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Pharmacokinetics and Anti-Inflammatory Activity of Andrographolide in Rats

L. Gondaliya Vaishali[1], J.H. Patel[1*], R.D. Varia[1], S.K. Bhavsar[1], Priti D. Vihol[2] and Falguni D. Modi[1]

[1]Department of Pharmacology and Toxicology, Veterinary College, Navsari Agricultural University, Navsari, Gujarat, India
[2]Department of Veterinary Pathology, Veterinary College, Navsari Agricultural University, Navsari, Gujarat, India
**Corresponding author*

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A B S T R A C T

The pharmacokinetics and anti-inflammatory activity of andrographolide (50 mg/kg) were studied following intramuscular administration in rats. Drug concentration in plasma was determined using High Performance Liquid Chromatography (HPLC). The mean peak plasma drug concentration of 3.17 ± 0.06 µg/mL was achieved at 4 h. The pharmacokinetic parameters like mean absorption half-life (t½α) (0.61 ± 0.07 h), elimination half-life (t½β) (1.3 ± 0.10 h), apparent volume of distribution (Vdarea) (6.9 ± 0.38 L/kg), area under plasma drug concentration-time curve ($AUC_{0-\infty}$) (13.7 ± 0.47 µg.h/mL), area under first moment curve (AUMC) (61.8 ± 3.44 µg.h^2/mL), total body clearance (Cl_B) (3.6 ± 0.12 L/h/kg) and mean residence time (MRT) (4.6 ± 0.09 h) were observed. Following intramuscular administration of andrographolide, no anti-inflammatory effect was found in rats.

Introduction

Pain management is most critical in the management of veterinary patient. In current scenario, long term medication with pain management drugs is associated with various side effects and expenses of various synthetic drugs are rising. So scientist interest is renewed in traditional systems of medicine to find out effective, safer and cheaper remedy for animals and humans. In this context it had been shown in several animal studies that extracts of *Andrographis paniculata* and its constituents, namely the diterpene lactone andrographolide, have anti-inflammatory properties and possibly associated with the inhibition of the PAF-mediated inflammatory response (Amroyan *et al.*, 1999) and the inhibition of nitric oxide synthesis in macrophages (Chiou *et al.*, 1998). Most of pharmacokinetic and anti-inflammatory studies of andrographolide were conducted following oral and intravenous route. However, in veterinary clinical medicine intramuscular route is most important route of drug administration. So aim of this study was

to evaluate pharmacokinetics and anti-inflammatory activity of andrographolide in rats following intramuscular route of administration.

Materials and Methods

Experimental animals

The experiment was conducted on male *albino Wistar* rats weighing between 250 to 350 grams. Rats were kept under constant observation for two weeks before the commencement of the experiment and subjected to clinical examination to exclude possibility of any diseases. The animals were divided into groups and kept in cages. Standard ration and water was provided *ad libitum.* The experimental protocol was approved by Institutional Animal Ethics Committee.

Drug and chemical

Pure Andrographolide (98%), meloxicam sodium salt hydrate (>98%) and lambda carrageenan was obtained from Sigma-Aldrich St. Louis, USA. Methanol, acetonitrile and ortho-phosphoric acid (HPLC grade) were purchased from Merck Specialties Private Limited, Mumbai.

Pharmacokinetic study and data analysis

Animals (n=24) were divided into six groups. Each group comprise of four animals. Multiple numbers of rats were used for serial collection of blood at alternating time points. An intramuscular injection of Andrographolide (50 mg/kg) was administered in the left gluteal muscle. Blood samples was collected in K3EDTA vials, at different time interval i.e. 0.75, 1, 2, 4, 6, 8, 12 hours from retro orbital plexus. Blood samples were centrifuged at 3000 rpm for 10 minutes at 4°C and plasma was transferred to cryo-vials and, stored at – 20°C. Samples were analyzed within 48 h to quantify andrographolide concentration using High Performance Liquid Chromatography.

Andrographolide concentration in plasma samples was determined by reverse-phase High Performance Liquid Chromatography (HPLC) after extraction, using reported assays (Levita, 2014) with minor modifications. The High Performance Liquid Chromatography (HPLC) apparatus of Shimadzu (Japan) comprised of binary gradient delivery pump (model LC - 20AP), Diode Array Detector (model SPD M20A), Auto Sampler (model SIL 20A**)** and reverse phase C18 column (250 x 4.6 mm ID).The HPLC data integration was performed using software Clarity. Plasma (100 µL) was deproteinized by addition of acetonitrile (100 µL) and vortexed for one minute. This was followed by centrifugation at 8000 rpm for 10 minutes. An aliquot of supernatant was collected in clean auto sampler vial. The mobile phase was a mixture of methanol and water (60:40 v/v) adjusted to pH 5.0 with ortho-phosphoric acid, filtered by 0.2 µ size filter (Axiva N66) and degassed by ultra-sonication. It was delivered at an isocratic flow rate of 1.0 mL/min at ambient temperature. The effluent was monitored at 223 nm wavelength and injection volume was 20 µL. various pharmacokinetic parameters were calculated from plasma concentration of andrographolide using software PK solution (Version 2.0).

Initial stock solution was prepared by dissolving 2 mg pure andrographolide powder in 2 mL drug free plasma. The assay was sensitive, reproducible and linearity was observed from 0.04 to 200 µg/mL. The mean correlation coefficient (R^2) was 0.99. The limits of detection and limits of quantification were determined to be 0.09 and 0.19 µg/mL, respectively. Precision and accuracy of the

assay were assessed using samples at concentration 50, 25, 1.56, 0.39 and 0.09 µg/mL. At all concentration studied, the C.V. was less than 6.7 %.

Anti-inflammatory activity of andrographolide

The carrageenan-induced paw edema test was performed as described by Suebsasana *et al.*, (2009) with minor modification. Experiment animals (n=18) were divided into three groups in each group (n=6) six animals. Group I animals were treated with vehicle control (normal saline). Group II animals were treated with Meloxicam (5 mg/kg) following single intramuscular administration. Group III animals were treated with andrographolide (50 mg/kg) following single intramuscular administration. Acuteinflammation was produced by sub plantar injection of 0.1ml of 1% suspension of lambda carrageenan in normal saline in the left hindpawoftherats30minaftertheadministration oftestagents.Volume of the edematous paw was measured using a digital plethysmometer after carrageenan treatment at 0, 1, 2, 3, 4 and 5 hours. The results obtained for the andrographolide-treated groups were compared with the control and the difference between the readings was taken as the indicator of edema.

Statistical analysis

Andrographolide plasma concentration and pharmacokinetic parameters of different treatment groups were compared by students' "t" test using Microsoft excel 2007.

Results and Discussion

Following single dose intramuscular administration of andrographolide in the rats, adverse reactions were not observed. The mean plasma concentration-time profile of andrographolide following intramuscular administration at 50 mg/kg body weight had presented graphically in figure 1.

Following intramuscular administration of andrographolide, the initial drug concentration of 0.27 ± 0.02 µg/mL was observed at 0.75 h. The mean peak plasma drug concentration of 3.17 ± 0.06 µg/mL was achieved at 4 h which declined rapidly to 1.41 ± 0.07 µg/mL at 6 h.

The drug concentration of 0.49 ± 0.05 µg/mL in plasma was detected at 8 h and beyond then the drug was not detected in plasma.

Pharmacokinetic parameters (Mean ± SE) calculated for intramuscular route of drug administration have been depicted in table 1. While the anti-inflammatory activity of andrographolide assessed using the carrageenan-induced paw edema test at different time intervals is shown in table 2.

Following intramuscular administration of andrographolide (50 mg/kg) in rats, the mean peak (C_{max}) plasma drug concentration of 3.17 ±0.06 µg/mL was achieved at 4 h (T_{max}). Similarly, C_{max} (3.0 ± 0.6 µg/mL at 1.67 h) was reported following oral administration of *Andrographis paniculata* extract (200 mg/kg) in rats (Pannossian *et al.*, 2000). However, the lower values of C_{max} (1.27 ± 0.2 µg/mL at 2.4 h, 0.115 µg/mL at 0.75 h and 1,62 ± 0.11µg/mL at 0.99 h) were reported following oral administration of *Andrographis paniculata* extract (20 mg/kg), andrographolide (30mg/kg) and andrographolide tablet (10mg/kg) in rats respectively (Pannossian *et al.*, 2000; Suo *et al.*, 2007; Bera *et al.*, 2014).

In accordance to present study plasma concentration was detected upto 8 h after oral administration of ethanolic extract of *Andrographis paniculata* (20 and 200 mg/kg) in rats (Pannosian *et al.*, 2000).

Fig.1 Semilogarithmic plot of andrographolide plasma concentration following single dose intramuscular administration of andrographolide (50 mg/kg) in rats.
Each points represents mean ± S.E

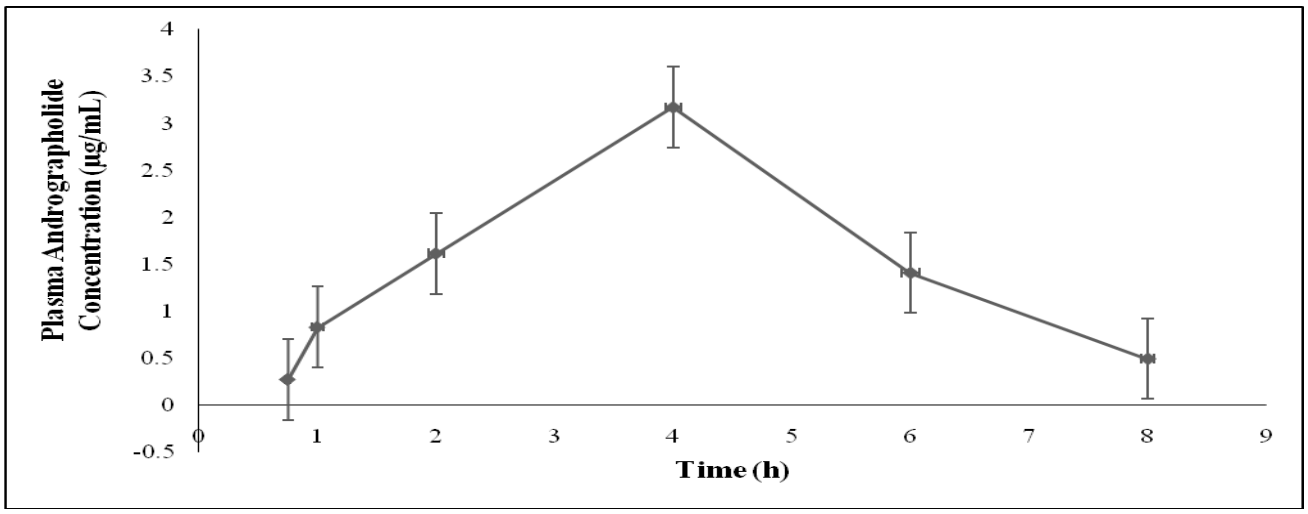

Table.1 Pharmacokinetic parameters of andrographolide (50 mg/kg) following intramuscular administration in rats

Pharmacokinetic Parameter	Unit	Mean ± S.E
K_a	h^{-1}	1.30 ± 0.24
β	h^{-1}	0.53 ± 0.04
$t_{½Ka}$	H	0.61 ± 0.07
$t_{½β}$	h	1.3 ± 0.10
C_{max}	µg/mL	3.1 ± 0.07
T_{max}	h	4.0 ± 0
$AUC_{(0-\infty)}$	µg.h/mL	13.7 ± 0.47
AUMC	$\mu g.h^2/mL$	61.8 ± 3.44
$Vd_{(area)}$	L/kg	6.9 ± 0.38
$Cl_{(B)}$	L/h/kg	3.6 ± 0.12
MRT	h	4.6 ± 0.09

Table.2 Anti-inflammatory activity of andrographolide

Treatment Group	**Volume of paw oedema after drug administration (mL). Mean ± S.E.**						**% inhibition in paw oedema after drug administration**				
	0 h	1 h	2 h	3 h	4 h	5 h	1 h	2 h	3 h	4 h	5 h
Saline (control)	0.60 ± 0.05	0.91 ± 0.04	1.09 ± 0.03	1.20 ± 0.02	1.31 ± 0.02	1.19 ± 0	-	-	-	-	-
Meloxicam (5 mg/kg BW)	0.64 ± 0.06	0.87 ± 0.04	0.74 ± 0.04**	0.69 ± 0.04**	0.63 ± 0.05**	0.72 ± 0.05 **	4.3	41.1	42.5	51.9	39.4
Andrographolide (50 mg/kg BW)	0.58 ± 0.01	0.86 ± 0.02	1.07 ± 0.01	1.19 ± 0.01	1.29 ± 0.01	1.17 ± 0.01	5.49	1.83	0.83	1.52	1.68

** Highly significant at $p<0.01$ when compared with control group

The mean absorption half-life ($t_{½Ka}$: 0.60 ± 0.07 h)of andrographolidein rats following single dose intramuscular administration in the present study was in accordance to absorption half-life (0.69 ± 0.3 h) reported following oral administration of ethanolic extract of *Andrographis paniculata* (20 mg/kg) in rats (Pannossian *et al.,* 2000). The elimination half-life ($t_{1/2\beta}$: 1.33 ± 0.10 h) of andrographolide in rats following single dose intramuscular administration in present study was found lower than elimination half-life (2.45 h, 3.1 ± 0.2 h and 2.5 ± 0.1 h;) reported following oral administration of andrographolide (30mg/kg) and ethanolic extract of *Andrographis paniculata* (20 and 200 mg/kg) in rats respectively (Bera *et al.,* 2014; Pannossian *et al.,* 2000). Mean apparent volume of distribution (Vd_{area}: 6.9 ± 0.38 L/kg) of andrographolide following intramuscular administration in rats was found higher than reported following oral administration (0.04 ± 0.005 and 0.06 ± 0.1) of *Andrographis paniculata* extract (20 and 200mg/kg) in rats respectively (Pannossian *et al.,* 2000). This finding was supported by higher value of AUC (13.7 ± 0.47 µg.h/mL) in comparison with findings reported following oral administration of andrographolide (30mg/kg) and ethanolic extract of *Andrographis paniculata* (20 and 200 mg/kg) in rats respectively (Bera *et al.,* 2014; Pannossian *et al.,* 2000). Moreover, low plasma protein (55%) binding efficiency of drug (Dey *et al.,* 2013) can also contribute high distribution of andrographolide in rats.

In the present study, the total body clearance (3.63 ± 0.12 L/h/kg) of andrographolide was in accordance with reported value of clearance (5.99 ± 1.74 L/h/kg) following intravenous administration of andrographolide (5 mg/kg) in rats (Yang *et al.,* 2013). However, lower value of total body clearance following oral administration (0.01 L/h/kg and 0.02 L/h/kg) of *Andrographis paniculata* extract (20 and 200 mg/kg) were reported in rats respectively (Pannossian *et al.,* 2000). The MRT(4.6 ± 0.09 h) values calculated following single dose intramuscular administration of andrographolide in present study was in accordance to MRT value (4.32 ± 0.2 h and 5.54 ± 0.1 h) reported following oral administration of ethanolic extract of *Andrographis paniculata* (20 mg/kg and 200 mg/kg body weight) in rats respectively (Pannossian *et al.,* 2000). The findings of present study indicate rapid clearance of andrographolide form body due to high total body clearance and low elimination half-life. It also indicates that andrographolide is distributed into vast area of body following intramuscular administration similar to oral administration.

In the present study, following intramuscular administration of andrographolide (50 mg/kg), no anti-inflammatory effect was found in rats. However, following intraperitoneal administration of andrographolide (4 mg/kg) significant reduction in paw volume was observed (Suebsasana *et al.,* 2009); following subcutaneous administration (10, 25 and 50 mg/kg) dose dependent anti-inflammatory effect was observed (Sulaiman *et al.,* 2010); following intraperitoneal administration of andrographolide sulfonic acid sodium salt in high (88 mg/kg) or low (22 mg/kg) doses significant reduction in rat paw swelling caused by the egg white was observed (Wen *et al.,* 2014) and following intraperitoneal administration of *Andrographis paniculata* extract (methanolic) for 5 consecutive days (10 mg/dose/animal) was found to inhibit carrageenan induced paw oedema formation compared to untreated controls group (Sheeja *et al.,* 2006). No anti-inflammatory effect in rats may be due to poor solubility of pure andrographolide, difference in vehicle used for administration and effective plasma concentration may be high.

It may be concluded that andrographolide is slowly absorbed, widely distributed and rapidly eliminated from the body. However, no anti-inflammatory effect was found in rats on comparison to meloxicam (upto 5 hours) (positive control) may be due to poor solubility of pure andrographolide, difference in vehicle used for administration and effective plasma concentration may be high.

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