is the mean of 3–4 mice. No significant difference was found in the uptake rates by the tissues between Aβ and Aβ–Cu.
3.6. The brain-to-blood efflux of human and rat Ab

The log of brain cpm at 0, 2, 5, 10, and 20 min after icv injection of either human or rat Ab is shown in Fig. 7. A significant relation existed between log cpm in brain versus time for Ab (rat: r = 0.52, p < 0.005; human: r = 0.61, p < 0.001) and Ab–Cu (rat: r = 0.44, p < 0.05; human: r = 0.53, p < 0.01). However, no statistically significant difference was observed between Ab and Ab–Cu in studies for either rat or human Ab.

4. Discussion

4.1. Toxicity studies

Using the 1-42 form of both rat and human Ab peptide, we studied the effect of Cu on (1) the cell death and (2) the thiol status alterations induced by both peptides.

As the first objective in our investigations, we studied the effect of Ab and Cu on the viability of the RBE4 cells. This is the immortalized rat brain capillary endothelial cell line, which is used as an in vitro BBB model [13]. The MTS assay was used to determine the cell viability after 24-h exposure to Ab or Ab–Cu complex. The complex was prepared so that Cu existed as a non-toxic Cu:Gly (1:6) adduct and did not add to the toxic effects of Ab when not bound to it [11]. Cu reduced the cell viability when bound to rat Ab peptide by approximately 33% when compared to control, but Cu did not have the same effect when bound to human Ab. We found no alterations in the viability of cells in the presence of Cu alone (Cu:Gly) and our results are consistent with those reported in a study by Huang et al. [11]. It is stated in the same study that human Ab binds to Cu with great affinity, compared to rat Ab, and leads to its aggregation and an increase in its toxicity. Here, we observed a greater toxicity with rat Ab–Cu than with human Ab–Cu. Huang et al. also reported a 70% decrease in the survival of primary neuronal cultures with Ab in the presence of Cu compared to a 40% decrease with Ab alone. The dissimilarities in the results observed in our study compared to Huang et al. study can be attributed to the differences in the cell lines and the dosage of Ab–Cu used. We assume that RBE4 cell line might be less sensitive to the low doses of Ab–Cu used in our study.

Cell death likely has several contributing factors, one of which is alteration in the thiol status. The thiol status in RBE4 cells was determined by measuring the GSH, CYS, and HCYS levels. Increased CYS and HCYS levels, with unchanged GSH levels found in a study [17]. Not many other studies have focused on the CYS and HCYS levels in a cell model after exposure to Ab. Here, we found that neither rat nor human Ab, Cu, or Cu in combination with the Ab’s showed any statistically significant effect on the GSH and CYS levels. Human Ab and Cu decreased HCYS levels, but their combination did not decrease HCYS more than Cu alone. Elevated HCYS levels in the plasma are considered a risk factor for AD [25]. The reason for this may be the elevated brain levels of Ab induced by increased serum HCYS levels as studied in hyperhomocysteinemic Alzheimer’s mouse model of amyloidosis [19]. A study of the serum of AD patients showed a positive relationship between CYS concentrations and AD [29], and increased HCYS concentrations were also observed in these patients. Above mentioned studies signify the relationship between the serum thiol levels (HCYS, CYS) and cerebral Ab levels or AD. Another study demonstrated that exposure of SH-SY-5Y human neuroblastoma cells to HCYS promotes apoptosis and increases the vulnerability of neurons to Ab-induced cell death [10]. On the other hand, we have studied the effect of Ab on HCYS levels and have observed that exposure to Ab decreased the HCYS levels of RBE4 cells. Also, in our study, a positive relationship existed between HCYS levels and the viability of cells exposed to rat and human Ab or Ab–Cu. However, further investigations are recommended to consider the presence of Cu and the source of Ab. Results from our study and from studies of the plasma samples of AD patients do not clarify whether the abnormalities in HCYS levels in the affected brain regions of AD precede and contribute to the neurodegenerative process.
4.2. Clearance studies

We have demonstrated the effects of copper on (1) the in vitro and in vivo clearance of both human and rat Aβ from brain (brain-to-blood passage), (2) the in vitro and in vivo unidirectional influx (blood-to-brain) rates of both human and rat Aβ, (3) the uptake rates of rat Aβ by different tissues, as measured by multiple-time regression analysis, and (4) the clearance from blood of rat Aβ.

Most of the cells in the body are thought to produce Aβ and this blood-borne peptide might contribute to the brain levels of Aβ. As a cofactor for several enzymes, the availability of copper enhances the interaction and complex formation between Aβ and copper in the blood. So there is a possibility that this complex might have access into the brain across BBB and contribute to the increased amyloid deposition as well as elevated concentrations of copper seen in the amyloid plaques.

The levels of Aβ peptide are in balance between its generation and clearance. To reduce the toxicity and damage associated with it, Aβ has to be removed efficiently from the brain. The LRP receptor that mediates the efflux of the peptide at the BBB seems to be impaired in AD [2,3,26]. Furthermore, it was shown that Aβ itself promotes the proteosome-dependent degradation of LRP [8,28]; thus, Aβ indirectly impairs the availability of its own transporter. Also, other changes in the primary structure of the peptide would alter its transport rate. It can therefore be expected that high affinity binding to metal...
ions of Aβ, with subsequent structural changes, would alter its transportation across the BBB. A recent study showed that aluminum increased the uptake of Aβ into the brain parenchyma, but did not affect its brain-to-blood efflux [4]. Here, we performed in vitro and in vivo experiments to test whether copper would have any similar effects. In vitro studies, copper had no statistically significant effect on the influx rate of rat Aβ, whereas, it significantly increased its efflux rate. Copper had no significant effect on the influx or efflux of human Aβ. This suggests that the three amino acids which differ between human and rat Aβ are important in mediating the Aβ–Cu interactions with LRP. In in vivo studies, copper had no statistically significant effect on the efflux of either rat or human Aβ peptide. This suggests that some endogenous factor in brain tissue counters the effect of copper seen in vitro on rat Aβ efflux.

The in vivo blood-to-brain influx can be divided into two steps: uptake from blood to the capillaries and transfer from the capillaries to the brain parenchymal space (brain cells and interstitial fluid space). This distinction is particularly important for Aβs because they are often sequestered by capillaries [32,14]. The capillary depletion-washout method can be used to subdivide these steps. In this technique, perfusion was used to washout all the vascular contents of the brain and also to remove any substances attached reversibly to the luminal surface of the capillaries. The materials left behind after washout were either sequestered by the endothelial cells or had crossed the BBB completely to enter the brain parenchymal space. Here, we observed that copper decreased the ability of Aβ peptide to cross the BBB and enter into the brain parenchyma. This was the opposite effect that aluminum has [4]. This may be because Cu is less effective in inducing amyloid aggregation/fibrillogenesis [23]. This might indicate a positive relationship between the extent of amyloid aggregation/fibrillogenesis and the access through BBB. About 40% more rat and human Aβ peptide entered into the brain when not bound to copper. Complexing with copper increased the sequestration of peptide by capillaries. This sequestration might increase the toxicity to the endothelial cells and decrease the amounts of the peptide to which brain cells were exposed.

The rate of unidirectional influx into the brain and uptake by other tissues, as measured by multiple-time regression analysis, was not altered for Aβ–Cu compared to Aβ, except for the kidney. The initial volume of distribution of Aβ–Cu into the serum increased significantly, compared to Aβ as determined by Vd, the apparent volume of distribution. Clearance rate from the blood did not differ for either compound. Also, Kf for other tissues, including brain, liver, kidneys, and heart, did not differ for Aβ–Cu and Aβ, whereas, a statistically significant decrease in the Kf for kidney was seen for Aβ–Cu. The clearance from the blood of rat Aβ–Cu (5.8 min) was not different from that for rat Aβ (6.8 min).

Based on the findings from our toxicity and clearance studies, we conclude that Cu does not have a significant effect on the thiol levels (except for HCY5 levels) or viability (except for rat Aβ–Cu) of RBE4 cells and it does not affect the clearance of Aβ from the brain. Therefore, as suggested by several studies, the toxicity of Aβ in the presence of copper may not be caused by its altered clearance. However, the presence of other metal ions, such as Fe and Zn, in the vicinity of Aβ along with Cu might have a cumulative effect on its aggregation, which would restrict the clearance. Studies relating the effects of these metal ions on amyloid aggregation/fibrillogenesis with that of the amount of the peptide entering into the brain through BBB are required to know the relationship between these two aspects. An opposite effect of Cu and Al on the transport of Aβ from capillaries into the brain parenchymal space suggests that Cu could have a protective effect on this aspect of Al–Aβ toxicity. It is also in need for research studies to focus on the individual roles of these metal ions in the clearance of Aβ in order to understand and resolve the underlying problem.

R E F E R E N C E S


