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Anti-nociceptive activity and toxicity evaluation of Cu(II)-fenoprofenate complexes in mice

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ABSTRACT

The proposed curative properties of copper(II)-non-steroidal anti-inflammatory drugs (NSAIDs) have led to the development of numerous copper(II)–NSAID complexes with enhanced anti-inflammatory activity. In this work, the antinociceptive and toxic effects of two new coordination complexes: $Cu_2(fen)_4(caf)_2$ [fen: fenoprofenate anion; caf: caffeine] and $Cu_2(fen)_4(dmf)_2$ [dmf: N-N′-dimethylformamide] were evaluated in mice. The antinociceptive effect was evaluated with two models: acetic acid-induced writhing response and formalin test. For the sub-acute exposure, the complexes were added to the diet at different doses for 28 days. Behavioral and functional nervous system parameters in a functional observational battery were assessed. Also, hematological, biochemical and histopathological studies were performed. $Cu_2(fen)_4(caf)_2$ and $Cu_2(fen)_4(dmf)_2$ significantly decreased the acetic acid-induced writhing response and the licking time on the late phase in the formalin test with respect to the control and fenoprofen salt groups. The sub-acute exposure to $Cu_2(fen)_4(caf)_2$ complex increased the motor activity, the number of rearings and the arousal with respect to the control and fenoprofen salt groups. These impaired parameters in mice exposed to $Cu_2(fen)_4(caf)_2$ can be attributable to the presence of caffeine as stimulating agent. On the other hand, all exposed groups decreased the urine pools in the functional observational battery and increased the plasmatic urea. These effects could be due to the decrease in the glomerular filtration caused by NSAIDs. In conclusion, both complexes $Cu_2(fen)_4(dmf)_2$ and $Cu_2(fen)_4(caf)_2$ were more potent antinociceptive agents than fenoprofen salt. Sub-acute exposure to different doses of these complexes did not produce significant changes in the parameters that evaluate toxicity.

1. Introduction

Fenoprofen is a non-steroidal anti-inflammatory (NSAID), analgesic, with antipyretic properties used to treat mild to moderate pain. It relieves symptoms of arthritis (osteoarthritis and rheumatoid arthritis), such as inflammation, swelling, stiffness and joint pain. Evidence suggests that NSAIDs act by inhibiting cyclooxygenase (COX) and, consequently, prostaglandin synthesis (Polisson, 1996). Cyclooxigenases COX-1 and COX-2 play different roles in the inflammatory process (Hao et al., 1999; Polisson, 1996). COX-1 acts to maintain normal physiological function by its control of the renal parenchyma, gastric mucosa, platelet, and most other mammalian tissues, while COX-2 produces the prostaglandins involved in inflammation and mitogenesis (Dillon et al., 2003; Hao et al., 1999). The inhibition of COX-1 leads to gastrointestinal ulcerogenic toxicity while a drug exerting the selective inhibition of COX-2 over COX-1 is considered a safer and more effective antiinflammatory agent (Polisson, 1996).

Copper is also believed to possess anti-inflammatory and analgesic effects (Blahova et al., 1994; Okuyama et al., 1987; Sorenson, 1989). Copper ions, as centers of active site of various metalloproteins, play a vital role in a number of widely differing biological processes like electron transfer, oxidation and dioxygen transport (Mirica et al., 2004; Rosenzweig and Sazinsky, 2006).

Experimental evidences proved that the coordination of NSAIDs to copper(II) ions improves the pharmaceutical activity of the drugs themselves and reduce their undesired collateral effects in human and animals (Agotegaray et al., 2010; Blahova et al., 1994; Cini et al., 2007; Korolkiewicz et al., 1989; Lewis, 1978; Sorenson, 1992; Sorenson et al., 1995).

It is also known that many copper(II)–NSAID complexes present superoxide dismutase (SOD) (Agotegaray et al., 2010; Devereux et al., 2007; Wangila et al., 2006) and catechol oxidase activity

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(Abuhijleh et al., 1992). These enzymes protect the living cell against various pathological conditions involving cardiovascular diseases, cancer, inflammation, diabetes and aging (Valko et al., 2007). Increasing attention has been paid to the SOD activity of copper(II)-complexes with anti-inflammatory properties because superoxide is implicated in the promotion of arthritis by the degradation of hyaluronic acid, which is essential for maintaining internal joint homeostasis (Auer et al., 1990; McCord, 1974). This is supported by the complete protection against this degradation by treatment with SOD (Czapski and Goldstein, 1998; McCord, 1974). Moreover, oxygen-derived free radicals play important roles in the pathogenesis of gastric mucosal injury (Nishiyama et al., 1996) and SOD exists in the mucosal tissues of the gastrointestinal tract where it is believed to protect against such damage (Klinowsky et al., 1996).

Because the cooper(II)–NSAID complexes have a more potent pharmacologic activity and lower collateral effects than their parent drugs, and taking into account that there are no reports on the antinociceptive and toxicological properties of copper(II)-fenoprofenate complexes, we present here the study of the analgesic activity employing the acetic acid writhing and formalin tests of two new complexes [$Cu_2(fen)_4(caf)_2$ and $Cu_2(fen)_4(dmf)_2$] as well as the results of the sub-acute exposure to different doses to evaluate their toxicity.

2. Materials and methods

2.1. Materials

Fenoprofen calcium salt, carboxymethylcellulose and Tween 80 were purchased from Sigma-Aldrich. All other reagents and solvents were of analytical grade and used without further purification.

2.2. Synthesis of the complexes

The complex $[Cu_2(fen)_4(dmf)_2]\cdot 2H_2O$ was prepared according to the following procedure: a 2.0 ml dmf solution containing 0.0811 g (0.150 mmol) of racemic fenoprofen calcium salt hydrate ($Ca(fen)_2\cdot H_2O$) was added to 3.0 ml of an ethanolic solution of $CuCl_2\cdot 2H_2O$ (0.0170 g, 0.100 mmol). The resulting green solution was stirred at room temperature for about one h and then kept at the same temperature overnight. The addition of an excess of water led to the immediate precipitation of a green complex with a glassy appearance, which gradually recrystallized at room temperature after three weeks. The bright green microcrystals obtained were filtered, washed with water and air dried. Yield: 64% (0.0387 g) elemental analysis, found: C, 62.5; H, 4.9; N, 1.7%. Calc. for $C_{66}H_{70}Cu_2N_2O_{16}$: C, 62.2; H, 5.4; N, 2.1%. A slow recrystallization of the complex in dmf/water yielded green single crystals without crystallization water which were studied by X-ray diffractometry. These results were published elsewhere (Agotegaray et al., 2008). The complex $[Cu_2(fen)_4(caf)_2]$ was prepared by the addition of a solution containing 50 mg (0.040 mmol) of $Cu_2(fen)_4(dmf)_2$ in 2 ml of acetone to 31 mg (0.160 mmol) of caffeine dissolved in 2 ml of hot ethanol under stirring. This procedure led to a resulting limpid, green solution. The slow diffusion of water resulted in the formation of two layers of solvents. After a few days at 4 °C green crystals were obtained, which were washed with water and air dried. Yield: 57% (0.0340 g). The well-shaped crystals could not be solved by X-ray crystallography due to molecular disorder but it was studied by spectroscopic and thermal techniques revealing the structure proposed. Analysis calculated for $C_{76}H_{72}Cu_2N_8O_{16}$: C, 61.6; H, 4.9; N, 7.6%. Found: C, 61.3; H, 4.8; N, 7.7%.

2.3. Experimental animals

Healthy CF1 female mice 8 weeks old were used. They were obtained from the colony of the animal facility from the Biology, Biochemistry and Pharmacy Department which were maintained under constant conditions of temperature (22 ± 1 °C) and humidity (70%), in a 12 h light:12 h dark cycle (light on at 6:00 h) during all the experiment. According to the body weight (approximately 30 g) they were randomly divided into different groups of 8–10 animals which were acclimatized for a week before starting the experiment. All animals had free access to tap water and standard diet (Ganave®, Ratas y Ratones, Alimentos Pilar S.A., Argentina) throughout the experiment. The care and the handling of the animals were in accordance with the internationally accepted standard Guide for the Care and Use of Laboratory Animals (2010) as adopted and promulgated by the National Institute of Health.

2.4. In vivo analgesic activity

2.4.1. Acetic acid-induced abdominal writhing

The test was performed as described by Collier et al. (1968) and Fontenele et al. (1996). Nociception was induced by an intraperitoneal (i.p.) injection of 0.6% acetic acid solution (10 ml/kg). Mice were orally treated by gavage with 26 mg/kg $Cu_2(fen)_4(dmf)_2$ and 31 mg/kg $Cu_2(fen)_4(caf)_2$, and 1 h later the acetic acid was injected. The vehicle used for dissolution of complexes was 0.05% CMC-Na and 0.1% Tween 80. The control group received the vehicle and the positive group received 21 mg/kg fenoprofen salt. Each quantity of drug administered was equivalent to the therapeutic dose of 20 mg/kg of fenoprofen.

Immediately after the injection of acetic acid, each animal was isolated in an individual box to be observed during 20 min. The number of writhing and stretching was recorded. A writhe is indicated by abdominal constriction and stretching by full extension of hind limb.

2.4.2. Formalin test

This test was carried out as described by Hunskaar and Hole (1987). Twenty microliters of 2.5% formalin was injected into the dorsal surface of the left paw of mice 1 h after oral administration by gavage of 26 mg/kg $Cu_2(fen)_4(dmf)_2$, 31 mg/kg $Cu_2(fen)_4(caf)_2$, 21 mg/kg fenoprofen salt or vehicle (0.05% CMC (carboxymethyl cellulose) and 0.1% Tween 80). The time that animals spent on licking and biting the injected paw was recorded. On the basis of the response pattern described by Tjolsen et al. (1992), two distinct periods of intensive licking activity were identified and scored separately. The nociceptive scores normally peaked 5 min after formalin injection (early phase) and 15–30 min after the injection (late phase). The early phase is due to a direct effect on nociceptors by the formalin. The late phase seems to be an inflammatory response (Tjolsen et al., 1992).

2.5. Sub-acute toxicity

The experiment was conducted according to the protocols described by OECD Guideline No. 407 (2008). Fenoprofen salt and complexes were incorporated into the standard aliment and small pieces of food made (approximately 0.2 g) with the dose for each mouse. Before daily administration, mice were left for two h fasting. The exposure was over a period of 28 days at a dose of 21 mg/kg and 42 mg/kg body weight/day for fenoprofen salt; 31 mg/kg and 62 mg/kg body weight/day for $Cu_2(fen)_4(caf)_2$, and 26 mg/kg and 52 mg/kg body weight/day for $Cu_2(fen)_4(dmf)_2$. Control animals received standard food.

During the exposure, all the animals were observed for signs of toxicity. At the end of the exposure, behavioral and functional parameters as well as motor activity were assessed for each mouse. Subsequently, blood samples were obtained for hematological analysis by retro-orbital bleeding (Fukuta, 2004). After that, mice were euthanized and blood samples were taken by cardiac puncture for biochemical analysis. Finally, necropsy observations and histopathological examinations were realized on several tissues.

2.5.1. Functional observational battery

On the 28th day of exposure, behavioral and functional parameters of the animals were evaluated through a functional observational