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A selective inhibitor of cyclooxygenase-2 reverses endotoxin-induced pyretic responses in non-human primates

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Abstract

The anti-pyretic effect of a selective cyclooxygenase-2 inhibitor, DFU (5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulfonyl)phenyl-2(5*H*)-furanone), was examined in conscious, un-restrained squirrel monkeys (*Saimiri sciureus*) using a radio telemetric system. Injection of bacterial endotoxin (lipopolysaccharide, 6 μg kg^{-1}, i.v.) in squirrel monkeys caused a gradual increase in core body temperature reaching a plateau of 2.07 ± 0.17°C above baseline at 2 h post-injection. Oral administration of DFU (1 mg kg^{-1}) reduced, and DFU (3 mg kg^{-1}) completely reversed the lipopolysaccharide-induced pyretic responses. The onset of action of DFU (about 30 min) is in good agreement with the pharmacokinetic profile of this compound in squirrel monkeys. The effect of DFU is comparable to that of a conventional non-selective non-steroidal anti-inflammatory drug (NSAID), diclofenac (3 mg kg^{-1}). Since the plasma levels achieved for DFU at the dose employed in the present study are below the threshold required for inhibition of cyclooxygenase-1, it is concluded that the anti-pyretic effect of DFU can be attributed predominantly to an inhibitory action on cyclooxygenase-2. Thus, lipopolysaccharide-induced pyresis in squirrel monkeys can be used as a model for evaluation of anti-pyretic activity of cyclooxygenase inhibitors.

Keywords: Cyclooxygenase 2; Cyclooxygenase 2 inhibitor; Pyresis; Telemetry; (Squirrel monkey)

1. Introduction

It is now well established that there are two major isoforms of cyclooxygenase. The constitutive enzyme, cyclooxygenase-1, has been well characterized and is believed to be involved in the maintenance of essential physiological functions such as platelet aggregation, cytoprotection in the stomach and maintenance of normal kidney functions. A second isozyme, cyclooxygenase-2, has also been described (for review, see DeWitt and Smith, 1995; Herschman, 1996) and it shares about 60% sequence homology with cyclooxygenase-1 at the amino-acid level. Cyclooxygenase-2 has been shown to be induced significantly in vivo under inflammatory conditions (Kennedy et al., 1993; Masferrer et al., 1994; Harada et al., 1994). This has led to the concept that cyclooxygenase-1 subserves a physiological homeostatic function whilst cyclooxygenase-2 plays a key role in pathological conditions. This has also provided a rationale for the development of selective inhibitors of cyclooxygenase-2 as a new class of anti-inflammatory agents which would possess a substantially improved side effect profile (e.g., markedly reduced propensity for gastrointestinal erosions) compared to current clinically available non-steroidal anti-inflammatory drugs (NSAIDs) which inhibit both cyclooxygenase-1 and cyclooxygenase-2 (Meade et al., 1993; Mitchell et al., 1993; Brideau et al., 1996). In most pre-clinical efficacy studies with selective cyclooxygenase-2 inhibitors, rodent models have been used. Functional models in other species have not been reported. In the present study, we describe a non-human primate model of endotoxin-induced pyresis in which telemetric measurements of core body temperature have been used to track the antipyretic activities of a non-steroidal antiinflammatory drug and, for the first time, a cyclooxygenase-2 inhibitor.

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2. Materials and methods

2.1. Experimental protocol

All experimental procedures were approved by the Animal Care Committee at the Merck Frosst Centre for Therapeutic Research according to guidelines established by the Canadian Council on Animal Care. Sterilized temperature probes (type TA10TA-F40; Data Sciences International, St. Paul, MN, USA, same below for telemetric equipments) were surgically implanted intra-peritoneally in the squirrel monkeys (*Saimiri sciureus*) (0.8–1.2 kg). These probes have built-in batteries that can be activated and deactivated by passing a magnet over the abdomen of the animals. The monkeys were allowed to recover from the surgery for at least 3 weeks before being used for further experimentation. In order to acclimatize the monkeys to a new environment and to being housed alone, the animals were placed individually in stainless steel cages ($24 \times 24 \times 33$ in) in a room with controlled temperature (25.5 ± 1.0°C) and humidity (45–60%) the evening before each experiment. Each cage is equipped with a signal receiver (model RLA2000) which picks up a radio signal from the temperature probe. The signal receivers were connected to a microcomputer via a consolidation matrix (BCM-100) and the signals from the probes were processed using a software package (Dataquest Labpro) obtained from the manufacturer. This system allows for the continuous monitoring of core body temperature in conscious, unrestrained monkeys. At approximately 09:00 h on the day of the experiment, the monkeys were restrained temporarily in restraint chairs and were given a bolus injection of lipopolysaccharide (from *Salmonella typhosa*, 6 μg kg^{-1}, dissolved in sterile saline; Sigma, St. Louis, MO, USA) via the femoral vein (time zero). The monkeys were returned to their cages and body temperature was recorded every 5 min. Two hours after injection of lipopolysaccharide, when the body temperature had increased by approximately 2°C, the monkeys were dosed orally with either vehicle (1% methocel; 3 ml kg^{-1} body weight), diclofenac (3 mg kg^{-1}; Sigma) or DFU (1 or 3 mg kg^{-1}; 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-ethylsulfonyl)phenyl-2(5*H*)-furanone; synthesized by the Medicinal Chemistry Department at Merck Frosst Centre for Therapeutic Research). Baseline temperature was determined by averaging all the temperature points during the hour before injection of lipopolysaccharide. The results are expressed as the difference between the body temperature and the baseline value since there is substantial variation in baseline temperature between individual monkeys (see Section 3).

2.2. Determination of plasma levels of DFU

In a separate study, two squirrel monkeys were dosed orally with DFU (5 mg kg^{-1}) and plasma samples were obtained at 15, 30, 60 and 120 min post-dosing. Plasma levels of DFU were determined by reverse-phase HPLC on a 4 μm Nova Pak C_{18} column (3.9×150 mm, Waters, Milford, MA, USA) following extraction with equal volume of acetonitrile. The solvent system was absolute methanol/10 mM phosphate buffer, pH 7.0 (45:55) at a flow rate of 1 ml min^{-1}; samples were monitored at 275 nm.

2.3. Statistics

Results are expressed as mean ± standard error of mean (S.E.M.). Difference between vehicle control and treatment groups were tested using one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparison. A P value less than 0.05 was considered statistically significant.

3. Results

The baseline temperature in 7 control monkeys was very stable since the monkeys had been acclimatized to the cages for at least 16 h before each experiment. The individual baseline temperature (average temperature for 12 time points recorded during the hour before injection of lipopolysaccharide) in each monkey was: 38.16 ± 0.02°C, 38.29 ± 0.01°C, 37.66 ± 0.08°C, 37.05 ± 0.08°C, 38.47 ± 0.04°C, 37.73 ± 0.07°C and 37.52 ± 0.05°C; reflecting a group mean of 37.84 ± 0.19°C ($n = 7$ monkeys). The body temperature, however, fluctuated substantially immediately

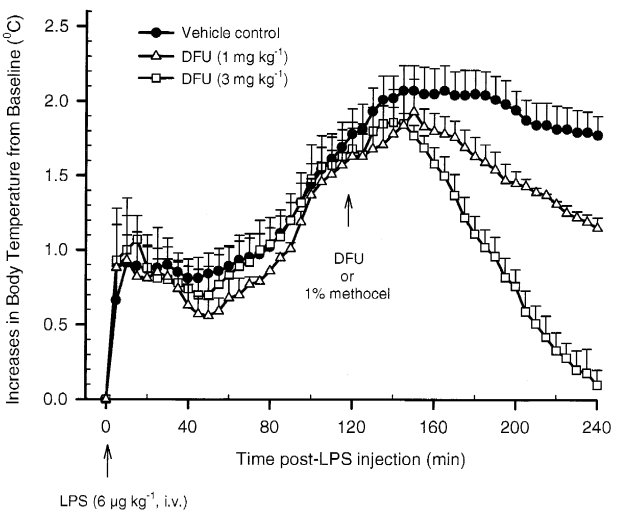

Fig. 1. Anti-pyretic effect of DFU in squirrel monkey. Lipopolysaccharide was injected at time zero and DFU or the vehicle was given orally 2 h after injection of lipopolysaccharide. Results are expressed as the mean increases (±S.E.M.) in body temperature over baseline value. Points in the DFU groups (1 or 3 mg kg^{-1}) beyond 180 min post-injection are significantly different from the matching vehicle control with $P < 0.05$. $n = 7$ (vehicle), 3 (DFU at 1 mg kg^{-1}), 4 (DFU at 3 mg kg^{-1}).

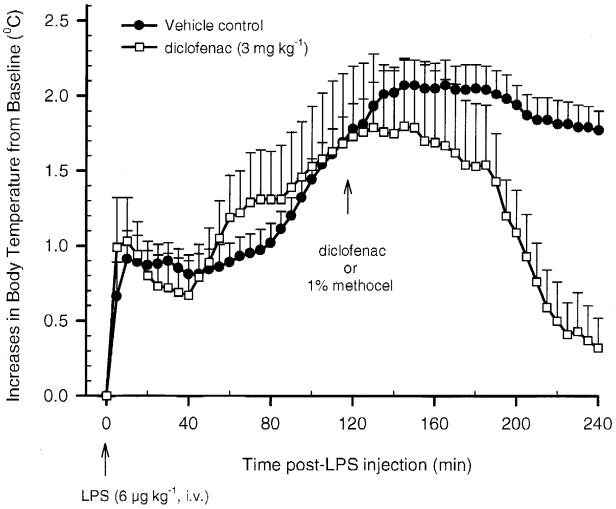

Fig. 2. Anti-pyretic effect of diclofenac in squirrel monkey. Lipopolysaccharide was injected at time zero and diclofenac or the vehicle was given orally 2 h after injection of lipopolysaccharide. The vehicle control group is the same group as shown in Fig. 1. Results are expressed as the mean increases (±S.E.M.) in body temperature over baseline value. Points in the diclofenac group beyond 205 min post-injection are significantly different from the matching vehicle control with $P < 0.05$. $n = 7$ (vehicle), 3 (diclofenac).

after intravenous injection of lipopolysaccharide (Fig. 1), possibly because of the activity and stress resulting from the handling of the animals. In the control (vehicle-treated) group, injection of lipopolysaccharide resulted in a gradual increase in body temperature, reaching an increase of 2.07 ± 0.17°C ($n = 7$) at 140 min post-injection. The elevation in body temperature was maintained at a reasonably steady level during the period between 140–240 min post-lipopolysaccharide injection. Oral administration of DFU (at 1 or 3 mg kg^{-1}) significantly reversed the elevated body temperature, starting within 30 min after oral dosing. At 240 min post-lipopolysaccharide injection, the mean increase in body temperature in the control group was 1.77 ± 0.13°C ($n = 7$), in the group treated with DFU at 1 mg kg^{-1} 1.15 ± 0.07°C ($n = 3$; 35% inhibition) and in the 3 mg kg^{-1} treated DFU group 0.10 ± 0.10°C ($n = 4$; 94% inhibition) (Fig. 1). Six hours after injection of lipopolysaccharide, the body temperature in all 3 groups had returned to the baseline level. The effect of DFU was comparable to that of a conventional NSAID, diclofenac (3 mg kg^{-1}, p.o.), which reversed the lipopolysaccharide-induced pyretic responses by 82% (temperature increase at 240 min was 0.32 ± 0.20°C, $n = 3$) (Fig. 2). In separate experiments, the plasma level of DFU (5 mg kg^{-1}, p.o.) in 2 monkeys reached a plateau 30 min (t_{max}) after oral administration and remained relatively constant for the remainder of the 2 h measurement period (Fig. 3).

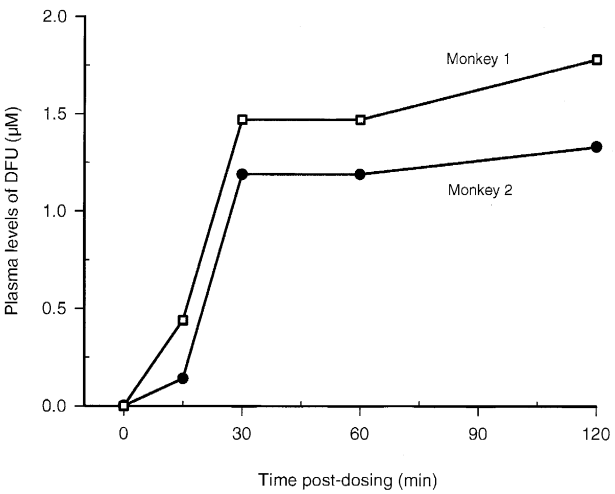

Fig. 3. Pharmacokinetics of DFU in squirrel monkey. Plasma levels of DFU in two monkeys dosed orally with DFU at 5 mg kg^{-1} are shown. Levels of DFU were determined by reverse-phase HPLC on a 4 μm Nova Pak C_{18} column (3.9×150 mm, Waters, Milford, MA, USA) following extraction with equal volume of acetonitrile. The solvent system was absolute methanol/10 mM phosphate buffer, pH 7.0 (45:55) at a flow rate of 1 ml min^{-1}; samples were monitored at 275 nm.

4. Discussion

Bacterial infection in experimental animals or human beings causes sepsis characterized, in part, by an increase in body temperature. This response is thought to be mediated by the production of endogenous pyrogens such as interleukin-1β and tumor necrosis factor-α which cross the blood-brain barrier and subsequently induce the release/synthesis of other pyrogenic mediators (for review, see Rosendorff and Woolf, 1979). There is now strong evidence to support a role for prostaglandins in the regulation of pyretic responses in pathological states such as that induced by bacterial endotoxin. Micro-injection of prostaglandins of the E series directly into the cerebroventricle or into the hypothalamic area of the brain causes a rise in body temperature in many species including rat (Williams et al., 1977; Horn et al., 1994), rabbit (Milton and Wendlandt, 1971; Laburn et al., 1975), cat (Milton and Wendlandt, 1971; Gollman and Rudy, 1988), pig (Parrott and Vellucci, 1996), sheep (Bligh and Milton, 1973) and monkey (Barney and Elizondo, 1978; Simpson et al., 1993). Prostaglandin-like material in the cerebrospinal fluid is enhanced during pyresis in experimental models (Feldberg et al., 1973; Phillip-Dormston and Siegert, 1974; Coceani et al., 1988). Conventional NSAIDs, which inhibit the production of prostaglandin E_2 by inhibiting both cyclooxygenase-1 and cyclooxygenase-2, are effective anti-pyretic agents in both laboratory animals and human beings. These data are highly supportive of a role for prostaglandins in mediating pyresis. Thus, an obvious question arises: which cyclooxygenase isozyme is principally involved in mediating the pyretic responses in pathological conditions. This question can now be addressed

since selective, bioavailable inhibitors of cyclooxygenase-2 are now available.

We have shown previously that the selective cyclooxygenase-2 inhibitor, L-745,337, can completely and dose-dependently reverse lipopolysaccharide-induced pyresis in rats (Chan et al., 1995). This is consistent with the observation that both cyclooxygenase-2 mRNA and protein are detectable in rat brain (Yamagata et al., 1993; Breder et al., 1995; Adams et al., 1996). In rats, it has been shown that cyclooxygenase-2 mRNA is induced in the preoptic area, a region thought to be involved in regulating body temperature, in lipopolysaccharide-treated but not in control animals (Cao et al., 1995). The time course of induction of cyclooxygenase-2 is in accord with the lipopolysaccharide-induced pyretic responses, supporting the involvement of prostaglandins generated via cyclooxygenase-2 in pyresis. In the present study, we have extended this observation in a non-human primate model of pyresis using a more selective cyclooxygenase-2 inhibitor, DFU. DFU is structurally different from L-745,337 and is more than 1000-fold selective for cyclooxygenase-2 (Riendeau et al., 1997). It has been reported that exposure of non-human primates such as the squirrel monkey (Lipton et al., 1979), rhesus monkey (Perlow et al., 1975) and baboon (Zurovsky et al., 1987) to bacterial pyrogen causes an increase in body temperature. In the present study, we have used radio telemetry to monitor core (intra-peritoneal) body temperature in conscious, un-restrained squirrel monkeys. Adoption of this experimental approach eliminates the ethical concern of restraining the monkeys for many hours and also reduces the procedure-induced artifacts which were, in fact, observed temporarily after intravenous injection of lipopolysaccharide. Oral administration of either DFU or the non-selective NSAID diclofenac reversed effectively the lipopolysaccharide-induced pyretic responses in squirrel monkeys. The onset of the action of DFU is relatively fast (approx. 30 min post-dosing). This observation is consistent with data from the pharmacokinetic studies of this compound that determined t_{max} to be 30 min after oral dosing. The effect of DFU can be attributed predominantly to inhibition of cyclooxygenase-2 since the plasma level of DFU (approx. 1.5 μM) at the dose range used in the present study is well below the threshold required to inhibit cyclooxygenase-1 (the IC_{50} for DFU to inhibit cyclooxygenase-1 and cyclooxygenase-2 in human whole blood is > 100 μM and 0.3 μM, respectively; Riendeau et al., 1997). Thus, it is an entirely reasonable conclusion that inhibition of cyclooxygenase-2 alone is sufficient to completely inhibit the pyresis caused by bacterial endotoxin.

In summary, we have demonstrated for the first time the anti-pyretic effectiveness of a cyclooxygenase-2 inhibitor in a non-human primate model. As selective cyclooxygenase-2 inhibitors are now in clinical development, it will be interesting to ascertain whether similar activity is evident in human subjects.

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