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Article

In vitro intestinal permeability studies, pharmacokinetics and tissue distribution of 6-methylcoumarin after oral and intraperitoneal administration in Wistar rats

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6-Methylcoumarin (6MC) is a semisynthetic coumarin with important *in vitro* and *in vivo* anti-inflammatory activity. In order to continue the pre-clinical characterization of this molecule, *in vitro* intestinal permeability, plasma profile and tissue distribution after oral administration in rats were studied. The permeability of 6MC was evaluated by the Caco-2 cellular model in both the apical-basal (A-B) and basal-apical (B-A) directions. The pharmacokinetics and biodistribution were evaluated in rats after oral and intraperitoneal administration at doses of 200 mg/kg. Transport experiments with Caco-2 cells showed that 6MC presented high permeability at all concentrations evaluated. This finding suggested that 6MC could be transported across the gut wall by passive diffusion. The plasma concentration-time curve showed that the maximum concentration (Cmax) was 17.13 ± 2.90 µg/mL at maximum time (Tmax) of 30 min for the oral route and Cmax 26.18 ± 2.47 µg/mL at 6.0 min for the intraperitoneal administration, with elimination constant of (K_e) 0.0070 min^{-1} and a short life half time of ($T_{1/2}$) lower that 120 min. The distribution study showed that 6MC has high accumulation in the liver, and widespread distribution in all the organs evaluated.

Uniterms: 6-Methylcoumarin/Pharmacokinetics/rats. 6-Methylcoumarin/distribution. Intestinal permeability/study. Intestinal permeability/study/*In vitro.*

INTRODUCTION

The oral route is the most common route for administration of new drugs, as it is considered the safest and most convenient. However, it also has limitations, as the drug must be absorbed from the site of absorption to the systemic circulation, and then distributed to the target organs, in order to produce its pharmacological effect (Hedaya, 2007).

According to the Biopharmaceutics Classification System (BCS) of the US Food and Drug Administration, drugs are classified based on two intrinsic properties that control their oral absorption: aqueous solubility and intestinal permeability (Amidon *et al.*, 1995). Knowledge of these drug properties not only assists in the classification of a drug in the BSC, but also guides the selection of candidate drugs during the drug development process (Griffin, O´Dricoll, 2008).

Solubility is one of the properties that most influences bioavailability due to its role in dissolution process according to the Noyes Whitney (Jambhekar, Breen, 2013). Since solubility is a thermodynamic

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parameter that defines the amount of material that can dissolve in a given solvent at equilibrium, it is one of the most critical and widely studied physical-chemical attributes of candidate drugs (Wei-Qin, 2008).

Permeability, meanwhile, is the property that determines the speed at which a dissolved drug passes through the intestinal wall and reaches the systemic circulation. Permeability is considered one of most important features in the absorption of drugs. This is a complex kinetic process dependent on several physiological, physiochemical properties of the drug, and on the biophysiochemical properties of a gastrointestinal barrier membrane. Different mechanisms of permeation through biological barriers have been described, the most important ones being passive diffusion (transcellular and paracellular), active uptake, and efflux transport (Fagerholm, 2007; Matsson *et al*., 2005).

Likewise, knowledge of the pharmacokinetic profiles allows us to characterize compounds in terms of their bioavailability and desirable action of duration. Four pharmacokinetic (PK) parameters are the most useful in characterizing the *in vivo* disposition of a compound: (i) clearance (*Cl*, units of volume/time), a measure of the ability of the body to eliminate a compound; (ii) volume of distribution (Vd, units of volume), the apparent volume/space in the body that contains the compound; (iii) half-life ($t_{1/2}$, units of time), the time taken for a compound to decrease to half of its initial concentration in the fluid or tissue in which it is measured in (e.g., plasma), and (iv) bioavailability (F, unitless, often expressed as %), the fraction of a compound that reaches the systemic circulation following non-intravenous administration (Fan, De Lannoy, 2014). Meanwhile, biodistribution studies describe the transit of a drug through the organism, the anatomic sites reached by the drug when it is in the systemic circulation, and the sites where it can accumulate (Hedaya, 2007).

On the other hand, some simple and complex coumarins have shown different biological activities, such as antibacterial, effects on the cardiovascular system, effects on the central nervous system, antioxidant activities, cytotoxicity, and anti-inflammatory properties (Grazul, Budzisz, 2009; Kidane *et al.*, 2004; Beillerot *et al.*, 2008; Anand, Singh, Singh, 2012; Sashidhara *et al.*, 2011; Kang *et al.*, 2009; Li *et al.*, 2011; Hoult, Paydt, Paya, 1996). Furthermore, 6-methylcoumarin (6MC, Figure 1), another simple coumarin, has shown important anti-inflammatory activity in *in vivo* and *in vitro* models (Cárdenas *et al.*, 2014).

Although 6MC has shown remarkable pharmacological effect, knowledge of its permeability, concentration-time and distribution is essential for understanding its biopharmaceutical profile. Thus, the aim of this work was to study the *in vitro* permeability and *in vivo* pharmacokinetics after oral and intraperitoneal administration in Wistar rats, in order to complete the biopharmaceutical characterization of 6MC.

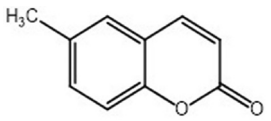


FIGURE 1 - Chemical structure of 6-methylcoumarin.

MATERIAL AND METHODS

Chemical reagents

6-Methylcoumarin (6MC, Sigma; St. Louis, MO, USA); trichloroacetic acid (Analytical grade, Merck, Darmstadt, Germany); acetic acid, methanol (HPLC grade, Merck, Darmstadt, Germany), and water HPLC grade were prepared by the MilliQ Plus system (Millipore Co., France).

Animals

Twelve-week-old Male Wistar rats were obtained from the animal house of the Pharmacy Department of the Universidad Nacional de Colombia. The assays were carried out in accordance with the international and local ethical guidelines on the use and care of laboratory animals. The local Research Ethics Committee (Act 03/2012 Faculty of Science) approved this study.

Drug analysis

For all the analyses, an Agilent 1100 Series HPLC chromatograph was used. For the permeability studies, a Phenomenex reversed phase column (Macclesfield, UK) Bondclone C-18 (150 x 3.9 mm; 10 µm) was used. The mobile phase was composed of A) water: methanol: acetic acid (95:5:1 v/v) and B): methanol: acetic acid (100:1 v/v); ratio 80:20 (A:B), under isocratic flow 1.0 mL/min. The analytical wavelength was 321 nm, and samples of 10 µL were injected.

For the pharmacokinetics and tissue distribution, a Phenomenex reversed phase column (Macclesfield, UK) Bondclone C-18 (150 x 3.9 mm; 10 µm) was used. The mobile phase was composed of A) water: methanol: acetic acid (95:5:1 v/v) and B): methanol: acetic acid (100:1 v/v) as gradient, for 32 min, at a flow rate of 1 mL/min, as

follows: 0 to 14 min: 0 % B to 50 % B; 14 to 23 min: 50 % B; 23 to 24 min: 50 % B to 0 % B; 24 to 32 min: 0 % B. UV detection was performed at 321 nm. The injection volume was 50 µL. The data were processed using the software program Value Solution Chemstation® (ChemStation for LC 3D systems Rev. B.03.02 [341]) (Hernández, Ospina, Aragón, 2014).

Caco-2 permeability studies

Cell culture

Caco-2 cells (ATCC:HTB-37) were cultured in high-glucose DMEM (Gibco, USA) supplemented with 10% fetal bovine serum and 1% non-essential amino acids (Gibco, USA), at 37 °C in a humidified 5% CO_2 atmosphere, until the cells reached 80-90% confluence. For the transport experiments, 100,000 cells (passages 113–115) were harvested and seeded on each polycarbonate insert (0.6 cm^2, 0.4 µm pore size; Millipore, USA), and allowed to grow and differentiate for 21–28 days prior to the experiments, as described previously by Kratz *et al.* (2012).

Transport experiments

The determination of *in vitro* intestinal permeability of 6MC was carried out under sink conditions in a series of pH-gradient bidirectional transport experiments with Caco-2 cells. Before the experiments, cell monolayers were rinsed with Hank's balanced salt solution (HBSS) and equilibrated for 30 min at 37 °C. The integrity of the monolayers was assessed before and after the experiments, by transepithelial electrical resistance (TEER) measurement. Only monolayers with TEER values above 200 Ωcm^2 were considered.

A stock solution of 6MC (10 mM DMSO) was diluted to final concentration of 10, 25, 50 or 100 µM in HBSS pH 6.5 (apical transport buffer) or pH 7.4 (basolateral transport buffer). Bidirectional experiments (apical-to-basolateral [AB] and basolateral-to-apical [BA]) were initiated by adding 6MC solutions to the donor compartment, and fresh buffer to the acceptor compartment. Caco-2 cell monolayers were incubated for 1 h at 37 °C under constant stirring (150 rpm). Receiver compartments were sampled at 0, 15, 30, 45 and 60 min, refilled with an equivalent amount of transport buffer, and samples were submitted to analysis by HPLC/UV. Apparent permeability coefficients (P_{app}) were calculated from the equation

$$P_{app} = \frac{\Delta Q}{\Delta t} \times \frac{1}{AC_0}$$

where $\Delta Q/\Delta t$ is the steady-state flux (mol/s), C_0 is the initial concentration in the donor chamber at each time interval (mol/mL), and A is the surface area of the filter (cm^2). Carbamazepine (CBZ) (50 µM) and hydrochlorothiazide (HCT) (200 µM) were used as controls. The data are presented as means ± SD of six independent monolayers.

Pharmacokinetic study

Male Wistar rats (12 weeks, 250 ± 10 g), with 5 animals for each administration route, were administrated, by oral gavage (p.o.) or intraperitoneal (i.p.) route, with 6MC at 200 mg/Kg, suspended in saline solution (NaCl 0.9 %) and tween-80. 200 mg/kg was chosen as doses, since in previous assays (data not shown) the biopharmaceutics parameter, Tmax, Cmax and AUC proved be linear in a range of dose of 100 to 400 mg/kg.

Blood samples, 400 µl, were collected from the retro-orbital sinus, at 2,6,10,15,20,25,30,45,60,120, 360 and 480 min after oral administration. After each sampling, the blood samples were centrifuged at 6000 rpm for 10 min, at 4 °C to separate the plasma, and then 200 µL of plasma was homogenized with 200 µL of trichloroacetic acid (20 %), and centrifuged at 13000 rpm for 10 min. The supernatant was recovered for 6MC quantification using the HPLC-DAD method previously described. The maximal observed plasma concentration (Cmax) and corresponding sampling time (Tmax) were determined by visual inspection of the data.

The apparent elimination rate constant (K_e) was estimated by linear regression of the log-transformed plasma concentrations during the terminal log-linear decline phase. The apparent terminal elimination half-life time ($t_{1/2}$) was calculated as $\ln 2/K_e$. The area under the 6MC plasma concentration-time curve from time zero to the last quantifiable point ($AUC_{0\text{-}t}$) was calculated using the linear trapezoidal rule. The AUC extrapolated to infinity ($AUC_{0\text{-}\alpha}$) was calculated as the sum of $AUC_{0\text{-}t}$, and the last quantifiable point was divided by the elimination rate constant. The apparent oral clearance (*Cl/f*) was calculated as the dose divided by $AUC_{0\text{-}\alpha}$. The apparent volume of distribution (*V/F*) was calculated as the apparent oral clearance divided by the elimination constant.

Tissue distribution study

Male Wistar rats (12 weeks, 250 ± 10 g) were administered 6MC at 200 mg/kg by oral gavage (p.o.), suspended in saline solution (NaCl 0.9 %). At 15, 30, 60 and 120 min after oral administration, the animals were sacrificed by cervical dislocation (five animals for each

sampling time). The organs (liver, heart, lung, kidney and spleen) were removed and washed with saline solution. The organs were then homogenized with a same volume of trichloroacetic acid (20 %) in a Polytron PT-10 – 35 (Kinematica, Newark, NJ, USA), and centrifuged at 13000 rpm for 10 min. The supernatanat was recovered for 6MC quantification.

Identification of the main 6-methylcoumarin metabolite

To determine possible metabolites of 6MC after oral administration, the plasma samples were analyzed by LC-ESI/MS. The system consisted of a Shimadzu HPLC equipped with two isocratic pumps, on-line degasser, a Rhodyne manual injector, a UV detector and a mass spectrometer (Shimadzu LCMS-2010EV). The chromatographic parameters were the same as those used in the HPLC-DAD analysis. LabSolution® V.3.0 software was used for the data acquisition and processing. Full scan mass spectra were recorded at between m/z 50 and 500 in positive mode. Nitrogen was used as nebulizer gas at 1 L/ min, capillary voltage was 4.500 V and detector voltage was 1.500 V. The collision dissolution line (CDL) and QarrayRF voltage were both 150 V. CDL and the Heat Block temperature as set at 250°C. Standard samples of coumarin and 6MC were analyzed following the same methodology.

Statistical analysis

All results were expressed as the arithmetic mean of the values obtained, ± the respective standard deviation. Simple comparisons between groups were performed using the Student's t-test, with a confidence level of 95%.

RESULTS

Permeability

Table I shows that 6MC presented a high P_{app} in the absorptive and secretory directions in Caco -2 cells. Given the finding of P_{app} values over 10^{-5}, it is suggested that 6MC is highly permeable in both directions. The efflux ratio ($P_{app\ BA}$/ $P_{app\ AB}$) of less than 2 in all cases indicates a passive diffusion mechanism and the absence of efflux. For all the concentrations evaluated, 6MC had high mass balance values, with average recovery of more than 70% in both the AB and BA directions.

The permeation of 6MC was performed in both A- B and B-A in the Caco-2 cellular model. Figure 2 shows the cumulative amount permeated (μM/cm^2) in the AB and BA directions. The relation of the concentration versus the cumulative amount permeated showed a linear relationship proportional to concentration (in both directions), with determinant coefficients of 0.9903 and 0.9853 for AB and BA direction, respectively. This means that there is no saturation of the acceptor medium in any concentration and time during the test time.

Pharmacokinetic

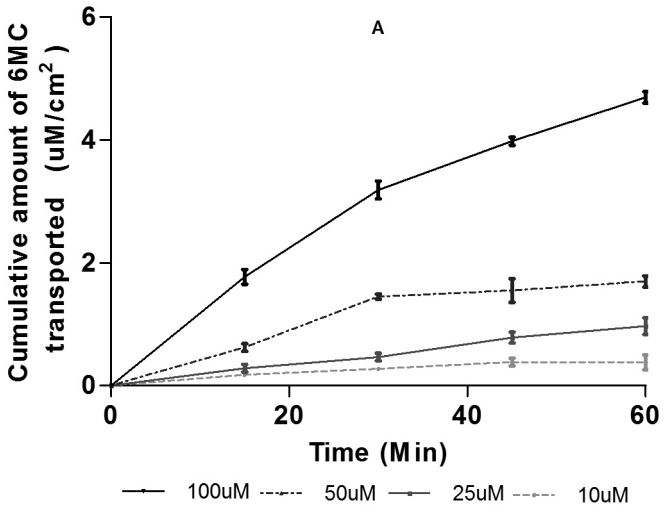
The plasma samples were analyzed using an HPLC-DAD validated method (Hernández, Ospina, Aragón, 2014). The mean plasma concentration *versus* time profiles of 6MC are shown in Figure 3. The pharmacokinetic parameters calculated for 6MC in plasma are summarized in Table II.

Plasma pharmacokinetics of 6-methylcoumarin in rats following oral administration. Data are expressed as mean ± SD of five animals. Dose administered 200 mg/kg.

TABLE I - Comparable P_{app} (cm/s) values for 6MC obtained in the absorptive (A-B), secretory (B-A) directions, and efflux ratio (P_{app} B-A/ P_{app} A-B)

	A-B		B-A		Efflux ratio
	Mean	SD	Mean	SD	
10 μM	9.1E-05	2.9E-06	1.3E-04	6.1E-05	1.48
25 μM	6.8E-05	2.4E-06	6.7E-05	1.4E-06	1.06
50 μM	6.4E-05	8.3E-06	6.0E-05	5.6E-07	0.94
100 μM	9.0E-05	4.4E-06	7.3E-05	1.5E-05	0.82
CBZ 50 μM	11.4E-05	2.4E-06			
HCT 200 μM	0.06E-05	0.3E-07			

The data are expressed as means ± SD of six independent monolayers. Carbamazepine (CBZ) (50 μM) and hydrochlorothiazide (HCT) (200 μM) were used as controls.



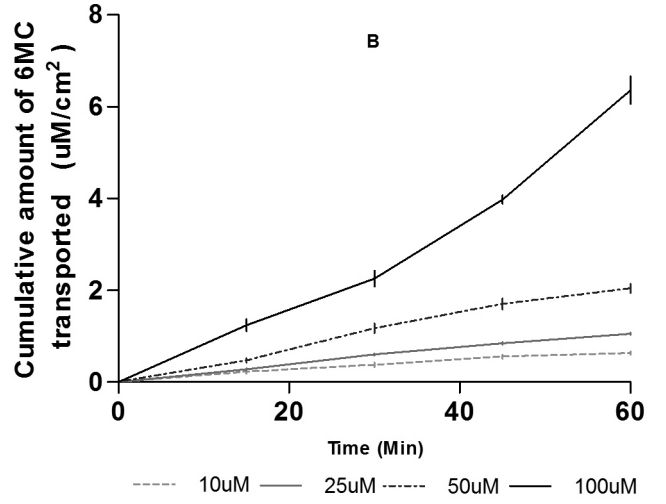


FIGURE 2 - Transport of 6-methylcoumarin across Caco-2 cells. Cumulative amount of 6-methylcoumarin transported across Caco-2 cells in the Apical to Basolateral (A) or Basolateral to Apical direction (B). Data are expressed as means ± SD of six independent monolayers.

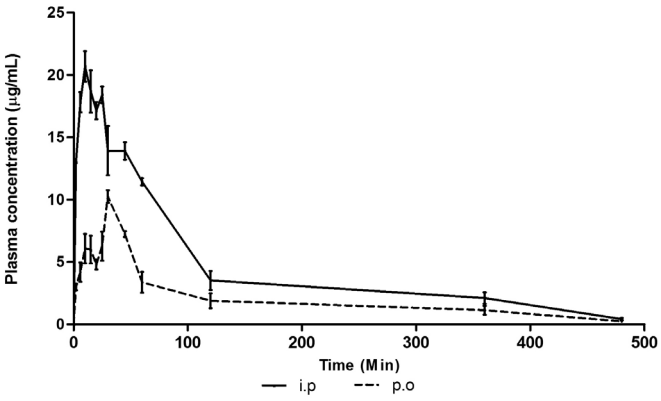


FIGURE 3 - Plasma concentration - time profile of 6-methylcoumarin after oral and intraperitoneal administration in Wistar rats.

Maximum concentration (Cmax), Maximun time (Tmax), Area Under Curve from time zero to the last quantifiable point (AUC_{0-480}), Area Under Curve extrapolated to infinite ($AUC_{0-\alpha}$), Elimination constant (K_e), Absortion constant (K_a), elimination half –time ($t_{1/2}$), Apparent oral clearance (Cl/F), Apparent volume of distribution (V/F). Cl/f and Vd were calculated taking into account the soluble 6MC in the volume administered**.** The data are generated using the mean values from individual rats ± SD (n=5).

As shown in Table II, the Cmax, Tmax, and ABC_{0-480} and $ABC_{0-\alpha}$ values for intraperitoneal administration are higher than those obtained after oral administration. This fact could be explained by that fact that in the intraperitoneal route, absorption occurs through the mesenteric vessels, draining to the portal vein, and thereby accessing the bloodstream in less time than by the oral route (Turner *et al*., 2011), making this a more rapid absorption route (Morton *et al.*, 2001).

TABLE II - Plasma pharmacokinetics parameters of 6-methylcoumarin after oral and intraperitoneal administration in Wistar rats, dose 200 mg/kg

	Oral	**Intraperitoneal**
AUC_{0-480} **(µg/mL*min)**	977.2 ± 276.5	2177.0 ± 331.6
$AUC_{0-\alpha}$ **(µg/mL*min)**	1015.4 ± 296.1	2234.9 ± 354.1
C max (µg/mL)	17.13 ± 2.90	26.18 ± 2.47
Tmax (min)	30	6.0
k_e **(min^{-1})**	0.0064 ± 0.0009	0.0076 ± 0.0007
K_a **(min^{-1})**	0.3311 ± 0.0489	
t ½ (min)	109.8 ± 15.0	92.3 ± 8.7
Cl/f (mL/min)	0.8 ± 0.3	0.4 ± 0.07
Vd (mL)	134.9 ± 30.4	48.8 ± 3.9
F	0.45	

TABLE III - The main coumarinic compounds identified by LC-ESI/MS analysis in plasma after oral administration of 6-methylcoumarin in Wistar rats

Peak	Rt (min)	$\lambda_{máx}$ (nm)	[M+H]+ (*m/z*)	Compound
1	12.5	278 and 321	147	Coumarin
2	14.6	278 and 321	161	6-methylcoumarin

Regarding the values obtained of k_e, $t_{1/2}$ and the Cl/f, no significant difference was found that could be attributed to the administration route. This fact is expected, since these are intrinsic parameters of the drug.

Besides 6MC, another majority compound was detected in the plasma after oral administration of 6MC. This compound could be related to gastrointestinal metabolism of 6MC, since it was not found in the blood samples analyzed after i.p. administration. Data from the LC-ESI/MS were used for preliminary identification of the main metabolite of 6-MC. The retention times (Rt), UV λ_{max} values and the molecular ions in positive mode are shown in Table III.

Distribution Tissue

Using a validated HPLC-DAD method, the concentration of 6MC after oral administration was determined in liver, heart, lung, kidney and spleen (Figure 4).

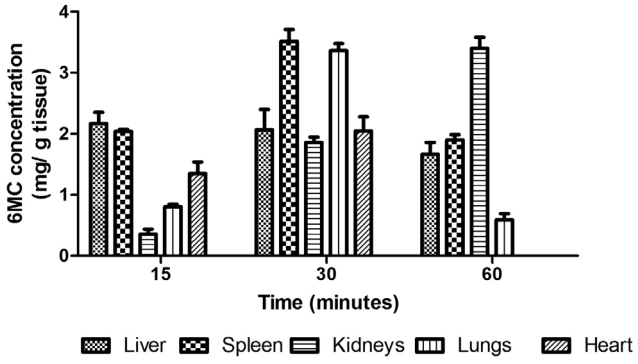


FIGURE 4 - Biodistribution profile of 6-methylcoumarin after oral administration in Wistar rats. Data expressed mean ± D.S of five animals. Dose administered 200 mg/kg.

In all the organs, 6MC concentrations were found to be in the range of between 0.35 ± 0.14 and 4.18 ± 0.03 µg per g of tissue. At 15 min, the highest concentrations were found in plasma and liver, and the lowest in kidneys, heart and lungs. At 30 min, the concentration increased proportionally in all the organs sampled. At 60 min, the distribution changed; in the kidney, the concentration of 6MC increased significantly, while the concentrations in the other organs decreased. At this same time point, the concentrations (µg/mL) were as follows; heart: 2.54 ± 0.10; plasma: 2.48 ± 0.62; spleen: 1.89 ± 0.15, lung: 0.59 ± 0.10 lung, kidney 3.40 ± 0.31, liver 1.45 ± 0.10. In addition, the 6MC fraction present in the evaluated organs was very low, since the total 6MC found in all the organs tested at 30 and 60 minutes was 0.104% and the 0.046% of the 6MC administered, respectively.

DISCUSSION

Ideal drug candidates should have adequate aqueous solubility and permeability in order to achieve effective concentration in the target tissue (Kratz *et al.*, 2012). In order to evaluate the *in vitro* intestinal permeability, transport experiments were performed on Caco-2 cells. These cells are able to fully polarize into differentiated monolayers, with well-established tight junctions and brush border membrane. They can also express several membrane transporters and metabolizing enzymes, allowing the measurement of functional permeability, both through passive diffusion and active transport (Kratz *et al.*, 2012). The apparent permeability of 6MC was high, with values of magnitude of 10^{-5}. This value is comparable with the P_{app} of other similar compounds such as umbelliferone, scopoletine, and coumarin, in which high permeability and no efflux were reported (Galkin, Fallarero, Vuorela, 2009).

The high intestinal permeability of a drug indicates that its transport across the gut wall does not represent a relevant restriction for its oral absorption. The linear increase in the amount of 6MC permeated, indicating that its transport across the gut wall, is probably mediated by passive diffusion, and the low efflux values in the different concentrations evaluated, indicating that efflux does not represent an interference for absorption (Galkin, Fallarero, Vuorela, 2009).

The BCS considers that a drug has high aqueous solubility if its maximum dose is completed dissolved in 250 ml of pH 1-7.5 buffered aqueous solution (Wei-Qin, 2008). According to this definition, it is suggested that 6MC has low solubility, since the dose of 6MC that

showed anti-inflammatory activity in rats is 200 mg/kg,(Cárdenas *et al.*, 2014) and its solubility is 0.57 ± 0.03 µg/mL in water and buffered solutions of pH 1.2, 6.8 and 7.4 (Cárdenas *et al.*, 2013). As consequence, 6MC can be classified as a class II compound (Low solubility/High permeability) accord to the BCS, when the absorption across the gut wall is limited mainly by solubility.

The concentration-time profiles (Fig 3) showed fast absorption, but low plasma concentration in relation to the dose administered (10.25 ± 0.72 y 26.18 ± 2.47 µg/mL oral and intraperitoneal via respectively). This fact may be related to the high clearance rate (*CL/F*) and possibly, a high rate of metabolism after administration of 6MC.

Regarding other coumarins orally administered in rats, 6MC have a similar $t_{1/2}$ to that reported for daphnoterine and daphnetin ($t_{1/2}$ of 93 ± 26 and ± 262.23 96.31 min respectively) (Wei *et al.*, 2015; Lin *et al.*, 2005; Zhang *et al.*, 2014). For coumarin, half-lives ($t_{1/2}$) of between 60-240 h were found in humans and other species, such as rats (Lake, 1999).

The absolute bioavailability is defined as the fraction of administered dose that reaches the systemic circulation, compared to the fraction of intravenously administered dose, while the relative bioavailability is the fraction of administered dose that reaches the systemic circulation compared to an administration route other than the intravenous one (Haidar, Kwon, Lionberger, 2008). Due to the low solubility of 6MC, its intravenous administration was not possible; therefore, the value reported for bioavailability in this study corresponds to the intraperitoneal route, a parenteral route with rapid absorption (Turner *et al.*, 2011). The relative bioavailability found for 6MC was 0.45 (45%) which is higher than that reported for coumarin administered orally in humans, which showed rapid absorption from the gastrointestinal tract and was rapidly metabolized by the first-pass effect, resulting in only 2-6 % found intact in the systemic circulation (Felter *et al.*, 2006). Similarly, other authors have described the behavior of high metabolization and low plasma concentrations of coumarin, indicating an absolute bioavailability of only 1.5 % (Hoult, Paydt, Paya, 1996).

As shown in Figure 4, in all the organs tested at 60 min, the 6MC concentration decreased to half the initial concentration after 30 min. This fact suggests that 6MC is rapidly eliminated from the systemic circulation ($t_{1/2}$ less than 2 hour and high apparent clearance) or extensively metabolized. Previous studies on the *in vivo* metabolism of coumarins reported that coumarins are metabolized mainly into two kinds of compounds: its hydroxyl derivatives, and its smaller acid derivatives, such as ortho-hydroxyphenyllactic acid, ortho-hydroxyphenylacetic acid and ortho-hydroxyphenylpropionic acid (Lake, 1999).

In this study, some peaks presented pseudo-molecular ions related to known coumarin metabolites. Although the highest peak observed (Rt 14.6 min) was the non-metabolized 6MC, metabolites could be observed and tentatively identified, such as coumarin (Rt = 12.5). These results suggest that demethylation is probably the primary biotransformation for 6MC in order to increase the polarity of the molecule. This demethylation reaction is reported for other compounds such as lobeglitazone (Song *et al.*, 2014), and for the Ostol, a coumarinic compound where the demethylation has been described as a phase I metabolic reaction of (Yuan *et al.*, 2009). Another peak (Rt = 11.8, [M+H]+ (*m/z*) 657.3) was observed, but was not possible to propose a structure, because the molecular weight is not comparable with the common metabolites of coumarin structure. The conjugation with glutathione (GSH) is common for coumarin (Lake, 1999).

The biodistribution after oral administration in the studied organs evidenced a high accumulation of 6MC in the plasma and in the most irrigated organs; similar behavior was found for 6MC biodistribution after intraperitoneal administration (Hernández, Ospina, Aragón, 2014). High levels of accumulation were found in the liver and kidney, which suggests that these organs are involved in the processes of metabolism, elimination and excretion of 6MC. The high accumulation in all organs evaluated is in concordance with the high *Vd* found both administration routes (p.o and i.p), indicating that 6MC is widely distributed throughout the body.

CONCLUSION

Some biopharmaceutical properties of 6MC were determined in order to increase the preclinical characterization of this promising drug. It was found that 6MC is highly permeable and according to the BCS, may be considered a compound class II. The biopharmaceutical and pharmacokinetic parameters were determined 6MC after oral and intraperitoneal administration in Wistar rats. It was found that the compound has a rapid removal times, as reflected in its short half-life of 110 min under constant removal of 0.0070 min^{-1}. A large volume of distribution was also observed in the biodistribution study, indicating extensive distribution. Coumarin was identified as the major metabolite 6-methylcoumarin, suggesting desmetililation as the first reaction 6MC biotransformation.

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CONFLICT OF INTEREST

The author(s) declare(s) that they have no conflict of interest to disclose.

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