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Research Paper

Preclinical pharmacokinetic and pharmacodynamic evaluation of thiazolidinone PG15: an anti-inflammatory candidate

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Abstract

Objectives Novel 5-benzilidene thiazolidinones have been synthesized and exhibited anti-inflammatory activity. In this work one of the compounds of the thiazolidinone chemical series, (5*Z*,*E*)-3-[2-(4-chlorophenyl)-2-oxoethyl]-5-(1*H*-indol-3-ylmethylene)-thiazolidine-2,4-dione (PG15) was investigated aiming to determine the drug's anti-inflammatory potential in pre-clinical studies.
Methods Methods used included the in-vitro inhibition of cyclooxygenase-1 and -2, in-vivo evaluation of anti-inflammatory activity by air pouch and peritonitis models and the pharmacokinetic profile after intravenous (3 mg/kg) and oral (3 and 6 mg/kg) dosing to rats.
Key findings A two-compartment model with a fast distribution and an elimination half-life of 5.9 ± 3.8 h described the PG15 plasma profile after intravenous dosing. PG15 showed an erratic and rapid absorption following oral administration with peak concentrations between 0.5 and 1 h. PG15 0.1 μM inhibited more than 30% and 13% of purified cyclooxygenase-1 and -2 activity *in vitro*, respectively. A lack of dose dependency was observed for the anti-inflammatory effect in the dose range investigated (0.8–50 mg/kg), with a maximum of 67.2 ± 4.6% inhibition of leucocyte migration in the carrageenan-induced air pouch model obtained with the 3 mg/kg dose, similar to that observed for indometacin 10 mg/kg.
Conclusions The erratic absorption of PG15 observed after oral dosing could explain the lack of anti-inflammatory dose dependency.
Keywords anti-inflammatory drug; PG15; pharmacodynamics; pharmacokinetics

Introduction

In drug discovery the idea has been considered that treatment should be applied to humans as a complex biological system, composed of cells and tissues networked by redundant, converging and diverging signalling pathways. Thus, better results are obtained when responses are evoked by multipoint interventions in more than one mechanism and in different targets,[1] introducing the concept of multi-target drugs, also known as dual or symbiotic drugs.[2] Using this concept, the molecular structure of the non-steroidal anti-inflammatory drug (NSAID) indometacin (Figure 1a) was chemically overlapped with the structure of the anti-diabetic peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist roziglitazone (Figure 1b), creating a new series of thiazolidinones aiming to reach two important targets in inflammation: the well know cyclooxygenases (COXs) and the recently discovered target of inflammation, PPAR-γ.[3]

In 2005 our group published the synthesis of the thiazolidinone 3-(4-chlorobenzyl)-5-(1*H*-indol-3-ylmethylene)-4-thioxo-thiazolidin-2-one) (Figure 1c) obtained by this approach and reported its activity against carrageenan-induced rat paw oedema, in which it exhibited 77.5% inhibition of the inflammatory stimulus 240 min after dosing.[4] This compound, that has an indol group attached to the thiazolidine central ring, was docked in the COX-2 channel using the FlexX program interfaced with Sybyl 7.2[5] with good bonding, exhibiting a free energy of –13 254 kcal/mol. Silva and co-workers (2003)[6] synthesized *N*-tryptophyl-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-2,4-thiazolidinedione (Figure 1d), which, against carrageenan-induced paw oedema, exhibited an average inhibition of 28.36% after a 100 mg/kg oral dose and did not show ulcerogenic activity on the gastric mucosa.

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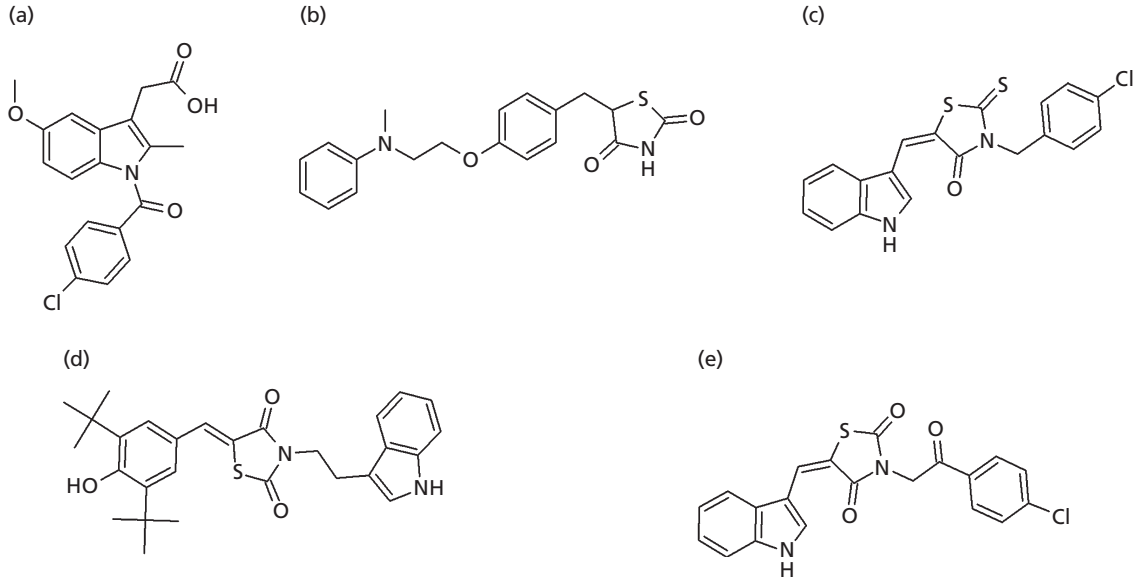

Figure 1 Chemical structure of indometacin (a), rosiglitazone (b), 3-(4-chlorobenzyl)-5-(1*H*-indol-3-ylmethylene)-4-thioxo-thiazolidin-2-one (c), *N*-triptofil-5-substituted-2,4-thiazolidinedione (d) and 5-(*Z,E*)-3-[2-(4-chlorophenyl)-2-oxoethyl]-5-(1*H*-indol-3-ylmethylene)-thiazolidine-2,4-dione (PG15) (e).

A structure–activity relationship study indicated that another compound of this series, 5(*Z,E*)-3-[2-(4-chlorophenyl)-2-oxoethyl]-5-(1*H*-indol-3-ylmethylene)-1,3-thiazolidine-2,4-dione (PG15) (Figure 1e), could be a better dual lead candidate.[7]

In this context, the aims of this work were to investigate the anti-inflammatory potential of PG15 in pre-clinical studies including the in-vitro inhibition of COX-1 and COX-2 and the in-vivo evaluation of anti-inflammatory activity by the air pouch and peritonitis models as well as to conduct a preliminary pharmacokinetic investigation of the drug after systemic and non-systemic dosing to rats.

Materials and Methods

Chemicals

PG15 was obtained by synthesis as described previously by Uchôa,[7] and was authenticated using mass and NMR spectroscopy. Carrageenan was acquired from Sigma (MO, US). Chlortalidone, nimesulide and dexamethasone were purchased from Galena (Campinas, Brazil) and indometacin and aspirin from Deg (São Paulo, Brazil). Celecoxib was acquired as commercial Celebra from Pfizer (Guarulhos, Brazil). COX inhibition reagents were supplied from Cayman Chemical Company (MI, US). Acetonitrile HPLC grade, ammonium hydroxide and polysorbate 80 were purchased from Merck (Darmstadt, Germany). HPLC water from Millipore's Milli-Q System was used throughout the analysis. Saline sterile solution was purchased from BBraun (São Gonçalo, Brazil). All other chemicals used in this study were of analytical grade.

Inhibition of purified cyclooxygenase-1 and -2

COX-1 and COX-2 assays were performed using the colorimetric ovine cyclooxygenase (COX) assay kit (Cayman Chemical Company, US). Inhibition of enzyme activity was determined by a colorimetric assay as previously described by Kulmacz and Lands.[8] The test measures the peroxidase component of cyclooxygenases, which is assayed by monitoring the appearance of oxidized *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm.

Different dilutions of PG15 were prepared in dimethyl sulfoxide (DMSO) and diluted further to derive the appropriate concentrations for testing. The assay was then performed as described in the assay kit booklet.[9]

In-vivo procedure

All in-vivo procedures were approved by the Universidade Federal do Rio Grande do Sul Ethics in Research Committee (protocol 2006/608) or the Universidade Federal de Pernambuco Ethics in Animal Experimentation Committee (protocol 23076.011488/2005-35). Male Wistar rats were purchased from Fundação para Produção e Pesquisa em Saúde–FEPPS (Porto Alegre, RS, Brazil). Male and female albino Swiss mice were purchased from the Aggeu Magalhães Bioterium (Recife, PE, Brazil). All animals were housed in accredited facilities under standard conditions for rodents. Animals were acclimatized for at least 3 days before use.

Carrageenan-induced air pouch inflammation

To induce air pouches, male and female Swiss mice (n = 10) were injected subcutaneously on the dorsal surface with 2.5 ml of air. After three days, the pouches were reinflated with 2.5 ml of air. On day six, the mice received vehicle or PG15 orally (0.8, 3, 12.5 or 50 mg/kg). One hour after drug administration, inflammation was induced by injecting 1 ml of carrageenan suspension (1% in saline solution) into the air pouch, as described by Klemm *et al.*[10] and adapted by Romano *et al.*[11] After 6 h, the mice were euthanized and the pouches were flushed with 3 ml of phosphate-buffered

saline (PBS) with heparin (10 IU/ml). Samples were diluted with Turk solution and leucocytes were counted in a Neubauer chamber under a microscope. The average numbers of leucocytes from treated groups were compared with the number of leucocytes of the control group, assumed to be 100%. From the 3 mg/kg group, samples of exudates were frozen at −32°C until analysis by liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS; described below under PG15 quantification in plasma) to quantify PG15 at the inflammation site.

Carrageenan-induced peritonitis

The carrageenan-induced peritonitis experiment was conduced in Male Swiss mice ($n = 6$) as describe by Oliani *et al.*[12] Mice were injected intraperitoneally with 0.25 ml of carrageenan (1% in saline solution) 1 h after receiving PG15 (3 mg/kg) or vehicle orally. The mice were euthanized 4 h after the induction of inflammation. The peritoneal exudates were flushed with 2 ml of heparinized PBS (10 IU/ml), collected and the total leucocyte count was determined. Aiming to quantify PG15 in the peritonitis exudates, samples were frozen at −32°C until analysis by LC-MS/MS (described below under PG15 quantification in plasma).

Pharmacokinetics

PG15 was administered to male Wistar rats, 290–325 g, by intravenous bolus injection (3 mg/kg, $n = 5$) or orally by gavage (3 mg/kg ($n = 3$) or 6 mg/kg ($n = 3$)). PG15 was administered as a 4 mg/ml suspension prepared with 10% ethanol and 10% polysorbate 80 in a 5% glucose solution. The maximum volume administered intravenously was 250 μl and orally, 500 μl.

After dosing, blood samples were collected into heparinized tubes by venopuncturing the lateral tail vein at scheduled time points up to 16 h (before dosing and at 5, 10, 15, 30 and 60 min, for intravenous administration; before dosing and after 15, 30, 45, 60 and 90 min, 3, 6, 9, 12 and 16 hours after oral dosing). Plasma was separated by centrifugation (10 min, 6800*g*, 4°C) and 100-μl samples were stored at −20°C until analysis.

From individual rat plasma profiles after intravenous and oral dosing, pharmacokinetic parameters were derived by non-compartmental and compartmental approaches. Non-compartmental analysis was conducted in Microsoft Office Excel 2007 using classical pharmacokinetic equations.[13] For compartmental analysis, plasma curve fitting was performed using Scientist v. 2.01 (MicroMath). The criteria employed to select the appropriate model to describe the profiles were: the model selection criterion (MSC), a modified Akaike Information Criterion (AIC) given by the program, the visual fitting of the model to the experimental data and the coherence of the pharmacokinetic parameters determined. The higher the MSC, the more appropriate is the selected model to describe the data.

PG15 quantification in plasma

On the day of analysis, samples were thawed, spiked with 10 μl of internal standard (IS) (chlortalidone 100 μg/ml), deproteinized with acetonitrile (300 μl) and centrifuged (10 min, 15 000 rev/min, 4°C). Twenty microlitres of the supernatant was used to quantify PG15 using an LC-MS/MS method previously validated according to Food and Drug Administration (FDA) guidelines (Uchôa, 2008).[7] Briefly, a reversed phase C18 Symmetry Waters column (75 × 4.6 mm, 3.55 μm particle size) kept at 40°C preceded by a C18 pre-column was eluted with a mobile phase consisting of acetonitrile–10 mM ammonium hydroxide (90:10%, v/v) with a flow rate of 0.3 ml/min. The LC-MS/MS system consisted of a Shimadzu LC coupled to a Micromass mass spectrometer with electrospray ionization in the negative mode. Detected fragments were 395.1 > 171.9 and 337.3 > 189.9 for PG15 and IS, respectively. The calibration curve was linear in the range of 10–1000 ng/ml; the lower limit of quantification was 10 ng/ml and the lower limit of detection was 2 ng/ml. The intra and inter-day precision expressed as the relative error was found to be less than 12.2 and 11.3%, respectively for all the concentrations investigated. Accuracy in the measurement of quality control (QC) samples was in the range of 86.9–113.1%. The same procedure was used to quantify PG15 in the exudate samples.

Statistics

Statistical comparisons between the vehicle-treated control and compound-treated groups were made by analysis of variance followed by Tukey's test. $P < 0.05$ was considered statistically significant for all in-vivo pharmacodynamic experiments. The results of the enzymatic inhibition assay were compared by Student's *t*-test ($\alpha = 0.05$). Pharmacokinetic parameters determined by both approaches for the intravenous group were compared by Student's *t*-test assuming equal variances ($\alpha = 0.05$). Analysis of variance followed by Tukey's test was used to compare the pharmacokinetic parameters determined for the different doses and routes of administration ($\alpha = 0.05$).

Results

Inhibition of purified cyclooxygenase-1 and -2

PG15 inhibited COX-1 and COX-2 at micromolar concentrations (Table 1) with no significant difference between the inhibition observed for the lower (0.01 μM) and the higher concentrations (1 μM) tested for each enzyme. Indometacin exhibited a higher affinity to COX-1 than PG15, reaching 100% of enzymatic activity inhibition at 1 μM. PG15,

Table 1 cyclooxygenase-1 and -2 inhibition by the thiazolidinone PG15, celecoxib and indometacin at 0.01 μM and 1 μM

Drug	Concentration (μM)	COX-1 inhibition (%)	COX-2 inhibition (%)
PG15	1	39.1 ± 3.6	10.1 ± 3.0
	0.01	30.0 ± 0.7	13.9 ± 3.7
Celecoxib	1	0	47.5 ± 1.0*
	0.01	0	11.6 ± 1.0*
Indometacin	1	100 ± 0.1*	0
	0.01	41.6 ± 0.1*	0

Data represent mean ± standard error; *$\alpha = 0.05$, comparing both doses of the same drug. COX, cyclooxygenase.

however, showed an inhibition of COX-2 activity similar to that observed for celecoxib at the lower concentration investigated (0.01 μM). The inhibitory concentration (IC50) of PG15 was not possible to calculate because when increased concentrations were used in the assay PG15 precipitated in the enzymatic reaction medium.

Inhibition of leucocyte migration in carrageenan-induced mouse air pouch

The inhibition of leucocyte migration, which expresses the anti-inflammatory activity of the drugs investigated, is presented in Table 2. For PG15 administered orally in the dose range of 0.8–50 mg/kg, a maximum of 67.2 ± 4.6% inhibition was reached with the 3 mg/kg dose, which was of a similar magnitude to that observed with indometacin, the reference drug.

Exudate samples analysed from the group that received a PG15 3 mg/kg oral dose 7 h after dosing showed a mean drug concentration of 84.9 ± 43 ng/ml (n = 6).

Carrageenan-induced peritonitis

The number of leucocytes 4 h after carrageenan-induced peritoneal inflammation in the control group of mice ($9.80 \times 10^6 \pm 0.94 \times 10^6$ leucocytes/ml) was reduced to $6.79 \times 10^6 \pm 0.76 \times 10^6$ leucocytes/ml after the oral administration of a 3 mg/kg dose of PG15. The count after indometacin (10 mg/kg) was $4.52 \times 10^6 \pm 0.61 \times 10^6$ leucocytes/ml. Assuming the control cells count as 100%, PG15 inhibited 30.7 ± 4.2% of leucocyte migration in the peritonitis model. Exudates analysed to quantify PG15 in inflamed peritoneal fluid 5 h after dosing exhibited average drug concentrations of 30.5 ± 7.7 ng/ml (n = 3) when a 3 mg/kg oral dose was administered.

Pharmacokinetics in rats

PG15 pharmacokinetics were evaluated in Wistar rats after 3 mg/kg intravenous dosing and 3 and 6 mg/kg oral dosing.

Table 2 Anti-inflammatory activity in the carrageenan-induced mouse air-pouch model exhibited by PG15 and standard NSAIDs given orally at respective effective doses

Compound	Dose (mg/kg)	PMNL/ml	Anti-inflammatory activity (%)[a]
PG15	0.8	$0.70 \pm 0.06 \times 10^6$	59.1 ± 3.5
	3	$0.56 \pm 0.08 \times 10^6$	67.2 ± 4.6[c]
	12.5	$0.83 \pm 0.08 \times 10^6$	51.5 ± 4.6
	50	$1.04 \pm 0.05 \times 10^6$	39.2 ± 2.9[b]
Aspirin	200	$0.45 \pm 0.03 \times 10^6$	73.7 ± 2.04[e]
Indometacin	10	$0.76 \pm 0.07 \times 10^6$	55.5 ± 4.3[f]
Dexamethasone	1	$0.40 \pm 0.1 \times 10^6$	76.6 ± 6.2[d]
Nimesulide	5	$1.16 \pm 0.1 \times 10^6$	32.2 ± 5.86[d]
Celecoxib	10	$0.27 \pm 0.06 \times 10^6$	84.2 ± 3.9[d]
Control	n.a.	$1.71 \pm 0.12 \times 10^6$	n.a.

PMNL, polymorphonuclear leucocytes; n.a., not applicable. PMNL count is expressed as total cell number by volume of exudate. Activity (%) was calculated considering the control group cells counting as 100%. [a]Values represent average ± standard error; [b]statistically different from other PG15 doses; [c]statistically different from PG15 12.5 and 50 mg/kg; [d]statistically different from all PG15 doses; [e]statistically different from PG15 12.5 and 50 mg/kg; [f]statistically different from PG15 50 mg/kg (α = 0.05, determined using analysis of variance followed by Tukey's test).

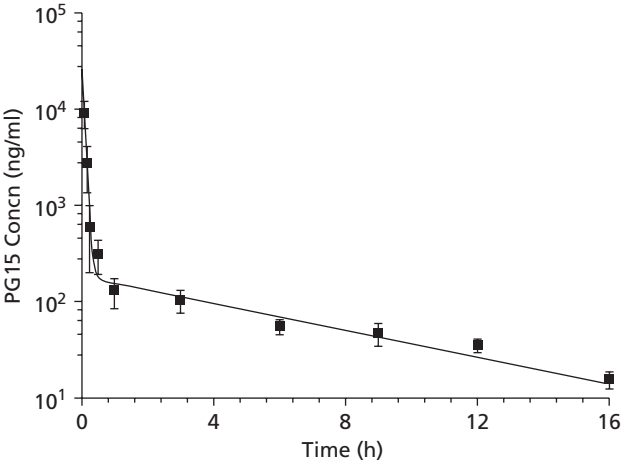

Figure 2 Mean plasma profile of PG15 in rats after a single 3 mg/kg intravenous dose. Values are average ± SE, n = 5.

After intravenous dosing, PG15 exhibited a rapid distribution, with plasma concentrations falling from 9000 ng/ml at 5 min after administration to 300 ng/ml at 30 min (Figure 2). After distribution, PG15 elimination was slow, characterizing the drug disposition in the body as a two-compartment model.

Pharmacokinetic parameters determined from individual plasma profiles after intravenous dosing are presented in Table 3. PG15 presented an average half-life of approximately 6 h, with a volume of distribution of 2.4 l/kg and a clearance of 0.9 l/h/kg. Pharmacokinetic parameters estimated by compartmental and non-compartmental approaches had no statistical difference, showing the suitability of the model selected to fit the experimental data. The goodness of fit, determined by the MSC, was in the range of range of 2.93–4.98, also confirming the model selected.

After oral dosing with 3 mg/kg PG15, the drug was rapidly absorbed showing peak plasma concentrations between 0.5 and 1 h (Figure 3a). The pharmacokinetic parameters determined by non-compartmental analysis for this dose are presented

Table 3 Pharmacokinetic parameters of PG15 following intravenous dosing of 3 mg/kg to Wistar rats

Parameter	Compartmental analysis
A (ng/ml)	71300 ± 67500
B (ng/ml)	162.2 ± 98.2
α (h^{-1})	21.28 ± 10.63
β (h^{-1}) or ke (h^{-1})	0.16 ± 0.09
$AUC_{0-\infty}$ (ng h/ml)	3949 ± 2068
Vc (l/kg)	0.11 ± 0.12
Vd_{ss} (l/kg)	2.4 ± 1.8
CL_{tot} (l/h/kg)	0.9 ± 0.5
t½α (h)	0.04 ± 0.03
t½β (h)	5.9 ± 3.8

A, intercept of the distribution phase; B, intercept of the elimination phase; α, distribution rate constant; β, elimination rate constant; AUC, area under the curve; Vc, volume of the central compartment; Vd_{ss}, volume of distribution at steady state; CL_{tot}, total clearance; k_e, elimination rate constant; t½α, distribution half-life; t½β, elimination half-life. Values represent average ± SD, n = 5.

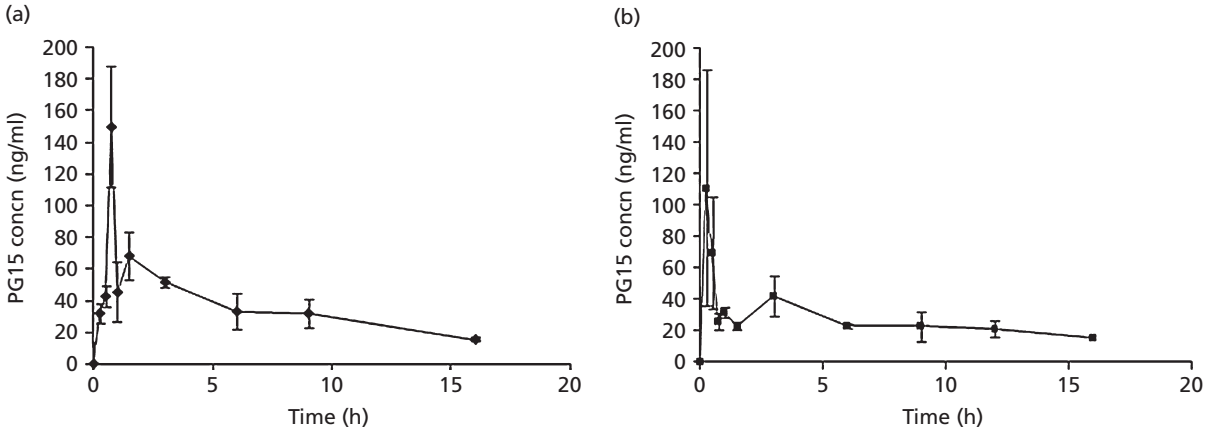

Figure 3 Mean plasma profiles of PG15 in rats after a single 3 mg/kg (a) or 6 mg/kg (b) oral dose. Values are average ± SE, $n = 3$.

in Table 4. The half-life, elimination rate constant and total clearance following oral and intravenous administration of a 3 mg/kg dose were statistically similar ($\alpha = 0.05$) (Tables 3 and 4).

Plasma profiles obtained after a 6 mg/kg dose were very erratic and variable (Figure 3b). The average profile was similar to that obtained after 3 mg/kg oral dosing. No pharmacokinetic model was capable of adequately fitting the individual profiles. The results of the non-compartmental analysis are shown in Table 4.

Discussion

The investigation of the pharmacological profile of PG15 as an anti-inflammatory candidate and the correlation of drug kinetics and dynamics was the aim of this investigation.

Inhibition of COX-1 and COX-2 evoked by PG15 showed that the drug is capable of inhibiting both COXs at the concentrations tested, exhibiting a slightly preference for COX-1. It was also noted that possibly the highest inhibition that the drug could reach was already obtained with the lower concentration.

Comparing with standard drugs tested in customary concentrations in the air pouch model, PG15 exhibited a higher activity than nimesulide, a COX-2 preferential inhibitor, similar activity to indometacin, a COX-1 preferential inhibitor, and a lower activity than aspirin, celecoxib and dexamethasone, COX-1 selective, COX-2 selective and phospolipase A2 inhibitors, respectively. Since the standard drugs tested, which act by different mechanisms, showed results compatible with those previously reported in the literature,[14] one can assume that the chosen experimental model was reliable to indicate that PG15 is an interesting anti-inflammatory candidate. It can also be seen that the effect obtained by PG15 was not dose-dependent in the dose range tested, because the highest effect was already reached with the 3 mg/kg dose.

Table 4 PG15 pharmacokinetic parameters determined by a non-compartmental approach following oral dosing to Wistar rats

Parameter	PG15 3 mg/kg	PG15 6 mg/kg
ke (h^{-1})	0.12 ± 0.01	0.19 ± 0.11
t½ (h)	5.8 ± 0.5	4.5 ± 2.2
$AUC_{0-\infty}$ (ng h/ml)	686 ± 117	465 ± 72
% $AUC_{extrapolated}$	18.6 ± 1.5	20.8 ± 13.0
C_{max} (ng/ml)	134.3 ± 53.4	150.8 ± 103.4
t_{max} (h)	0.75 ± 0.00	0.42 ± 0.02[a]
CL_{tot} (l/h/kg)	0.76 ± 0.1	0.8 ± 0.1
Bioavailability	0.17	0.06

k_e, elimination rate constant; t½, elimination half-life; AUC, area under the curve; C_{max}, plasma peak concentration; t_{max}, time to peak concentration; CL_{tot}, total clearance. Values are average ± SD, $n = 3$/group. [a]α, 0.05, comparing doses.

The leucocyte migration to inflammatory sites observed in this inflammation model is a gradual process, which is dominated in its early phases by chemokine- and cytokine-mediated neutrophil recruitment.[15] It has been reported that after a lag time of about 2 h, neutrophils steadily accumulate in the carrageenan induced air pouch up to 8 h. Similarly, the exudate concentrations of interleukin-8 increase after a 2-h lag time reaching a peak at 8 h.[16] It is also known that tumour necrosis factor is a mediator of inflammation in the carrageenan-induced mouse air pouch model.[11] This suggests that PG15 could act by interfering in chemokine- and cytokine-mediated leucocyte recruitment. It is important to note that the production of PGE_2 is induced in the air pouch model, a process which is largely derived from COX-2 originating in the lining tissue.[17] Another prostaglandin derived from COX-2, PGF_2, is responsible for stimulating leucocyte migration to inflammation sites,[18] demonstrating a good relationship between COX-2 inhibition and the reduction of leucocyte count in the pouch exudate. Thus, the in-vitro inhibition of COX-2 observed with PG15 is certainly one of the mechanisms responsible for the anti-inflammatory activity determined in the air pouch model.

It is not possible to discard the possibility that PG15 also acts by activating PPAR, since PPAR ligands also repress expression of cell adhesion molecules on endothelial cells and the secretion of chemokines by epithelial and other cells, decreasing the recruitment of leucocytes to the site of inflammation.[19]

It can be seen from Table 2 that PG15 exhibits an important anti-inflammatory activity, comparable with that

of reference NSAIDs such as indometacin. However, when PG15 inhibition of COX-2 *in vitro* is compared with the activity presented by reference drugs such as celecoxib, inhibition was not so impressive and it was achieved only at a relatively high concentration. Celcoxib IC50 for COX-2 was 0.04 μM[20] while PG15 maximal inhibition attained was around 10% with a 10 μM concentration. So, the in-vitro inhibition observed corroborates the hypothesis that PG15 activity *in vivo* is due not only to COX inhibition but also to an associated mechanism.

The lower anti-inflammatory activity observed in the peritonitis model compared with the air pouch model may be a reflection of the drug concentrations obtained in each site. According to Sedgwick *et al.*,[21] the air pouch model induces the proliferation of cells that stratify on the surface of the cavity to form a structure similar to the synovia. In the peritonitis model, the inflammatory site corresponds to cells of the normal peritoneal cavity and recruited cells. Therefore, the inflammatory sites investigated have low tissue similarities, which was reflected in the penetration behaviour of the drug, where PG15 concentrations determined in the air pouch model were around 2.8 times those determined in peritonitis.

Pharmacokinetic study demonstrated that PG15 after 3 mg/kg intravenous dosing exhibited a two-compartment model profile, where high concentrations immediately after injection decreased about 30 times in 30 min. Although PG15 showed plasma profiles compatible with first-order elimination after 3 mg/kg intravenous dosing, data from intravenous administration of 15 mg/kg indicate that the drug probably presents saturable elimination at higher doses.[7]

After PG15 3 mg/kg oral dosing the drug was rapidly absorbed, although from Figure 3 it can be seen that the first hour after dosing was not well-characterized, making it difficult to fit the data to either a one- or two-compartment model, although a distribution phase could be visualized in the plots.

PG15 plasma concentrations after intravenous 3 mg/kg dosing were extremely high in the first 15 min after administration and declined very quickly. These high concentrations resulted in a greater overall body exposure to PG15 after systemic administration, expressed by an area under the curve (AUC) of 4025 ± 1496 ng h/ml; in comparison, the AUC observed after the same dose was administered orally was 686 ± 117 ng h/ml, leading to a bioavailability of 17%.

Aiming to evaluate whether PG15 exhibits linear pharmacokinetics in the dose range investigated in the pharmacodynamic studies, an oral dose of 6 mg/kg was evaluated. Comparing the profile of PG15 6 mg/kg with that obtained after oral dosing of 3 mg/kg one can observe that doubling the dose did not result in an overall increase in body exposure to the drug. The AUC for both groups were statistically similar ($\alpha = 0.05$) due to the high interindividual variability, although the 6 mg/kg dose presented an average AUC (465 ± 72 ng h/ml) smaller than the AUC determined for the smaller dose (686 ± 117 ng h/ml), resulting in a bioavailability of 6%.

An erratic absorption of PG15 after oral administration can be observed in the plasma concentration profiles (Figure 3a, b), by the elevated variability of the pharmacokinetic parameters determined as well as by the fact that the profile of neither dose could be adequately fitted to any compartment model investigated. Similar variability was not observed after intravenous administration. PG15 erratic absorption can be attributed to its poor solubility at the site of absorption due to its high lipophilicity (LogP = 4.05, calculated by Tekto's method).[22] The low solubility of the drug in aqueous media, even at different pHs, probably renders the drug to be poorly and non-homogenously absorbed along the gastrointestinal tract.

Erratic absorption behaviour, similar to that observed for PG15, has been reported for drugs like 5-fluorouracil and cannabinoids in clinical trials. Oral administration of 5-fluorouracil gives rise to erratic plasma values due to greater variability in absorption, whereas a 96-h intravenous infusion showed constant concentrations of the drug in plasma.[23] After oral administration of the highly lipophilic cannabinoid drugs dronabinol and nabilone, Guzman[24] reported that the absorption was slow and erratic probably due to degradation in the acid pH of the stomach and variable individual rates of first-pass metabolism in the liver. Some patients showed more than one plasma peak after oral dosing, which made it more difficult to control the drug's effects. Other routes of cannabinoid administration, such as intravenous, rectal and sublingual, circumvent the aforementioned problems of oral administration by producing single, rapid and high drug plasma peaks, confirming that the oral route is the cause of the profiles and not the drug *per se*.

The observed pharmacokinetic lack of linearity and erratic profiles after oral dosing are in accordance with the anti-inflammatory results obtained in the air pouch model, where no significant difference in activity was observed when increasing the dose from 0.8 mg/kg to 3 mg/kg. Moreover, when the dose was increased from 3 mg/kg to 12.5 or 50 mg/kg, the activity was reduced, probably as result of absorption difficulties.

It is also important to note that the PG15 concentration present at the inflammatory site of the air pouch model measured 7 h after a 3 mg/kg oral dose was of the same order of magnitude (83.85 ± 43.46 ng/ml) as that observed in plasma at the same time (approximately 42 ng/ml), demonstrating that the drug in plasma equilibrates with drug in the inflammatory exudates and that plasma concentrations can be used as a surrogate for inflammatory tissue concentrations.

Summarizing, the pharmacokinetic evaluation of PG15 in rat plasma reveals an interesting profile for a COX inhibitor. The drug presented a half-life of around 6 h, a volume of distribution of approximately 2.5 l/kg and a clearance of 0.9 l/h/kg. After oral dosing, PG15 exhibited a low and erratic absorption, probably due to its low solubility in water, associated or not with first-pass metabolism. These hypotheses should be further investigated.

Plasma concentrations detected in the pharmacokinetic study contribute to explaining the lack of dose–effect relationship observed in the pharmacodynamic studies. To deal with PG15 absorption problems, pharmaceutical technology knowledge can be applied. Further investigations should pursue the preparation of co-crystals, metastable polymorphs, high-energy amorphous forms or ultrafine particles,[25] preparation of microparticles,[26] incorporation into micro- or nanoparticles[27] or even incorporation into carrier systems as cyclodextrin

clusters, which are successful strategies that have been employed previously in similar situations.

Conclusions

PG15 was found to be effective in two mice models used to establish anti-inflammatory activity. The efficacy of PG15 was similar to that of indometacin, and the potency was generally comparable. In-vitro COX inhibition showed that PG15 is not COX-selective, leading to classification of the drug as a non-selective NSAID. The pharmacokinetic profile of PG15 revealed oral absorption problems when the dose is increased and further studies with a view to improve drug absorption should be conducted. The combination of results described in this manuscript suggested PG15 as a potential leading compound for an anti-inflammatory candidate that can be further optimized.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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