Research Article

Antinociceptive Activity of Metamizol Metabolites in a Rat Model of Arthritic Pain

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ABSTRACT The aim of the present study was to evaluate the antinociceptive activity of the main metamizol (MET) metabolites, 4-methylaminoantipyrine (MAA), 4-aminoantipyrine (AA), 4-formylaminoantipyrine (FAA), and 4-acetylaminoantipyrine (AAA) using the "pain-induced functional impairment in rat" model (PIFIR model). The antinociceptive efficacies of MAA and AA were 288.3% h and 281.1% h, respectively, close to the efficacy of MET (333.80% h). The effective dose to attain 50% of the maximum response (ED_{50}) values for MET, MAA and AA were 126.1, 124.9, and 110.7 mg/kg, respectively. FAA and AAA were essentially inactive in this experimental model. Part of the antinociceptive effect showed by MET in this study might be attributed to the effect of the metabolites MAA and AA on cyclooxygenases COX-1 and COX-2 activity. Drug Dev Res •• : ••–••, 2013.

Key words: metamizol; metabolites; synthesis; antinociception; PIFIR model

INTRODUCTION

Metamizol (MET) is a nonsteroidal anti-inflammatory pro-drug that is an effective analgesic, antipyretic, and antispasmodic agent. Initially, MET was classified as a peripherally acting drug as its major metabolites act as cyclooxygenase (COX) inhibitors [Brune and Alpermann, 1983; Brogden, 1986]. However, MET is much more effective as an antipyretic and analgesic drug than as anti-inflammatory agent in vivo, thus it has been proposed that MET inhibits prostaglandin synthesis centrally but not peripherally [Campos et al., 1999] and that its antinociceptive effect is mediated by central mechanisms [Carlsson et al., 1986; Tortorici et al., 1996]. Other authors have suggested that COX-2 through its peroxidase function, is the target [Aronoff et al., 2006] or that another variant of COX-1, the putative COX-3 located in the central nervous system (CNS), is the enzyme inhibited by MET [Chandrasekharan et al., 2002] although the relevance

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of this isoform in humans has been questioned [Kis et al., 2005]. Other mechanisms such as the opioidergic system [Vanegas and Tortorici, 2002] and the arginine–nitric oxide–cyclic guanosine monophosphate pathway [Duarte et al., 1992] have been proposed to explain the antinociceptive action of MET in different animal models. Recently, it was found that the endogenous cannabioid system is also involved in the analgesic effect of MET [Rogosch et al., 2012].

Numerous studies related to the pharmacological effects of MET and its mechanisms of action have been reported; however, little information about pharmacodynamics and/or pharmacokinetics of its pure metabolites exists. After administration, MET undergoes hydrolysis by a nonenzymatic mechanism to yield the metabolite, 4-methylaminoantipyrine (MAA), which is transformed in the liver by cytochrome CYP3A4 to 4-aminoantipyrine (AA) and by oxidation to 4-formylaminoantipyrine (FAA). AA is then acetylated to 4-acetylaminoantipyrine (AAA) by the polymorphic N-acetyl-transferase enzyme. The antinociceptive and anti-inflammatory effects of MET and its metabolites, MAA, AA, FAA, and AAA, have been studied in the acetic acid writhing test for analgesic activity, in a test for reversal of yeast-induced motor impairment, a test of anti-inflammatory activity [EMEA, 2003], and in the adjuvant arthritis model in rat [Weithmann and Alpermann, 1985]. The "pain-induced functional impairment in rat" model or PIFIR model [López-Muñoz et al., 1993] has been widely used in our laboratory to evaluate the antinociceptive activity of MET, either alone or in combination with other drugs [López-Muñoz, 1994; Domínguez et al., 2000; Hernández-Delgadillo et al., 2002; López-Muñoz et al., 2008; Domínguez-Ramírez et al., 2010]. Nevertheless, the antinociceptive activity after administration of pure MET metabolites has not yet been evaluated for arthritic pain using this model. While AA can easily be obtained, the metabolites, MAA, FAA and AAA, are not commercially available. Hence, the objectives of this study were to synthesize these metabolites by simple and efficient reactions and to evaluate their antinociceptive activities, along with AA metabolite, using the PIFIR model.

METHODS AND MATERIALS

Compounds

MET was purchased from Sanofi-Aventis (México City, México); AA (CAS no. 201-452-3) and uric acid were purchased from Sigma (St. Louis, MO, USA). MET metabolites, MAA, FAA, and AAA were synthesized in our laboratory at the Universidad Autónoma Metropolitana-Xochimilco.

Synthesis of Metabolites

Synthesis of three of the main metabolites of MET (MAA, FAA, and AAA) was developed in the laboratory using simple and fast reactions [Fieser and Jones, 1955; Yoshioka and Ogata, 1977] in order to have enough quantities for use in pharmacodynamic studies. Synthesized metabolites purity determined by melting point, infrared (IR) and Hydrogen-1 nuclear magnetic resonance (^{1}H-NMR) spectrophotometry. Melting points were determined on a Büchi B-540 melting point apparatus (Büchi Labortechnik AG, Fawil, Switzerland) and are uncorrected. IR spectra were recorded on a Perkin Elmer FT-IR-1600 spectrometer (Perkin Elmer Inc., Waltham, Ma, USA) with KBr disks. ^{1}H-NMR spectra were measured on a Mercury Plus AS400 NMR System (Agilent Technologies, Palo Alto, California, USA) spectrometer. Chemical shifts are given in ppm relative to Me_4Si ($\delta = 0$). Synthesis routes for each metabolite are shown in Figure 1 and are described as follows:

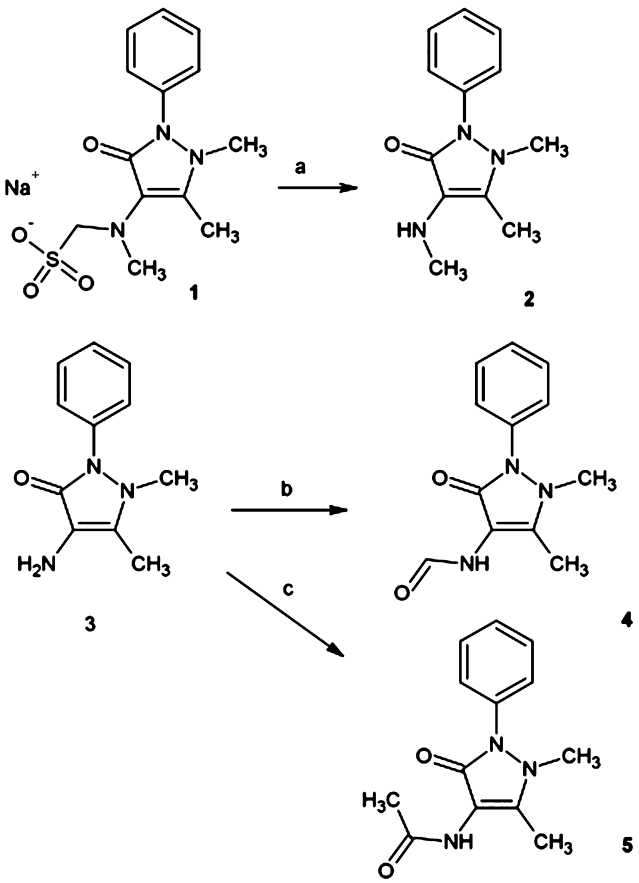

Fig. 1. Metamizol metabolites synthesis. (**1**) metamizol (MET); (**2**) 4-methylaminoantipyrine (MAA); (**3**) 4-aminoantipyrine (AA); (**4**) 4-formylaminoantipyrine (FAA); (**5**) 4-acetylaminoantipyrine (AAA). Reagents: (**a**) HCl, MeOH, 60°C; (**b**) HCO_2H, Δ; (**c**) AcO, H_2SO_4, 50°C.

2-Phenyl-1,5-dimethyl-4-(methylamino)-1,2-dihydro-3H-pyrazole-3-one synthesis (MAA)

A suspension of MET sodium salt **1** (20 g; 0.056 moles) in MeOH (160 mL) was treated with concentrated HCl solution (20 mL) at 60°C for 3 h, the solvent was eliminated under vacuum. The solid residue was then basified to pH 8–10 with 10% of potassium hydroxide (KOH) solution and was extracted with ethyl acetate (EtOAc), washed with brine, and dried with anhydrous Na_2SO_4, and the solvent was evaporated in vacuum (Fig. 1). The crude product was recrystallized from hexane-diethyl ether (50:50, v/v) to give 10.7 g (86.7%) of MAA **2**, beige or light yellow powder, mp 65 ± 2°C. IR (KBr) ν: 3342.67 (NH), 1671.11 (C = O), 1647.90(C = O), 1595.11, 1498.20 (C = C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.36-7.48 (m, 4H, C_6H_5), 7.21-7.25 (m, 1H, C_6H_5), 2.85 (s, 3H, CH_3-N), 2.817(s, 3H, CH_3-N), 2.24 (s, 3H, CH_3C=).

N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-yl) formamide synthesis (FAA)

A solution of 10 g of AA **3** in formic acid (75 mL) was stirred at 100°C. The reaction advance was monitored by thin layer chromatography (TLC), (MeOH-EtOAc 1:4, v/v). The cold mixture was poured into 40 mL of ice water and treated with 20% NaOH solution to pH 5–6 (Fig. 1). The precipitate was separated by suction filtration to give 8.7 g (76.5%) of beige solid of FAA **4**, mp 195–197°C. IR (KBr) ν: 3380.21 (NH), 3056.0 (H-C=), 1690.01 (C = O), 1645.51 (C = O), 1620.70, 1591.70, 1481.3 (C = C). ^{1}H-NMR ($CDCl_3$) δ: 9.13 (bs, 1H, NH), 8.24 (d, J = 1.6 Hz, H-C = O), 7.31-7.49 (m, 5H, C_6H_5), 3.10 (s, 3H, CH_3-N), 2.25 (s, 3H, CH_3C=).

N-(2-phenyl-1,5-dimethyl-3-oxo-2,3-dihydro-1H-pyrazole-4-yl)acetamide synthesis (AAA)

AA **3** (5 g, 0.025 moles) was slowly added to acetic anhydride (4.3 mL). The temperature was controlled at 50°C, and the advance of reaction was monitored by TLC (MeOH-EtOAc 1:4, v/v). After disappearance of AA, the mixture of reaction was poured into 40 mL of ice water and neutralized with $NaHCO_3$. The mixture was extracted with $CHCl_3$ (3 × 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under vacuum. The resulting solid was purified by suspending in EtOAc for 18 h and then collected by vacuum filtration to give 9.5 g (79.87%) of a white solid AAA **5**, mp 203–205°C. IR (KBr) ν(cm^{-1}): 3313.21 (NH), 1678.55 (C = O), 1657.00 (C = O), 1634.75, 1529.45, 1492.08 (C = C). ^{1}H-NMR ($CDCl_3$) δ: 9.44 (bs, 1H, NH), 7.43-7.48 (m, 2H, C_6H_5), 7.29-7.36 (m, 3H, C_6H_5), 3.09 (s, 3H, CH_3-N), 2.20 (s, 3H, CH_3C = O), 2.05 (s, 3H, CH_3-C = C).

PHARMACODYNAMIC STUDIES

Experimental Animals

Male Wistar rats [Crl:(WI)fBR] from our own breeding facility (Universidad Autónoma Metropolitana-Xochimilco, México) weighing between 180 and 210 g were used in this study. Animals were housed in groups of six per cage in a room under controlled temperature (22–24°C) and with a 12-h light/dark cycle. Rats were provided with standard chow (Purina Laboratory Rodent Diet 5001, Pet Food, México City, México) and water *ad libitum*. Twelve hours before the experiments food was withheld, animals had free access to water. Experiments were performed during the light phase and animals were used only once. All experimental procedures and protocols were approved by the local Institutional Animal Care and Use Committee, in accordance with the Mexican Federal Regulations for the care and use of laboratory animals, Mexican Ministry of Health (NOM-062-ZOO-1999) and adhere to the Guide for Care and Use of Laboratory Animals, Washington, D.C. [2011]. The number of experimental animals was kept to a minimum. At the conclusion of the study, rats were euthanized with CO_2 to avoid unnecessary suffering.

Measurement of Antinociceptive Activity

Antinociception was assessed using the PIFIR model [López-Muñoz et al., 1993]. Animals were lightly anesthetized with ethylic ether in an anesthesia chamber (glass dryer Pyrex saturated with ether vapors). Nociception was induced by an intra-articular injection with 50 µL uric acid (30%) into the right knee joint. Immediately afterward, an electrode was attached to each hind-paw of the animals. Rats were allowed to recover from anesthesia and then placed on a stainless steel cylinder of 30 cm diameter. This cylinder was rotated at 4 rpm for periods of 2 min every 30 min in order to force the animals to walk. The time of electrode contact on the cylinder was recorded with a computer-controlled data acquisition system.

After uric acid injection, rats developed progressive dysfunction of the injured limb. The time of contact of the injured hind limb reached zero 2.5 h after injection with uric acid. At this time, MET or its metabolites, previously dissolved in 20% dimethylsulfoxide and 0.9% saline solution, were subcutaneously (s.c.) administered to the animals. The time of electrode contact was recorded at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 h after

administration. Data were expressed as the percentage of the functionality index (FI %), i.e., the time of contact of injected limb divided by the time of contact of the control left paw ×100. Recovery of the contact time of the injured limb (FI %) was considered as expression of the antinociceptive effect. For the purpose of this study, inducing nociception in the experimental animals was unavoidable. However, care was taken to avoid unnecessary suffering.

Experimental Design

Animals were randomly distributed into groups of six animals each, and the antinociceptive effects of MET and its four metabolites were evaluated. As previously reported, a dose of 562.3 mg/kg of MET produces almost 100% of antinociceptive effect with the PIFIR model [López-Muñoz, 1994], showing no adverse effects. Consequently, the effect of all compounds was evaluated initially at that dose. Metabolites that showed antinociceptive effect at this dose (562.3 mg/kg) and MET were administered at five additional doses (these doses were increased at logarithmic intervals of 0.25 units: 31.6, 56.2, 100, 177.8, and 316.2 mg/kg) to obtain their corresponding dose–response curves (DRCs). Controls consisted of the corresponding vehicles.

Data analysis and Statistics

Data were expressed as the percentage of the functionality index (FI %, the time of contact of the injected foot divided by the time of contact of the control left foot multiplied by 100). Temporal effect courses were constructed by plotting the antinociceptive effect (FI %) vs. time (h) for each treatment, and pharmacodynamic parameters were obtained from these data. The cumulative antinociceptive effect elicited by MET and its metabolites during the whole observation period (4 h) was determined as the area under the effect-time curve (AUC; % h), estimated by the trapezoidal method rule [Rowland López and Tozer, 2010]. The maximal observed effect (E_{max}), expressed in terms of FI %, the time required to reach this response (T_{max}), and the effect observed at the end of the experiment (E_{4h}) after each treatment, were directly obtained from temporal effect courses. The results were compared by one-way ANOVA followed by Tukey's or Dunnett's T3 test. Significant differences between means were indicated by $P < 0.05$. DRCs were constructed by plotting AUC vs. log dose for each compound. DRCs were fitted to a three-parameter logistic function using the Sigma-Plot Program v. 11.0. Antinociceptive efficacy (AUC_{max}) and ED_{50} values were estimated from the fitted equation.

RESULTS

MET Metabolites Synthesis

MET metabolites (MAA, FAA, and AAA) synthesis using simple and fast reactions was successful, with moderate yields (79.9–86.7%) of the pure compounds.

Antinociceptive Activity

The cumulative antinociceptive effect during the observation period (4 h) was determined as the area under the effect-time curve (AUC) in order to analyze the whole antinociception effect elicited by MET and its metabolites (MAA, AA, FAA, AAA) at the highest dose of 562.3 mg/kg, s.c. Figure 2 shows the antinociceptive effects elicited by MET and its metabolites at this dose. The ordinate axis corresponds to the AUC (% h), for the total antinociceptive effect shown by the drug (s) during a 4h-period. The antinociceptive effects of MAA and AA at 562.3 mg/kg dose were almost the same ($P > 0.05$), 267.46 ± 15.62 and 306.14 ± 15.62 % h respectively, as for MET (309.49 ± 21.20 % h). On the contrary, AAA and FAA had negligible antinociceptive activity at this dose. Additional temporal courses of the antinociceptive effect were obtained after s.c. administration of 31.6, 56.2, 100, 177.8 and 316.2 mg/kg doses of MET and the two active metabolites MAA and AA. Antinociception for AAA and FAA was not studied at lower doses than 562.3 mg/kg considering the null response obtained at the highest dose.

The pharmacodynamic parameters of AUC, E_{max}, T_{max} and E_{4h} were obtained from the temporal courses for antinociceptive effect of MET and its active metabolites, administered at six different doses. Pharmacodynamic parameters were compared by one-way

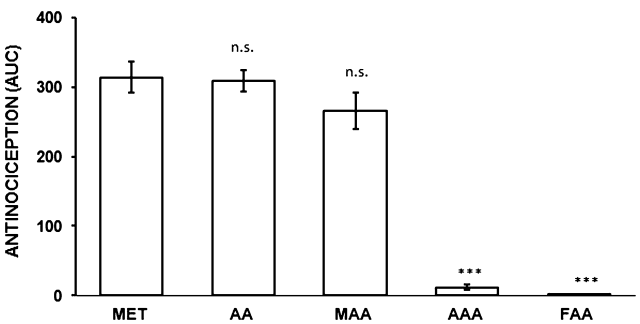

Fig. 2. Overall antinociceptive effect after administration of a single dose of 56.3 mg/kg of metamizol (MET), 4-methylaminoantipyrine (MAA), 4-aminoantipyrine (AA), 4-formylaminoantypirine (FAA) and 4-acetylaminoantipyrine (AAA) in arthritic rats. Data from temporal courses of the effect were transformed in AUC (FI% h). Values are mean AUC ± S.E.M. ($n = 6$). $^{***}P < 0.001$ metabolite vs. MET; $^{n.s}P > 0.05$ metabolite *vs.* MET.

TABLE 1. Pharmacodynamic parameters for metamizol (MET) and its metabolites 4-methylaminoantipyrine (MAA) and 4-aminoantipyrine (AA) after s.c. administration of six doses in arthritic rats. Data are expressed as the mean of six rats ± SEM

Treatment Dose	AUC (% h)	E_{max} (IF%)	T_{max} (h)	E_{4h} (IF%)
31.6 mg/kg				
MET	7.84 ± 3.82	6.67 ± 2.81	0.58 ± 0.20	1.75 ± 0.98
MAA	13.46 ± 2.11	8.09 ± 0.63	1.42 ± 0.24	0.74 ± 0.45
AA	3.11 ± 1.53[b]	5.60 ± 3.01	0.92 ± 0.62	0.13 ± 0.13
56.2 mg/kg				
MET	90.71 ± 10.07	39.81 ± 4.35	1.08 ± 0.33	15.25 ± 4.43
MAA	76.09 ± 13.40	28.04 ± 6.69	1.42 ± 0.54	14.83 ± 5.43
AA	15.83 ± 2.97[a,b]	12.66 ± 2.39[a]	0.92 ± 0.15	0.21 ± 0.17[a]
100 mg/kg				
MET	150.26 ± 20.36	61.48 ± 8.01	1.75 ± 0.38	24.90 ± 5.27
MAA	117.11 ± 22.52	35.53 ± 6.68	2.58 ± 0.49	27.01 ± 5.95
AA	143.19 ± 24.26	54.29 ± 6.95	1.25 ± 0.40	21.32 ± 4.81
177.8 mg/kg				
MET	197.97 ± 30.95	72.60 ± 5.00	1.92 ± 0.49	40.24 ± 12.83
MAA	122.44 ± 21.89	55.59 ± 7.07	1.42 ± 0.27	19.92 ± 5.62[a]
AA	213.98 ± 26.96	73.29 ± 9.58	1.50 ± 0.32	38.97 ± 7.41
316.2 mg/kg				
MET	262.25 ± 21.62	93.41 ± 6.21	1.58 ± 0.52	66.20 ± 10.49
MAA	230.42 ± 25.19	70.58 ± 7.00[a]	2.92 ± 0.54	65.16 ± 5.93
AA	233.69 ± 11.53	77.53 ± 4.45	0.92 ± 0.24[b]	46.91 ± 7.09
562.3 mg/kg				
MET	309.49 ± 21.20	101.96 ± 3.52	2.08 ± 0.45	80.07 ± 7.20
MAA	267.46 ± 27.85	88.00 ± 8.48	3.00 ± 0.47	75.09 ± 10.52
AA	306.14 ± 15.62	100.10 ± 3.57	1.58 ± 0.42	71.25 ± 7.95

[a]$P < 0.05$ MAA vs. MET, AA vs. MET. ANOVA followed by Tukey's or Dunnett's T3 test; [b]$P < 0.05$ MAA vs. AA. ANOVA followed by Tukey's or Dunnett's T3 test.

analysis of variance (ANOVA) followed by the Tukey's or Dunnett's T3 test for multiple comparisons. The results are shown in Table 1.

Statistical differences in AUC (whole antinociception) were only found between AA and MAA at a dose of 31.6 mg/kg and between MAA and MET at a dose of 56.2 mg/kg ($P < 0.05$). E_{max} and E_{4h} were also lower for AA than for MET at this dose ($P < 0.05$). The duration of the effect (E_{4h}) was also lower for MAA than for MET at a dose of 177.8 mg/kg, and E_{max} was lower for MAA than for MET at a dose of 316.2 mg/kg ($P < 0.05$). T_{max} was only lower for AA than for MAA at a dose of 316.2 mg/kg. For the rest of the parameters, no differences between metabolites and MET were found ($P > 0.05$).

Finally, AUCs calculated from temporal courses of the antinociceptive effect of MET, MAA, and AA were used to construct the DRC for each compound (Fig. 3). The *y*-axis corresponds to the AUC (%·h) of temporal course (whole antinociceptive effect showed for the compound during 4 h); the *x*-axis is the dose on logarithmic scale of the administered compound. DRCs were adequately fitted to a three-parameter logistic function ($R^2 \geq 0.98$). As can be observed, in all cases, the AUC increased in a dose-dependent manner.

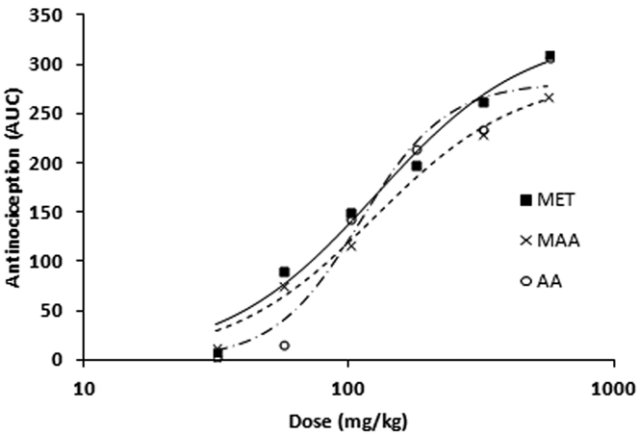

Fig. 3. Dose–response curves for metamizol (MET) and its metabolites 4-methylaminoantipyrine (MAA) and 4-aminoantipyrine (AA) in the PIFIR model. The response (antinociception) is expressed on the *y*-axis as the area under the curve (AUC) of the functionality index over the 4-h observation period (% h) vs. the dose on the *x*-axis (logarithmic scale). Symbols represent experimental data, lines correspond to fitted data. Each point represents the mean of six animals. Error bars were omitted for clarity.

Values of antinociceptive efficacy (maximal global effect) estimated from DRCs were 333.8, 288.3 and 281.1% h for MET, MAA, and AA, respectively. ED_{50} values of 126.2, 124.9, and 110.7 mg/kg were obtained for MET, MAA, and AA, respectively. Therefore, both metabolites exhibited similar efficacy and potency to MET in this model.

DISCUSSION

The ability of MET to inhibit COX-1 and COX-2 activities has been evaluated using various cyclooxygenase sources [Campos et al., 1999] with different animal models of inflammatory pain [Brogden, 1986; Tatsuo et al., 1994; EMEA, 2003] and in humans [Hinz et al., 2007].

Several authors have pointed out that antinociceptive activity produced by MET is mainly due to the activity of its metabolite MAA on both cyclooxygenases, thus inhibiting prostaglandins synthesis [Brogden, 1986; Levy et al., 1995]. It has been pointed that MET elicits a substantial and virtually equipotent inhibition of COX isoforms via MAA. Given the COX-2 suppression by MET, a significant portion of its analgesic action was ascribed to peripheral mechanisms [Hinz et al., 2007]. In view of the observed COX-1 suppression, other physicochemical factors rather than differential COX-1 inhibition could explain gastrointestinal tolerability of MET. In addition, ex vivo inhibition of COX enzymes was correlated with MAA plasma concentrations found in volunteers receiving a single dose of 500 or 1000 mg of MET [Hinz et al., 2007]. AA, which is the oxidation product of MAA, constitutes the other active metabolite of MET. AA has shown lower activity than MAA in other animal models [EMEA, 2003]. MAA was around 50 times more active than MET as an inhibitor of cyclooxygenases, and AA was less active than MET; the other two metabolites, FAA and AAA, were almost inactive. On the other hand, ED_{50} values of 90, 99, and 104 mg/kg were obtained in the acetic writhing test, for MET, MAA, and AA, respectively [Brogden, 1986; EMEA, 2003]. The results obtained with the PIFIR model, revealed that both metabolites, MAA and AA, show practically the same antinociceptive effect (efficacy and potency) as MET in arthritic rats. No statistical differences were found for most of the pharmacodynamic parameters obtained from metabolites temporal courses when compared with MET parameters. In a few cases, the effect observed with MAA or AA differed from that of MET, especially at the lowest doses. It is important to mention that even though AA shows practically the same antinociceptive effect of MET in this and other models, it has not been therapeutically put to use because of its toxicity [Teng et al., 2011]. However, none of the animals used in this study showed any signs of abnormal behavior or other adverse effects that could be ascribed to treatment with the test substances. The other two metabolites FAA and AAA showed negligible activity at the highest dose (562.3 mg/kg), which was consistent with the results obtained in other experimental models [EMEA, 2003].

As the AUC value represents the integrated antinociceptive effect during the observation period and thus includes both the maximal response and the duration of action, this expression was used for the construction of the DRCs [López-Muñoz et al., 1993]. Values estimated for antinociceptive efficacy (AUC_{max}) from DRCs for MET, MAA and AA are near the maximum antinociceptive effect that can be attained with the PIFIR model (i.e. 375% h). Antinociceptive efficacies of MET, MAA, and AA are also near the efficacy of morphine (310.2 ± 25.3% h) reported in the PIFIR model (López-Muñoz et al., 1993). MET is a pro-drug, which is immediately transformed into its active metabolite MAA which, in turn, is transformed to AA metabolite; so both metabolites contribute to the effect attained when administering the parent drug to rats.

As MET is more effective as an antipyretic and analgesic drug than as an anti-inflammatory agent *in vivo*, it was proposed that it inhibits prostaglandin synthesis centrally but not peripherally [Campos et al., 1999]. The PIFIR model [López-Muñoz et al., 1993] provides a model of inflammatory and chronic pain similar to that of clinical gout. Both COX isoforms (COX-1 and COX-2) contribute to the local inflammatory response in this model indicating that they may have a role in the maintenance of physiological homeostasis. It has also been suggested that the therapeutic benefits of NSAIDs are mainly due to inhibition of both isoforms [Ventura et al., 2000, 2002]. The results obtained in our study suggest that the effect observed for MET with the PIFIR model can primarily be attributed to the activity of MAA and AA metabolites as COX-1 and COX-2 isoform inhibitors, although other mechanisms of action previously described for MET could also contribute to the efficacy of the metabolites MAA and AA and they still remain to be confirmed.

Recently, two unknown metabolites of MET were isolated and identified as the arachidonoyl amides of MAA and AA. These metabolites were positively tested for cannabinoid receptor binding (CB1 and CB2) and cyclooxygenases inhibition (COX-1 and COX-2) suggesting that the endogenous cannabinoid system is also involved in the analgesic effect of MET [Rogosch et al., 2012]. Therefore, it is expected that these metabolites also contribute to the global antinociceptive activity of MET and its metabolites. Finally, it can be expected that antinociceptive effect observed with the PIFIR

model after administration of MET will be related to the pharmacokinetics of both metabolites, MAA and AA [Hinz et al., 2007].

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