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Anti-inflammatory effects of cerebrocrast in a model of rat paw edema and on mononuclear THP-1 cells

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Abstract

Cerebrocrast (IOS 1.1212; 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid di(2-propoxyethyl) diester) is a novel derivative of 1,4-dihydropyridine, which does not antagonize Ca^{2+} influx in neuronal tissues. Since several classical dihydropyridines possess anti-inflammatory properties, we first studied the effects of cerebrocrast in a model of rat paw edema induced by carrageenan. Cerebrocrast had a preventative effect in this model of inflammation, with maximal activity (32–45% inhibition) in the 0.1–0.25 mg kg^{-1} range. It was ineffective when added after the injection of carrageenan. Subsequent in vitro experiments showed that cerebrocrast in the micromolar range inhibited secretion of interleukin-1β, interleukin-6 and neurotoxic products by cells of the human monocytic THP-1 line while failing to affect secretion of tumor necrosis factor (TNF-α). It also lacked any direct neuroprotective effect against toxic secretions from stimulated THP-1 cells. The data obtained suggest that cerebrocrast may be useful not only in various inflammatory disorders of peripheral tissues, but also in treating neurodegenerative diseases, where inflammatory mechanisms in general and microglial activation, in particular, are thought to play an important role.

Keywords: Dihydropyridine; Inflammation; Cytokine; Neuroprotection; Mononuclear phagocytes; Microglia

1. Introduction

Cerebrocrast (IOS 1.1212; 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid di(2-propoxyethyl)diester) is a novel derivative of 1,4-dihydropyridine. Previous pharmacological studies with this model compound have shown that it radically differs from classical dihydropyridines, which are typically Ca^{2+} channel antagonists, since it does not block Ca^{2+} influx in neuronal tissues. Cerebrocrast has been reported to have long-lasting memory-improving and neuroprotective effects in several in vivo models (for a review, see Klusa, 1995). Cerebrocrast has also been shown to affect the numbers and functional activity of T cells in a rat model of diabetes (Briede et al., 1999a,b) as well as to elevate corticosterone levels in rat plasma (Liutkevicus et al., 1999). The latter two observations, taken together with the fact that several other dihydropyridine derivatives exhibit anti-inflammatory actions (e.g. Fukuzawa et al., 1997; Kumar and Knaus, 1994; Oyanagui and Sato, 1991), prompted us to study whether cerebrocrast possesses anti-inflammatory properties. First, we explored whether cerebrocrast has any preventative or therapeutic effect in a rat model of paw edema. Secondly, we studied in vitro pharmacological effects of cerebrocrast on several functional parameters of mononuclear phagocytes. For these experiments, cells of the human monocytic THP-1 line were used as surrogate representatives. Due to metabolic and morphological similarities, this cell line has been accepted as a good model of human monocytes/macrophages (Prieto et al., 1994; Tsuchiya et al., 1982; Yates et al., 2000). The effects of cerebrocrast were studied on the secretion of three pro-inflammatory cytokines: interleukin-1β, interleukin-6 and tumor necrosis factor-α (TNF-α). We also evaluated the effects of this drug in an assay system, where human neuronal SH-SY5Y cells are exposed to toxic secretions from human THP-1 monocytic cells. This model

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was developed previously (Klegeris et al., 1999) in order to simulate the in vivo human microglial activation and neuronal damage observed during neurodegenerative disease processes.

2. Materials and methods

2.1. Animals

Adult male (150–180 g) Wistar rats, obtained from the Laboratory Animal Center, Institute of Immunology, Vilnius, Lithuania, were used for the experiments. The animals were maintained in an environment with controlled temperature (22 ± 2 °C). Food and water were provided ad libitum. All procedures were carried out in accordance with guidelines of the European Union and were approved by the Ethics Committee on Animal Experimentation, Institute of Immunology, Vilnius, Lithuania.

2.2. Reagents

Cerebrocrast (IOS 1.1212; 4-[2-(difluoromethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid di(2-propoxyethyl) diester, molecular weight 511.6) was synthesized at the Latvian Institute of Organic Synthesis (Riga, Latvia) and was used as a suspension in olive oil for in vivo experiments, or dissolved in dimethyl sulfoxide for in vitro use. The following substances were obtained from Sigma: bacterial lipopolysaccharide (from *Escherichia coli* 055:B5); λ-carrageenan; diaphorase (EC 1.8.1.4, from *Clostridium kluyveri*, 5.8 Units mg^{-1} solid), dimethyl sulfoxide; *p*-iodonitrotetrazolium violet; L-glutamic dehydrogenase (EC 1.4.1.3 from bovine liver, 30 Units mg^{-1} solid), NAD^+, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), phosphatase substrate Sigma 104 and prednisolone. Human recombinant interferon-γ was purchased from Bachem California (Torrance, CA). Antibodies used in enzyme-linked immunoabsorbent assays (ELISA) were as follows: for interleukin-1β capture, a rabbit polyclonal (1:1,000 dilution, a gift from Dr. H. Ziltener, The Biomedical Research Centre, Vancouver, BC, Canada); for interleukin-1*β* detection, mouse monoclonal (1:50, obtained from Dr. A.E. Berger, Upjohn, Kalamazoo, MI); for interleukin-6 capture, a rat monoclonal (1:500 dilution, PharMingen, San Diego, CA); for interleukin-6 detection, a rabbit polyclonal (1:2,000, ICN Biomedicals, Costa Mesa, CA); for TNF-α capture, a rabbit polyclonal (1:200); and for TNF-α detection, a mouse monoclonal (1:2,000, both from Chemicon, Temecula, CA). The alkaline phosphatase-labeled anti-mouse and anti-rabbit antibodies (1:3,000) were supplied by GIBCO BRL, Life Technologies. Human recombinant cytokines used for ELISA calibrations were from Biosource International (Camarillo, CA) (TNF-α) and Bachem California (interleukin-1β and interleukin-6).

2.3. Model of rat paw edema and cerebrocrast treatment

Experiments were performed as described by Winter et al. (1962). The animals were anaesthetized and injected subcutaneously with carrageenan (100 μl of 2% solution in saline) into the right paw. Twenty-four hours later, differences in the weight of the injected vs. the uninjected paw were determined as an indicator of the inflammation (paw edema) (Winter et al., 1962). Anti-inflammatory properties of cerebrocrast were studied by injecting various doses of this drug ($0.01-1.0$ mg kg^{-1}) i.p. either 3 h before or 3 h after administration of carrageenan. The control rats were injected with the same volume of solvent (0.5 ml olive oil). Prednisolone (100 mg kg^{-1}) served as a positive control.

2.4. Cell culture

The human monocytic THP-1 cell line was obtained from the American Type Culture Collection. The human neuroblastoma SH-SY5Y cell line was a gift from Dr. R. Ross, Fordham University, NY. These cells were grown in Dulbecco's modified Eagle's medium-nutrient mixture F12 ham supplemented with 10% fetal bovine serum (GIBCO BRL, Life Technologies, Burlington, ON, Canada). Both cell lines were used without initial differentiation. The viability of monocytic cells in the presence of cerebrocrast was monitored visually with a phase-contrast microscope and also by the release of lactate dehydrogenase, and by MTT assay, which detects live cells (see below). The latter two assays were performed after 24-h incubation with the drug.

2.5. Measurement of cytokines interleukin-1β, interleukin-6 and TNF-α

The concentrations of interleukin-1β, interleukin-6 and TNF-α in cell-free supernatants were measured after 48-h incubation by an ELISA as described previously for interleukin-1β (Klegeris et al., 2000). Briefly, cells were seeded into 24-well culture plates (0.6 ml, 5×10^5 cells ml^{-1}). They were exposed to a combination of 0.5 μg ml^{-1} lipopolysaccharide and 150 Units ml^{-1} interferon-γ and after 48-h incubation, the concentration of cytokines in cell-free supernatants was measured as follows. Capture antibodies were diluted in 0.1 M bicarbonate coating buffer, pH 8.2. Aliquots (50 μl) were added to each well of 96-well plates (Easy Wash, Corning) and incubated overnight at 4 °C. Nonspecific binding sites were blocked by incubation of the wells with 200 μl of 3% bovine serum albumin in phosphate-buffered saline for 2 h at room temperature. Samples and recombinant cytokine standards diluted in phosphate-buffered saline/3% bovine serum albumin were added at 100 μl per well, and plates were incubated overnight at 4 °C. Detection antibodies were diluted in phosphate-buffered saline/3% bovine serum albumin and added at 100 μl to each well. Plates were incubated for 1 h at room temperature. The alkaline phosphatase-labeled antibody was

added (1:3,000 dilution) in phosphate-buffered saline/3% bovine serum albumin at 100 μl per well, followed by 45 min of incubation at room temperature. After each of the above experimental steps, plates were washed 2–8 times with 0.5% Tween in phosphate-buffered saline, pH 7.0. Absorbance at 405 nm was read after 120-min incubation of wells with substrate buffer containing 1 mg ml^{-1} Sigma 104 phosphate substrate in 0.1 M diethanolamine buffer, pH 9.8. A microplate reader with a 405 nm filter was used to measure absorbance of each sample. Concentrations of cytokines in the experimental samples were calculated according to the absorbances obtained from wells containing standards of recombinant cytokine. For each set of experiments, standard curves were run, where concentrations of cytokines were reduced to levels that were indistinguishable from readings obtained with media alone. These blanks values were subtracted from readings of experimental samples. The detection limits, which correspond to media alone + 2 standard deviations were 10.4 ± 3.4 mUnits ml^{-1} for interleukin-1β; 2.4 ± 0.4 Units ml^{-1} for interleukin-6; and 2.2 ± 0.8 ng ml^{-1} for TNF-α.

2.6. Cytotoxicity of THP-1 cells towards SH-SY5Y neuroblastoma

The cytotoxicity experiments were performed as described previously (Klegeris et al., 1999). Briefly, human monocytic THP-1 cells were seeded into 24-well plates at a concentration of 4×10^5 cells per well in 0.8 ml of Dulbecco's modified Eagle's medium-nutrient mixture F12 ham medium containing 5% fetal bovine serum. The cells were incubated in the presence or absence of cerebrocrast for 30 min prior to the addition of an activating stimulus (0.5 μg ml^{-1} lipopolysaccharide with 150 Units ml^{-1} interferon-γ). After 24-h incubation, 0.4 ml of cell-free supernatant was transferred to each well containing SH-SY5Y cells. The cells had been plated 24 h earlier at a concentration of 2×10^5 ml^{-1} in 0.5 ml of Dulbecco's modified Eagle's medium-nutrient mixture F12 ham medium containing 5% fetal bovine serum. After 72 h of incubation, the neuronal culture media were sampled for lactate dehydrogenase to determine release from dead cells, while evaluation of surviving cells was performed by the MTT assay as described before (Klegeris et al., 1999). Cerebrocrast was added either to THP-1 cell cultures 30 min before their stimulation, or directly to SH-SY5Y cells at the time when THP-1 cell supernatants were transferred to wells with neuronal cells. Values obtained in the presence of cerebrocrast were normalized against values obtained with comparably stimulated THP-1 cells in the absence of the drug.

2.7. Statistical analysis

Data are presented as means ± standard error of the mean (S.E.M.). The data were evaluated statistically by either the single factor analysis of variance (ANOVA) or the randomized blocks design ANOVA, followed by the Fisher's least significant differences test for multiple comparisons. In those cases, where data are presented as a percentage of control values, statistical analyses were performed before transformation of data.

3. Results

Anti-inflammatory properties of cerebrocrast were evaluated first by using a model of rat paw edema. Fig. 1 shows that this compound, when added 3 h before carrageenan injection, in a dose dependent manner, reduced paw edema development (Fig. 1A). The maximal activity (32–45% reduction in paw weight difference) was observed in the 0.1–0.25 mg kg^{-1} range. Doses below and above this range were less effective. Cerebrocrast suppressed edema only

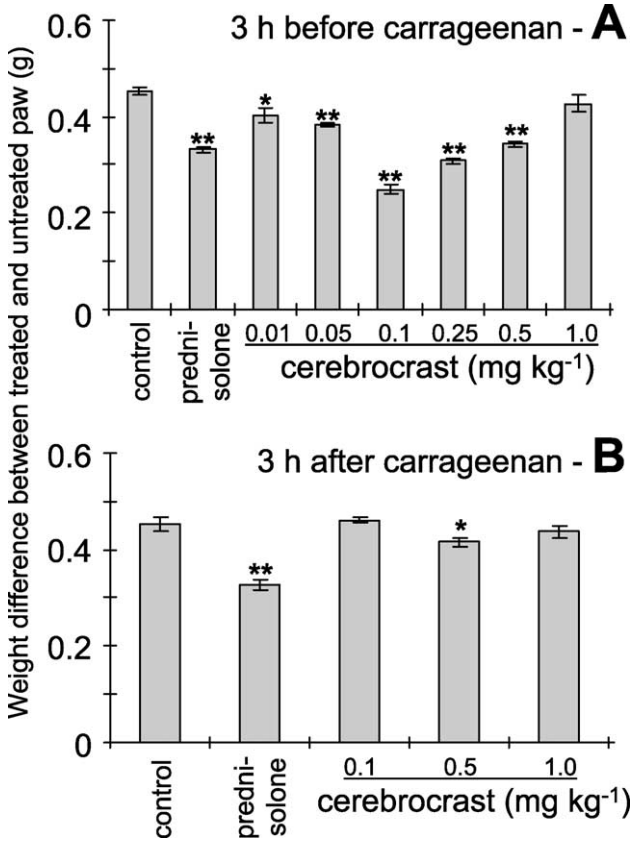

Fig. 1. Cerebrocrast inhibits rat paw edema when added before and not after carrageenan treatment. Cerebrocrast at the concentrations shown on the abscissa or prednisolone (100 mg kg^{-1}) was administered i.p. either 3 h before (A) or 3 h after (B) carrageenan was injected into one paw. Weight of paws was determined 24 h later and weight differences between treated and untreated paws were calculated. The number of animals used for each data point from left to right was as follows: A: 24,14,5,5,13,5,13,13; B: 11,8,8,8,8,8. Statistical significance was first assessed by the single factor ANOVA (A: $F = 37$, $P < 0.0001$; B: $F = 23$, $P < 0.0001$), followed by Fisher's least significant differences test for multiple comparisons; ** $P < 0.01$ and * $P < 0.05$, significantly different from animals treated with vehicle solution only.

when it was administered before carrageenan treatment, since administration of this compound after carrageenan injection did not reduce the development of paw edema (Fig. 1B). The steroidal anti-inflammatory drug prednisolone, which was used at a single dose of 100 mg kg^{-1} as a positive control, inhibited significantly edema formation when added either before or after injection of carrageenan (27–28% reduction in paw weight difference).

Subsequently the anti-inflammatory effects of cerebrocrast were studied on mononuclear THP-1 cell functions in vitro. First, it was established that at the concentrations tested (0.1 to 20 μM) cerebrocrast did not affect THP-1 cell viability after 24-h incubation. This was determined by examining cells under phase-contrast microscope, and by measuring lactate dehydrogenase activity in cell supernatants as well as by performing MTT assay on live cells (data not shown). Table 1 shows that cerebrocrast in a concentration-dependent manner inhibited secretion of interleukin-1β and interleukin-6, while the secretion of another cytokine TNF-α was not affected. Statistically significant inhibitory effects could be observed at concentrations above 2 μM, where cerebrocrast inhibited interleukin-1β secretion by up to 66%, and interleukin-6 secretion by up to 42%. Cerebrocrast was also tested for its ability to inhibit THP-1 cell toxicity towards neuronal SH-SY5Y cells (Fig. 2). When added to THP-1 cells before their stimulation with lipopolysaccharide and interferon-γ, cerebrocrast in a concentration-dependent manner inhibited the toxic action of monocytic cells. This effect could be observed at a concentration equal to or higher than 1 μM and reached 47% inhibition according to measurement of lactate dehydrogenase release from damaged cells (Fig. 2A). A similar concentration dependency was registered by using the MTT assay, an independent measure of live cells (Fig. 2B). This assay showed a 59% increase in viable cells in the presence of 20 μM cerebrocrast when compared to cells treated with dimethyl sulfoxide vehicle only. Fig. 2 also shows that cerebrocrast was ineffective when added to SH-SY5Y cells directly at the time these cells were exposed to supernatants from cultures of stimulated THP-1 cell.

Table 1
Inhibition of THP-1 cell cytokine secretion by cerebrocrast

Cerebrocrast (μM)	Interleukin-1β (Units ml^{-1})	Interleukin-6 (Units ml^{-1})	TNF-α (ng ml^{-1})
Stimulated with lipopolysaccharide and interferon-γ			
20	0.43 ± 0.10 (5)[a]	30.1 ± 5.8 (4)[b]	11.7 ± 2.4 (3)
10	0.66 ± 0.18 (5)[a]	31.7 ± 7.2 (4)[b]	11.7 ± 2.6 (3)
2	0.85 ± 0.23 (5)	39.4 ± 6.2 (4)	11.6 ± 3.1 (3)
1	0.96 ± 0.38 (4)	45.7 ± 12.2 (4)	12.8 ± 3.7 (3)
0	1.27 ± 0.36 (5)	52.2 ± 15.3 (4)	12.7 ± 3.1 (3)
Unstimulated			
0	0.001 ± 0.001 (5)	1.7 ± 1.0 (4)	5.4 ± 0.3 (3)

Human monocytic THP-1 cells (2.5×10^5 per well) were plated in 24-well plates in the presence or absence of cerebrocrast for 30 min before a combination of lipopolysaccharide (0.5 μg ml^{-1}) and interferon-γ (150 Units ml^{-1}) was added. After 48 h incubation, the levels of three different cytokines in cell-free supernatants were measured by ELISA. Data are presented as mean ± S.E.M. and the number of independent experiments is shown in parentheses.

[a] $P<0.01$ significantly different from the control samples containing lipopolysaccharide and interferon-γ only, Fisher's least significant differences test for multiple comparisons.

[b] $P<0.05$ significantly different from the control samples containing lipopolysaccharide and interferon-γ only, Fisher's least significant differences test for multiple comparisons.

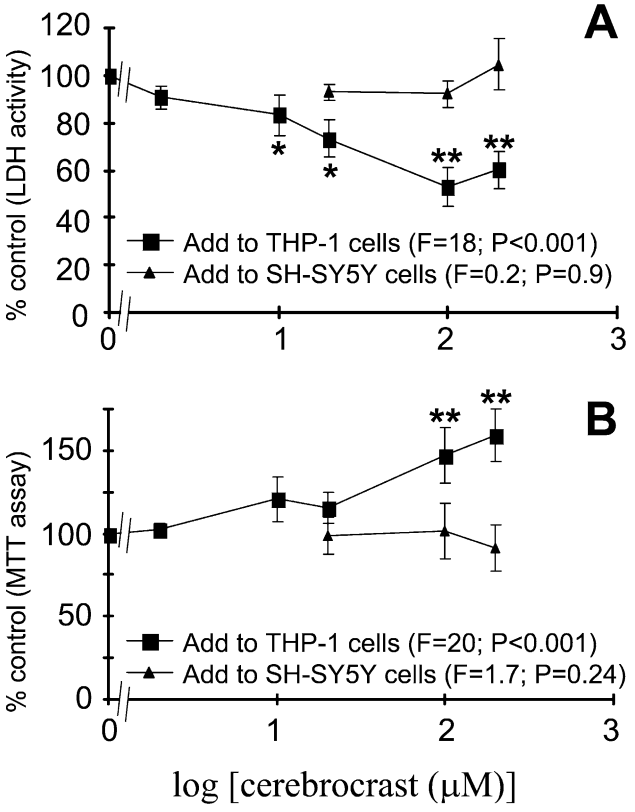

Fig. 2. Inhibition of human monocytic THP-1 cell toxicity towards human SH-SY5Y neuroblastoma cells by cerebrocrast. Cerebrocrast was added to either THP-1 cells 30 min before stimulation with lipopolysaccharide (0.5 μg ml^{-1}) and interferon-γ (150 Units ml^{-1}) (-■-, $N=5-10$), or directly to SH-SY5Y cells at the time the cell-free supernatants of THP-1 cells were transferred to the wells containing the neuronal cells (-▲-, $N=3-4$). Viability of SH-SY5Y cells was assessed after 72 h by measuring the lactate dehydrogenase (LDH) activity in the supernatants (A), and also by MTT assay (B). Data are normalized against control values obtained in the absence of cerebrocrast and presented as mean ± S.E.M. Significance levels for the concentration-dependent effect of the drug were calculated by randomized blocks design ANOVA, followed by Fisher's least significant differences test for multiple comparisons; ** $P<0.01$ and * $P<0.05$, significantly different from control samples incubated in the absence of cerebrocrast.

4. Discussion

The present results show that cerebrocrast at the doses of 0.01–0.5 mg kg^{-1} significantly inhibits development of rat paw edema when administered 3 h before injection of

carrageenan. The most effective concentration range was 0.1–0.25 mg kg^{-1}, where this preventative effect was comparable to that achieved by the steroidal anti-inflammatory drug prednisolone (100 mg kg^{-1}). Interestingly the effective doses for nonsteroidal anti-inflammatory drugs (NSAIDs) in the model of carrageenan-induced rat paw edema have been reported to be at or above the concentrations used for cerebrocrast (Inoue et al., 1994; Amann and Schuligoi, 2000). Thus, Inoue et al. (1994) measured the following ED_{30} values (mg kg^{-1}): diclofenac, 0.7; ketoprofen, 1.1; piroxicam, 1.3; naproxen, 1.8; indomethacin, 2.3; and aspirin, 167.0. Cerebrocrast, however, did not show a significant therapeutic effect, since its administration 3 h after carrageenan injection did not inhibit formation of paw edema. The in vitro experiments showed that cerebrocrast in the micromolar range inhibited secretion of interleukin-1β, interleukin-6 and neurotoxic products by human THP-1 mononuclear cells, while failing to affect secretion of TNF-α. It also lacked any direct neuroprotective effect, since no inhibition could be observed when cerebrocrast was added to neuronal SH-SY5Y cells directly at the time of their exposure to toxic secretions from THP-1 cells.

The selective inhibitory action of cerebrocrast on the secretion of interleukin-1β and interleukin-6, but not TNF-α levels, suggests that this substance affects selective intracellular pathways that are necessary for the production of some interleukins, but not TNF-α. A divergence of intracellular pathways that eventually lead to selective cytokine production has been well characterized for both lipopolysaccharide and interferon-γ signaling (Akira et al., 2001; Benveniste et al., 1995; David, 1995). Furthermore, we and others have observed a similar divergence before, where the secretion of interleukin-1β is inhibited, while that of TNF-α is not by ligands of peripheral benzodiazepine receptors as well as calmodulin inhibitors (reviewed in Klegeris et al., 2000). Furthermore, inhibitory actions of interleukin-10 have been reported to affect secretion of interleukin-6, without affecting interleukin-1β and TNF-α levels, also illustrating the complexity of THP-1 cell intracellular pathways engaged by lipopolysaccharide stimulation (Murthy et al., 2000).

The mechanism by which cerebrocrast exerts these effects is not known. The anti-inflammatory action of cerebrocrast on paw edema development observed in this study was achieved at the same dose range (up to 0.5 mg kg^{-1}), where cerebrocrast affects T cell functions (Briede et al., 1999a,b). In this dose range, it also influences some behavioral parameters and has a protective effect in certain neurodeficiency animal models (for a review, see Klusa, 1995). These include neurodeficiencies induced by cycloheximide administration, hypoxia and paternal alcoholization. It also had a normalizing effect on cholesterol-induced accelerated aging, which might indicate an anti-atherogenic potential. Atherosclerosis is believed to be promoted by inflammation (Ross, 1999). This concentration range is lower than that (5 mg kg^{-1}) required to induce changes in rat plasma corticosterone levels (Liutkevicus et al., 1999), which would suggest that the anti-inflammatory action of cerebrocrast is not likely to be mediated by steroids. Cerebrocrast also did not mimic steroid action since, unlike prednisolone, it failed to inhibit edema formation when administered after carrageenan injection. The possibility that cerebrocrast has several distinct mechanisms of action in vivo is also suggested by the fact that its anti-inflammatory effect seemed to diminish at higher doses, thus making the dose–response curve U-shaped. This could happen if at higher doses cerebrocrast affects mechanisms that oppose its anti-inflammatory activity. Correa et al. (1997) have already reported that corticotropin-releasing factor exhibits similar action in rat paw edema induced by carrageenan, *Naja naja naja* phospholipase A_2, or histamine: pretreatment with low doses inhibited the edema, while higher doses had no anti-inflammatory activity.

The anti-inflammatory activity of cerebrocrast is unlikely to be restricted to this particular model of inflammation, since this compound also was effective in a mouse model of contact hypersensitivity induced by 1-fluoro-2,4-dinitrobenzene (DNFB) as described by Hiltz and Lipton (1990). When administered at a dose of 0.5 mg kg^{-1} 2 h before 1-fluoro-2,4-dinitrobenzene treatment cerebrocrast had a small (15.6%), but statistically significant inhibitory effect, while it again was ineffective when administered after 1-fluoro-2,4-dinitrobenzene. Further experiments are needed in order to assess the effective range of doses for cerebrocrast in this and other in vivo models of inflammation.

It has been noted before that cerebrocrast exerts its activity through mechanisms that differ from those of typical Ca^{2+} channel antagonists of the 1,4-dihydropyridine series, since it does not block Ca^{2+} entry in neuronal tissues (see Klusa, 1995). The data from this study points out two further discrepancies supporting a distinct mechanism of action for cerebrocrast. First, although dihydropyridines have been reported before to have an anti-inflammatory action measured by a reduction of rat paw edema, the concentrations required to achieve such inhibition are much higher (100 mg kg^{-1} range) in the case of such drugs as nifedipine, nicardipine and nimodipine (Oyanagui and Sato, 1991) as well as in the series of novel compounds described by Kumar and Knaus (1994). Secondly, such dihydropyridines as amlodipine and nicardipine have been shown to inhibit TNF-α production both in vivo and in vitro (Fukuzawa et al., 2000), while cerebrocrast had no effect on secretion of this cytokine. Interestingly, doses at which TNF-α inhibition was observed, were much higher than those necessary for Ca^{2+} channel blocking (Boyd et al., 1987). Therefore, Ca^{2+} channel blocking mechanisms are suggested not to be involved in TNF-α inhibition. Although the exact molecular mechanism by which cerebrocrast exerts its activity is not known, it appears not to be mediated by Ca^{2+} channel blockade, and not to involve steroids. One possible mode of action may involve adenosine A_3 receptors, since recent data show that several dihydropyri-

dines are good ligands for these receptors, which are also expressed by immune cells (for a review, see Jacobson, 1998). Further research is needed in order to explore this possibility.

The data obtained show that the nonclassical dihydropyridine cerebrocrast exerts anti-inflammatory activity by preventing formation of rat paw edema in vivo, and by inhibiting secretion of two pro-inflammatory cytokines interleukin-1β and interleukin-6 in vitro. Furthermore, this drug reduces neurotoxic secretion of mononuclear phagocytes. Since human neurodegenerative diseases, such as multiple sclerosis and Alzheimer's disease, are known to involve inflammatory mechanisms in general and microglial activation in particular (see McGeer and McGeer, 1999; Neuroinflammation Working Group, 2000), the data obtained in this study warrants further studies exploring the suitability of cerebrocrast for treatment of not only various inflammatory disorders of peripheral tissues, but also some neurodegenerative diseases.

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References

Akira, S., Takeda, K., Kaisho, T., 2001. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat. Immunol. 2, 675–680.

Amann, R., Schuligoi, R., 2000. Inhibition of carrageenan-induced edema by indomethacin or sodium salicylate does not prevent the increase of nerve growth factor in the rat hind paw. Neurosci. Lett. 278, 173–176.

Benveniste, E.N., Huneycutt, B.S., Shrikant, P., Ballestas, M.E., 1995. Second messenger systems in the regulation of cytokines and adhesion molecules in the central nervous system. Brain Behav. Immun. 9, 304–314.

Boyd, R.A., Giacomini, J.C., Wong, F.M., Nelson, W.L., Giacomini, K.M., 1987. Comparison of binding activities and negative inotropic potencies of the 1,4-dihydropyridine calcium channel blockers in rabbit myocardium. J. Pharmacol. Exp. Ther. 243, 118–125.

Briede, J., Daija, D., Bisenieks, E., Makarova, N., Uldrikis, J., Poikans, J., Duburs, G., 1999a. Effects of some 1,4-dihydropyridine Ca antagonists on the blast transformation of rat spleen lymphocytes. Cell Biochem. Funct. 17, 97–105.

Briede, J., Daija, D., Stivrina, M., Duburs, G., 1999b. Effect of cerebrocrast on the lymphocyte blast transformation activity in normal and streptozotocin-induced diabetic rats. Cell Biochem. Funct. 17, 89–96.

Correa, S.G., Riera, C.M., Spiess, J., Bianco, I.D., 1997. Modulation of the inflammatory response by corticotropin-releasing factor. Eur. J. Pharmacol. 319, 85–90.

David, M., 1995. Transcription factors in interferon signaling. Pharmacol. Ther. 65, 149–161.

Fukuzawa, M., Satoh, J., Ohta, S., Takahashi, K., Miyaguchi, S., Qiang, X., Sakata, Y., Nakazawa, T., Takizawa, Y., Toyota, T., 2000. Modulation of tumor necrosis factor-α production with anti-hypertensive drugs. Immunopharmacology 48, 65–74.

Hiltz, M.E., Lipton, J.M., 1990. Alpha-MSH peptides inhibit acute inflammation and contact sensitivity. Peptides 11, 979–982.

Inoue, K., Fujisawa, H., Motonaga, A., Inoue, Y., Kyoi, T., Ueda, F., Kimura, K., 1994. Anti-inflammatory effects of etodolac: comparison with other non-steroidal anti-inflammatory drugs. Biol. Pharm. Bull. 17, 1577–1583.

Jacobson, K.A., 1998. Adenosine A_3 receptors: novel ligands and paradoxical effects. Trends Pharmacol. Sci. 19, 184–191.

Klegeris, A., Walker, D.G., McGeer, P.L., 1999. Toxicity of human THP-1 monocytic cells towards neuron-like cells is reduced by non-steroidal anti-inflammatory drugs (NSAIDs). Neuropharmacology 38, 1017–1025.

Klegeris, A., McGeer, E.G., McGeer, P.L., 2000. Inhibitory action of 1-(2-chlorophenyl)-N-methyl-N-(1 methylpropyl)-3-isoquinolinecarboxamide (PK 11195) on some mononuclear phagocyte functions. Biochem. Pharmacol. 59, 1305–1314.

Klusa, V., 1995. Cerebrocrast. Drugs Future 20, 135–138.

Kumar, P., Knaus, E.E., 1994. Synthesis and antiinflammatory activity of 5-(1,6-dihydropyridyl)-tetrazol-2-acetic acids, esters and amides. Drug Des. Discovery 11, 15–22.

Liutkevicus, E., Ulinskaite, A., Meskys, R., Kraujelis, K., Duburs, G., Klusa, V., 1999. Influence of different types of the 1,4-dihydropyridine derivatives on rat plasma corticosterone levels. Biomed. Lett. 60, 39–46.

McGeer, P.L., McGeer, E.G., 1999. Inflammation of the brain in Alzheimer's disease: implications for therapy. J. Leukocyte Biol. 65, 409–415.

Murthy, P.K., Dennis, V.A., Lasater, B.L., Philipp, M.T., 2000. Interleukin-10 modulates proinflammatory cytokines in the human monocytic cell line THP-1 stimulated with *Borrelia burgdorferi* lipoproteins. Infect. Immun. 68, 6663–6669.

Neuroinflammation Working Group, 2000. Inflammation and Alzheimer's disease. Neurobiol. Aging 21, 383–421.

Oyanagui, Y., Sato, S., 1991. Inhibition of nilvadipine of ischemic and carrageenan paw edema as well as of superoxide radical production from neutrophils and xanthine oxidase. Arzneim.-Forsch. 41, 469–474.

Prieto, J., Eklund, A., Patarroyo, M., 1994. Regulated expression of integrins and other adhesion molecules during differentiation of monocytes into macrophages. Cell. Immunol. 156, 191–211.

Ross, R., 1999. Atherosclerosis: an inflammatory disease. N. Engl. J. Med. 340, 115–126.

Tsuchiya, S., Kobayashi, Y., Goto, Y., Okumura, H., Nakae, S., Konno, T., Tada, K., 1982. Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester. Cancer Res. 42, 1530–1536.

Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenin-induced oedema in the hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 111, 544–547.

Yates, S.L., Burgess, L.H., Kocsis-Angle, J., Antal, J.M., Dority, M.D., Embury, P.B., Piotrkowski, A.M., Brunden, K.R., 2000. Amyloid β and amylin fibrils induce increases in proinflammatory cytokine and chemokine production by THP-1 cells and murine microglia. J. Neurochem. 74, 1017–1025.