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Cobalt(II) β-ketoaminato complexes as novel inhibitors of neuroinflammation

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ABSTRACT

Neuroinflammation contributes to the pathogenesis of neurological disorders including stroke, head trauma, multiple sclerosis, amyotrophic lateral sclerosis as well as age-associated neurodegenerative disorders including Alzheimer's and Parkinson's diseases. Therefore, anti-inflammatory drugs could be used to slow the progression of these diseases. We studied the anti-neuroinflammatory activity of four novel square planar cobalt(II) compounds bearing tetradentate β-ketoaminato ligands with variation in the number of $-CF_3$ ligand substituents, as well as their corresponding unmetallated organic ligands. Cobalt (Co) complexes were consistently more active than their corresponding ligands. One of the complexes, L^3Co at concentrations (1–10 μM) that were not toxic to cells, significantly reduced cytotoxic secretions by human monocytic THP-1 cells, astrocytoma U-373 MG cells, and primary human microglia. This anti-neurotoxic action of L^3Co was reduced by SP600125 and PD98059, selective inhibitors of c-Jun NH2-terminal kinase (JNK) and extracellular signal regulated kinase (ERK) kinase (MEK)1/2 respectively. L^3Co had no effect on secretion of monocyte chemotactic protein-1 (MCP-1) by THP-1 cells, but it inhibited the NADPH oxidase-dependent respiratory burst activity of differentiated human HL-60 cells. L^3Co upregulated heme oxygenase-1 (HOX-1) expression by THP-1 cells, which may be one of the molecular mechanisms responsible for its anti-inflammatory properties. Two of the Co compounds tested showed activity only at high concentrations (50 μM), but L^2Co was highly toxic to all cell types used. Select Co complexes, such as L^3Co, may exhibit pharmacological properties beneficial in human diseases involving neuroinflammatory processes. Further studies of the *in vivo* efficacy, safety and pharmacokinetics of L^3Co are warranted.

1. Introduction

Data accumulated during the last two decades show that neuroinflammatory processes play an important role in the pathogenesis of both acute and chronic neurological disorders (Chang et al., 2009; Frautschy and Cole, 2010; Lee et al., 2011; Skaper, 2007; Wunder et al., 2009). Therefore, identification and development of new broad spectrum anti-inflammatory agents capable of reducing neuroinflammation are needed. A number of different strategies could be used to search for such agents, but quite often the assays used involve two of the glial cell types, microglia and astrocytes. These cells are believed to play a central role in initiating and regulating neuroimmune reactions. They are capable of secreting both neurotrophic and neurotoxic substances (Chang et al., 2009; Heneka et al., 2010; Klegeris and McGeer, 2005; Lull and Block, 2011). Reduced neurotrophic support or increased release of neurotoxins could lead to neuronal damage. Reactive oxygen species generated by glial NADPH-dependent oxidase are an example of neurotoxins (Lull and Block, 2011; Simonyi et al., 2010). In addition, glial cells secrete a plethora of cytokines, which regulate neuroimmune responses (Heneka et al., 2010; Lee et al., 2011).

Inorganic and bioinorganic medicinal agents have made a growing contribution to medical science and human health in the past half century. Metallocomplexes with platinum (cisplatin), bismuth (bismuth subsalicylate in Pepto-Bismol) and lithium carbonate are already widely used clinically (Thompson and Orvig, 2003). Cobalt (Co)-containing drugs and compounds have been less studied, however Co plays an essential role in the body by being present in the metallocomplex vitamin B12 (cobalamin) (Dali-Youcef and Andres, 2009). Some Co(III) complexes possess antiviral (Schwartz et al., 2001) and antimicrobial properties (Konstantinovic and Cakic, 2010; Rupesh et al., 2006). They also exhibit antiproliferative activity against several different tumor cell types (Jung et al., 1997; Klanicova et al., 2006; Ott et al., 2005, 2008). Recent studies have reported anti-inflammatory activity of several structurally different Co metallocomplexes by using both *in vitro* (Rupesh et al., 2006) and *in vivo* (Hunoor et al., 2010; Konstantinovic and Cakic, 2010) measurements.

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We synthesized and characterized a series of square planar Co(II) compounds bearing tetradentate β-ketoaminato ligands with variation in the number of $-CF_3$ ligand substituents (Gurley et al., 2011). The current *in vitro* study assessed the anti-inflammatory potential of these compounds with particular emphasis on their ability to ameliorate neuroinflammatory reactions. We studied four different Co complexes (Fig. 1A) and their corresponding organic ligands (Fig. 1B) for their ability to reduce cytotoxic secretions by monocytic and astrocytic cells (Hashioka et al., 2009; Klegeris and McGeer, 2005; Lee et al., 2011). We also measured secretion of monocyte chemotactic protein-1 (MCP-1) since this chemokine has been implicated in neuroimmune reactions (Conductier et al., 2010; Sokolova et al., 2009). We studied the effects of the compounds on phagocyte NADPH-oxidase mediated respiratory burst (Lull and Block, 2011; Simonyi et al., 2010). Real-time quantitative polymerase chain reaction (RT-qPCR) technique was used to measure expression of heme oxygenase-1 (HOX-1) by monocytic cells in the presence of one of the compounds.

2. Materials and methods

2.1. Reagents

The following substances were used in various assays and were obtained from Sigma-Aldrich (Oakville, ON, Canada): bacterial lipopolysaccharide (LPS, from *Escherichia coli* 055:B5), diaphorase (EC 1.8.1.4, from *Clostridium kluyveri*, 5.8 U/mg solid), dimethyl sulfoxide (DMSO), Triton X-100, luminol sodium salt, N-formyl-met-leu-phe (fMLP), *p*-iodonitrotetrazolium violet, NAD^+, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), a highly selective, cell-permeable inhibitor of p38 mitogen-activated protein kinases (p38 MAPK, SB202190), a selective inhibitor of c-Jun NH2-terminal kinase (JNK, SP600125), and a selective cell-permeable inhibitor of MAPK/extracellular signal regulated kinase (ERK) kinase (MEK)1/2 (PD98059).

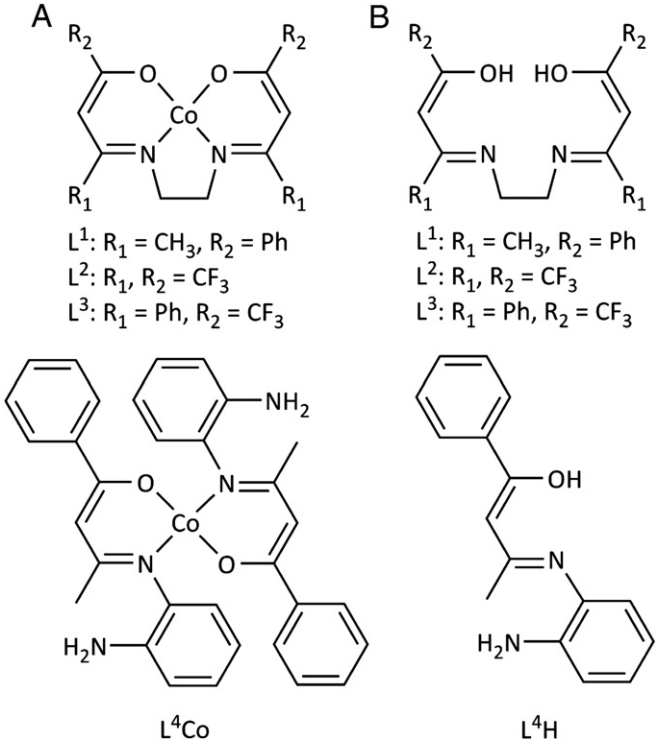

Fig. 1. Chemical structures of the Co complexes L^1Co, L^2Co, L^3Co and L^4Co (A) and their corresponding ligands L^1H_2, L^2H_2, L^3H_2 and L^4H_2 (B).

Human recombinant interferon (IFN)-γ, anti-human MCP-1 antibodies and recombinant protein standards used in the MCP-1 enzyme-linked immunoabsorbent assay were purchased from Peprotech (Rocky Hill, NJ, USA). Four different Co-containing metallocomplexes (Fig. 1A) and their corresponding ligands (Fig. 1B) were synthesized and characterized as previously described (Gurley et al., 2011).

2.2. Cell culture

The human monocytic THP-1, the human astrocytic U-373 MG and human promyelocytic HL-60 cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The human neuroblastoma SH-SY5Y cell line was a gift from Dr. R. Ross, Fordham University, NY. Microglia were prepared from human post-mortem brain frontal cortex tissue samples according to a published procedure and were used in experiments after 12 days in culture (Walker et al., 2009). Human brain tissue samples were obtained from the Banner Sun Health Research Institute Brain Bank, Sun City, AZ, USA through their Brain and Body donation program with informed consent and the approval of the Banner Sun Health Corporation Institutional Review Board (IRB). Microglia were prepared from a case that was assessed by a neuropathologist and determined to have no neuropathological abnormalities. All cells were grown in Dulbecco's modified Eagle's medium-nutrient mixture F12 ham (DMEM-F12) supplemented with 10% fetal bovine serum (FBS) and an antibiotic solution (100 U/ml penicillin, 100 μg/ml streptomycin) supplied by Thermo Scientific HyClone (Logan, UT, USA). The cell lines were used without differentiation, except HL-60 cells, which were treated with DMSO as described below (see Section 2.7).

2.3. Effects of compounds on THP-1, U-373 MG and human microglia viability and MCP-1 secretion from THP-1 cells

Human monocytic THP-1 cells were seeded into 24 well plates at a concentration of 5×10^5 cells/ml in 0.9 ml of DMEM-F12 containing 5% FBS. Human astrocytic U-373 MG cells were seeded onto 24-well plates at a concentration of 2×10^5 cells/ml in 0.8 ml of DMEM-F12 containing 5% FBS and were incubated for 24 h before use in experiments to allow the cells to adhere to the bottom of the well. Human microglia were plated at 7.5×10^4 cells per well in DMEM-F12 containing 5% FBS. Following 48 h incubation, media were removed and replaced with fresh medium. Cells were incubated in the presence or absence of various compounds or their vehicle solution (DMSO) for 15 min prior to the addition of the activating stimulus (1 μg/ml LPS with 300 U/ml IFN-γ for THP-1 cells and microglia or 300 U/ml IFN-γ for U-373 MG cells). The final concentration of DMSO in cell culture medium did not exceed 0.5%. It has been shown that DMSO affects a number of different parameters in various cell types (Chang et al., 2001; Gurtovenko and Anwar, 2007; Santos et al., 2005); therefore, preliminary experiments were performed which showed that at this concentration DMSO had no effect on cellular parameters studied.

The effects of selective inhibitors of JNK (SP600125), MEK1/2 (PD98059) and p38 MAPK (SB202190) were also investigated. THP-1 cells were incubated with various concentrations of the MAPK inhibitors or their vehicle solution (DMSO) for 15 min prior to the addition of 10 μM L^3Co. Following 15 min incubation, the activating stimuli were applied (1 μg/ml LPS with 300 U/ml IFN-γ). After 24 or 48 h incubation, 100 μl of cell culture media was sampled for lactate dehydrogenase (LDH) to determine the percentage of dead cells, while the evaluation of the surviving cells was performed by the MTT assay. The concentration of MCP-1 (ng/ml) in THP-1 cell supernatants was measured in 100 μl of cell-free culture media samples by enzyme-linked immunoabsorbent assay according to the protocol provided by the supplier of the antibodies (Peprotech).