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1,1′-Xylyl bis-1,4,8,11-tetraaza cyclotetradecane: A new potential copper chelator agent for neuroprotection in Alzheimer's disease. Its comparative effects with clioquinol on rat brain copper distribution

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Abstract—Dysfunction of copper metabolism leading to its excess or deficiency results in severe ailments. Recently, neurodegenerative disorders such as Alzheimer's disease have been associated with copper metabolism. Compounds having the ability to reduce copper levels in brain or to affect its distribution could have neuroprotective effects, mainly through a downregulation of the transcription of amyloid peptide precursor (APP). We report here the biological effect of compound 1,1′-xylyl bis-1,4,8,11-tetraaza cyclotetradecane, which specifically affects copper concentration in the brain cortex region. Its copper homeostatic activity is compared with that of clioquinol, a well-known drug, which has been recently reported as an active Aβ-peptide clearance drug in vivo for Alzheimer's patients.

A central, unresolved question in the pathophysiology of Alzheimer's disease (AD) relates to the role of metal ions in plaque formation and neurodegeneration. AD plaques containing fibrils composed of the 39–42 residue amyloid-β (Aβ) peptides are thought to be linked to neurodegeneration in AD.[1,2] Metal ions have been proposed to play a significant role in the assembly and neurotoxicity of AD fibrils.[3] Administration of a metal ion chelator decreases deposition of Aβ in the brains of transgenic mice[4] and releases soluble Aβ from preformed amyloid deposits,[5] supporting the hypothesis that metal ions are incorporated in plaque architecture in vivo. Selective chelating therapy to combat AD is a promising strategy. It has been observed through Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) that patients with low Cu levels had significant by higher ADAS-cog values than patients with medium Cu levels, who exhibited lower ADAS-cog scores. This finding supports the hypothesis of a mild Cu deficiency in most AD patients.[6]

An hydrophobic moderate metal chelator (5-chloro-7-iodo-8-hydroxy-quinoline, known as clioquinol), termed as a metal–protein-attenuating compound (MPCA), has exhibited a promising effect in a phase II clinical trial of moderately severe AD patients.[7] More recently, it has been shown that in mammals clioquinol–copper complexes can form in the instestinal tract and cross the blood–brain barrier (BBB) to enter the brain, and this explains why soluble copper and zinc levels were increased by 15% in mice brain upon clioquinol treatment. Therefore, clioquinol–copper complexes could selectively and markedly elevate copper levels in the brain of individuals with AD and counterbalance the changes in copper levels observed in AD; most probably mediated through the amyloid peptide precursor (APP) export function.[8] It should be underlined that clioquinol was removed from the US market in 1971 because of a link with subacute myelo-optic neuropathy (SMON), an

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uncommon neurological syndrome largely confined to Japan.[9] These issues may be important in understanding the integration of copper homeostasis in AD and with other physiological processes such as aging.

Because of their interesting biological and physicochemical properties, saturated macrocyclic polyamines such as 14-membered tetramine derivatives have attracted the attention of biologists, mainly through the discovery and development of bicyclam, a chemokine receptor antagonist, which has highlighted the therapeutic potential of this compound in HIV infection,[10] inflammatory diseases, cancer and stem-cell mobilization.[11] In the present work, our aim was to study the influence of such bicyclam analogues **1** and **2** (named as JLK 169 and JLK 1291) on the distribution of copper in rat brains in comparison with other metal chelating agents such as **3** (Clioquinol) or **4** (D-Penicillamine), and to discuss the role of bicyclam analogues in the copper homeostasis.

Bicyclam **1** was synthesized according to known procedure.[12] Its Cu^{2+} complex **2** was synthesized as described under Ref. 13. Compounds **3** and **4** were commercially available. The structures of analogues **1**–**4** are presented in Figure 1.

All animal experiments are described under Ref. 14. Copper ion determination was carried out using atomic absorption as described under Ref. 17.

Compounds **1**–**4** were injected in rats according to the above animal experiment protocol. The obtained results are summarized in Table 1.

As is observed, **4** did not affect copper concentrations in blood or CSF or indeed in the studied brain regions. It is known that **4**–Cu(I) complexes have very poor aqueous solubility in the pH range of 1.9–7.6 due to the formation of neutral complexes.[18] Moreover, **4** which preferentially binds Cu(I) is less likely to extract Cu(II) involved in non redox structural capacity in proteins.[19] In contrast, **1** mainly affects copper concentration in the brain cortex, compared to its corresponding copper complex **2** or to **3**, while copper concentrations in blood, CSF or corpus callossum are similar to those found in untreated rats.

Figure 2 shows the comparative effects of **1** and **3** on copper ion distribution in various brain regions and blood and CSF with respect to the control.

It can be observed that 1 h after injection the effects of **1** and **3** are quite similar since both significantly decreased by about 70% copper concentration in the CSF with respect to the control and only very slightly in blood. They do not affect copper concentration in the corpus callosum. (It should be remembered that copper concentration in CSF does not necessarily correlate with copper concentration in brain department). In contrast, the effect of **1** compared to that of **3** on the observed increase of copper concentration in cortex is significantly different since **3** has a very low effect on copper cortex concentration, while in contrast **1** increased copper concentration with respect to the control by about 90%. It should be underlined that our results concerning **3** are different from those of other reports[20] which indicated a 15% overall increase of copper in whole mice brain upon **3** injection.

When copper analysis was performed on tissue samples or blood and CSF of animals decapitated 2 h, instead of 1 h, after drug treatment (**1** and **3**), the effects on copper distribution in the cortex or in CSF observed after 1 h were almost abolished, with respect to the control.

Moreover, copper–bicyclam complex **2** injected for 1 h has almost no significant effect on copper concentration on the whole brain samples as well as in CSF with respect to the control.

Several parameters need to be taken into account to explain the observed differences in the effects of these two drugs on copper distribution in the brain.

- First, the binding affinities for copper between **1** and **3** are quite different. Their log K_s values are, respectively, 27 and 10[21]:

  (K_s constant being defined as follows:

  $K_s = [\text{complex}]/[\text{ligand}] \cdot [Cu^{2+}]$)

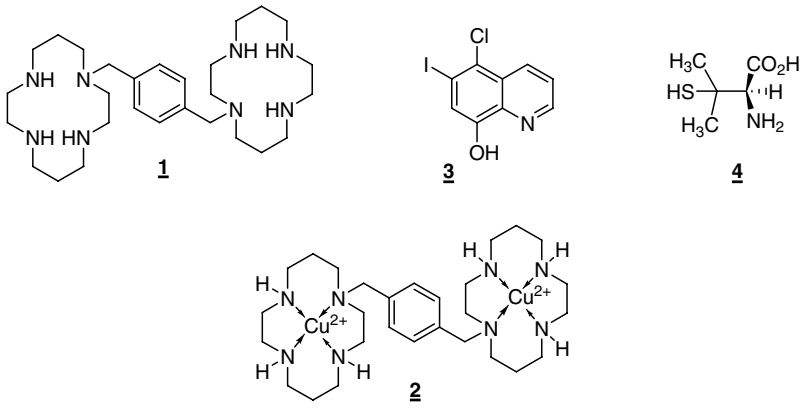

Figure 1. Molecular structures of the different metal chelators.