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Assessing Minipigs as Superior Non-Rodent Pre-Clinical Models: Insights from Plasma Protein Binding and Metabolism of Marketed NSAIDs Compared Across Species

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ABSTRACT

As per regulatory authorities' requirements, pre-clinical studies need to be conducted in at least one rodent and one non-rodent species. Usually, dogs are considered the non-rodent pre-clinical species of choice even though minipigs and monkeys are physiologically closer to humans than dogs. The aim of this study was to demonstrate that minipigs may be a better model for pre-clinical studies compared to dogs for some drug classes. In the present in vitro study, plasma protein binding and metabolic stability in liver microsomes of nine marketed non-steroidal anti-inflammatory drugs (NSAIDs) was evaluated in minipig, dog, monkey, and human species. Eight out of nine tested NSAIDs showed statistically similar plasma protein binding in minipig and human plasma which was different from dog and monkey plasma. Similarly, drug metabolism assays showed similar metabolism in minipig and human liver microsomes, which was different compared to dog and monkey liver microsomes. The results from both the assays showed greater similarity between minipigs and humans suggesting the use of minipig species as a better pre-clinical non-rodent model for NSAIDs instead of the conventional dog species. Additionally, the use of the more accessible minipig species may help in saving time and resources during pre-clinical studies and may help the safety studies in humans during later stage clinical trials.

Keywords: Minipig; plasma protein binding; equilibrium dialysis; NSAIDs.

INTRODUCTION

Successful pre-clinical studies during drug development are a steppingstone in a drug's success story. The selection of pre-clinical species is a critical factor in a new drug development program as it affects the safety in humans and the design of further clinical studies. As per regulatory requirement, a new chemical entity (NCE) must be preclinically tested in at least one rodent and one non-rodent species before conducting clinical studies in humans to ensure safety in humans. For pre-clinical studies, dogs and monkeys (non-human primates) are traditionally used as non-rodent species. Dogs are accessible, easy to handle, and extensive prior toxicological safety assessment data exists in literature. Monkeys are less accessible but are closest to humans in terms of genetic similarity. However, use of dogs and monkeys is restricted by societal constraints, certain physiological differences, as well as ethical issues. Minipigs are the other alternate non-rodent species for pre-clinical studies.

They have many anatomical, physiological and biochemical similarities with humans relative to other non-rodent species [1, 2-13]. Since then, use of minipigs have been increased in biomedical research. In the European Union (EU), currently more than 60,000 pigs are used per

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year for scientific procedures [7].

Gottingen minipigs are the most widely used. In the early 1960s, Göttingen University first developed the Göttingen strain [7]. The benefit of the Göttingen strain is that it is a white non-pigmented and small-size minipig (adult average body weight of 30–40 kg). It is of very high parasitic and microbiological quality [6-7].

The cardiovascular, digestive, and urogenital systems of the minipig are highly comparable to the human systems [1-9]. The anatomy of the skin is relatively close to human skin and hence it can also be used for dermal studies. Metabolic activity and enzymatic processes in minipigs have close parallels to the humans. The minipigs are sensitive to a wide variety of chemicals and drugs which have been advantageous over the traditional non-rodent species in relation to specific responses to particular drug classes. Minipigs can be used for all routes of administration as parenterally or orally, and in many cases for metabolic or pharmacological reasons minipigs are preferable to dogs or non-human primates [5-6].

Moreover, pharmacokinetics of drug candidates in humans has shown more resemblance to minipigs than to dogs. This result is primarily due to the species differences observed in metabolizing enzymes and drug transporters. Cytochrome P450 pattern of enzymes in minipigs has been reported and has shown similarities to humans [5]. Similarly, expression of P-gp and CYP3A in livers and small intestines of foetal, neonatal, juvenile, and adult Göttingen minipigs has been assessed by immunohistochemical methods and shown to be comparable to humans [5].

There are important differences between minipigs and dogs which in some cases favour the use of minipigs instead of the conventionally used dogs as a non-rodent model in safety evaluation [6]. Minipigs are the recommended non-rodent species for drug candidates that are metabolized by aldehyde oxidase (AO), N-acetyltransferases (NAT1 and NAT2) or cytochrome P450 (CYP2C9) enzymes which are not expressed in dogs [13]. Another advantage of using minipigs is the lack of emesis which is a major drawback in dogs for certain drugs like anticancer and NSAIDs. Emesis in dogs during pre-clinical studies can generate incorrect or unreliable data which may later affect the clinical studies in humans [37].

Even though minipig species has great potential based on the genetic background data, it is still necessary to corroborate these findings using experimentation. The current manuscript presents an in vitro comparison of plasma protein binding and metabolism in dog, minipig, monkey and human liver microsomes for different NSAIDs. Although the data of plasma protein binding and metabolism of these NSAIDs are available in dogs and humans, the data from minipigs and monkeys are not available. Based on the results, three non-rodent species dogs, minipig and monkey are evaluated to select the better non-rodent model for preclinical studies.

METHODS

Reagents used.

NSAIDs in active pharmaceutical ingredient (API) form of celecoxib, diclofenac, ibuprofen, ketoprofen, flurbiprofen, indomethacin, meloxicam, piroxicam and naproxen were purchased from Sigma-Aldrich (St. Louis, Missouri). All other chemicals were of reagent grade and purchased from commercial sources. Dog plasma (Beagle, male) and minipig plasma (Gottingen, male) were purchased from Palamur Biosciences (Hyderabad, India). Monkey plasma (Cynomolgus, male) was procured from BioIVT, NY, USA and human plasma (Caucasian, male) was obtained from Bio Ally (Bangalore, India). Plasma used in the assays was a pool of three donors and was collected in K_2EDTA anticoagulant. Dog, minipig, human and monkey liver microsomes were purchased from Sekisui Xenotech LLC (Kansas, USA).

Plasma Protein Binding by Equilibrium Dialysis Method

The 96-well equilibrium dialysis apparatus (HTD 96B, Gales Ferry, Connecticut, USA) and cellulose membranes

(12-14 KDa molecular weight cutoff) were purchased from HT Dialysis (Gales Ferry, Connecticut, USA). The cellulose membranes were regenerated by soaking in Milli-Q water for 15 min, followed by 15 min in 25% methanol and finally 15 min in 100 mM sodium phosphate buffer (pH 7.4). The equilibrium dialysis apparatus was assembled by placing the regenerated membranes between rows of wells of Teflon bars. The apparatus was tightly clamped to prevent any leakage during dialysis [19-22].

The pH of blank plasma from all species was adjusted to 7.4 using 0.1 N HCl and preincubated for 15 min at 37 °C. DMSO stock solutions of the compounds were spiked in the blank plasma in 1.5 mL Eppendorf microfuge tubes (0.4% final DMSO concentration) to get 10 µM final concentration. These tubes were mixed by inversion 5-6 times. Aliquots (120 µL per half-cell) of plasma spiked with drug were loaded into the donor chamber of the dialysis plate and remaining plasma in tubes were incubated at 37°C for 6 h with 5% carbon dioxide atmosphere. In receiver chamber, 120 µL of blank sodium phosphate buffer (100 mM, pH 7.4) was added. The Dialyser was sealed with adhesive sealer (Axyseal, Axygen, USA) and dialysis was conducted for 6 h at 37 °C, 60 rpm and 5% CO_2 atmosphere. After incubation for 6 h, aliquots from the donor and receiver chambers were pipetted and collected in acetonitrile containing internal standard. Similarly, aliquots from plasma tubes incubated for 6 h were collected in acetonitrile containing internal standard. LC-MS/MS was used to analyse these samples.

Binding of warfarin in human plasma (literature value approx. 1% unbound) [19,25] in one well of each row was tested as positive control to ensure membrane integrity, adequate clamp pressure and no leakage in each row of the dialysis plate.

Acceptance criteria for plasma protein binding assay: To accept and consider the compound as stable in plasma, 100 ± 30% criteria were considered at 6 h compared to 0 h samples concentration. To rule out any non-specific binding of the compound to the membrane or apparatus the percent recovery had to be within 70-130% [19].

percent unbound are reported for the compounds where percent CV (coefficient of variation) was <30% and at least 4 replicates out of 6 replicates were acceptable.

The positive control warfarin in human plasma run along with the compounds showed percent unbound of approximately 1% [19,25].

Procedure for Drug Metabolism Assay

Liver Microsomes Assay: In a 96 deep well plate 50 mM sodium phosphate buffer (pH 7.4), liver microsomes (final concentration 0.5 mg/mL), and nicotinamide adenine dinucleotide phosphate (NADPH, final concentration 1 mM) were added. The plate was kept in an incubator shaker for preincubation at 37°C, 60 rpm for 10 min. After 10 min, the compound stock solutions prepared in DMSO was spiked in the reaction mixture to obtain final compound concentration of 0.5 µM. After spiking of the compound, reaction mixture was mixed well and aliquots were taken at the time points of 0, 5, 10, 20 and 30 min and immediately quenched with acetonitrile containing internal standard rolipram/flufenamic acid.

Similarly, control experiment without NADPH was performed parallelly to investigate non-CYP metabolism. Samples were collected at 0 and 30 min and immediately quenched with acetonitrile containing internal standard rolipram/flufenamic acid.

Verapamil (highly metabolised in dog, minipig, monkey and human liver microsomes) was used as positive control along with the compounds to ensure that the liver microsomes used in the assay were metabolically active. Acceptance criteria for metabolic stability assay was that the percent remaining for positive control verapamil must be ≤ 25% at 30 min in dog, minipig, monkey and human liver microsomes.

Bioanalysis

Bioanalysis of metabolic stability and plasma protein binding samples was conducted on LC-MS/MS (Applied Biosystems API 4000 triple quadrupole, AB Sciex®, Ontario, Canada) using a Thermo C18, 4.6x50 mm, 5 µm

column. Acetonitrile with 0.1% formic acid (mobile phase B):5 mM ammonium formate with 0.1% formic acid (mobile phase A) at 70:30 ratio was used for analysis using positive mode (MH+) ionisation for diclofenac, ibuprofen, ketoprofen, meloxicam and piroxicam. Rolipram was used as internal standard for diclofenac, ibuprofen, ketoprofen, meloxicam and piroxicam analysis.

Acetonitrile (mobile phase B):5 mM ammonium acetate (mobile phase A): 70:30 was used for compounds analysis using negative mode (MH^-) for celecoxib, flurbiprofen, indomethacin and naproxen. Flufenamic acid was used as internal standard for celecoxib, flurbiprofen, indomethacin and naproxen analysis.

Each analytical run had twelve calibration curve standards, and two sets of quality control (QC) samples run at three different concentrations, low, medium, and high-quality control (LQC, MQC and HQC). LQC was selected as five times the lower limit of quantification (LLOQ), HQC was 75% of the upper limit of quantification and MQC was one intermediate quality control selected from the calibration curve standards. Batch was accepted when at least 67% of overall calibration curve met ± 20% accuracy criteria and 50% of QCs at each concentration level of QC met ± 20% accuracy criteria.

Data Analysis for Plasma Protein Binding Samples

Percent unbound of the compounds was determined using the concentrations in buffer samples (receiver compartment) and in plasma samples (donor compartment) collected from the HT Dialysis® apparatus after 6 h of dialysis using equation no 1 below.

$$\%Unbound = \left\{\frac{[Compound]_{receiver}}{[Compound]_{donor}}\right\} \times 100 \qquad (1)$$

$$\%Bound = 100 - \%Unbound$$

Percent recovery was calculated to evaluate non-specific binding by adding the concentrations in the receiver compartment and donor compartment samples and comparing that with the 6 h stability samples using equation no 2 below.

$$\%Recovery = \left\{\frac{[Compound]_{receiver} + [Compound]_{donor}}{[Compound]_{6\,h}}\right\} \times 100 \qquad (2)$$

Data Analysis of Metabolic Stability Samples: Percent remaining of parent compound at different time points was calculated using area ratio at that time point compared to zero min area ratio. The data was fitted to a one phase exponential decay equation ($A = A_0e^{-kt}$) using GraphPad Prism® software. The half-life ($t_{1/2}$) generated by the software are reported. Intrinsic clearance CL_{int} was calculated using the below equation no. 3, where k = decay rate constant (min^{-1}).

$$CL_{\text{int}} = \frac{k \times \text{volume of reaction mixture } (\mu L)}{\text{protein content (mg)}} \qquad (3)$$

Statistical Analysis: A two tailed t-test was conducted to check the percent unbound value observed was significant (S) or non-significant (NS). GraphPad Prism® software was used to calculate the p-value. The means of percent unbound considered significantly different when the p value was <0.05. p-value >0.05 was considered as non-significant difference.

RESULTS

Plasma Protein Binding: Total nine NSAIDs (celecoxib, diclofenac, ibuprofen, flurbiprofen, ketoprofen, meloxicam, piroxicam, indomethacin and naproxen) were tested for in vitro plasma protein binding in dog, minipig, monkey and human plasma to compare the binding, to find the similarity and to select the best non-rodent preclinical model. Eight out of nine NSAIDs showed similar binding in minipig and human plasma.

All nine compounds were stable up to 6 h at 37°C in dog, minipig, monkey and human plasma so the percent unbound observed is acceptable and reliable. The literature values available for human plasma binding for these reported NSAIDs are consistent with the observed values in this study [19,25]. The average percent remaining at 6 h was >95 % for all the compounds.

Percent recovery was 70-130% indicating that there was no non-specific binding of the compounds to the cellulose membranes or any leakage in HT Dialysis® apparatus during dialysis period of 6 h.

The percent unbound, percent stability and percent recovery in dog, minipig, monkey and human with standard deviation (SD) are reported in Table 1 and Table 2.

Table 1. Percent Unbound of the NSAIDs with SD in Dog, Minipig, Monkey and Human Plasma

% Unbound				
Compound (NSAIDs) Name	**Dog**	**Minipig**	**Human**	**Monkey**
Celecoxib	2.17 ± 1.06	1.28 ± 0.65	1.50 ± 0.22	2.42 ± 0.72
Diclofenac	1.96 ± 1.27	1.06 ± 0.57	0.86 ± 0.90	0.99 ± 0.40
Ibuprofen	0.41 ± 0.15	0.13 ± 0.06	0.56 ± 0.34	0.43 ± 0.31
Flurbiprofen	3.60 ± 1.68	0.54 ± 0.54	0.36 ± 0.08	0.51 ± 0.28
Ketoprofen	5.91 ± 1.93	1.30 ± 0.96	2.47 ± 0.80	7.06 ± 0.72
Meloxicam	2.44 ± 0.38	0.68 ± 0.55	0.25 ± 0.10	0.69 ± 0.28
Piroxicam	5.16 ± 0.69	1.29 ± 0.52	0.43 ± 0.01	0.74 ± 0.16
Indomethacin	1.84 ± 0.74	1.22 ± 0.18	0.69 ± 0.16	0.62 ± 0.55
Naproxen	2.07 ± 1.36	0.5 ± 0.43	0.68 ± 0.34	0.48 ± 0.49
Warfarin (Positive Control)*	-	-	1.45 ± 0.40	-
* Warfarin binding (approx. 1% unbound as per literature) in human plasma at 10 µM was run as positive control to check the validity of dialysis apparatus and membrane integrity.				

Table 2. Percent Stability and Percent Recovery of the NSAIDs in Dog, Minipig, Monkey and Human Plasma at 6 h, 37°C

% Stability and % Recovery at 6 h, 37 °C								
Species	**Dog**		**Minipig**		**Human**		**Monkey**	
Compound (NSAIDs) Name	**%Stability**	**%Recovery**	**%Stability**	**%Recovery**	**%Stability**	**%Recovery**	**%Stability**	**%Recovery**
Celecoxib	95	103	98	102	96	98	113	86
Diclofenac	96	107	99	105	95	104	93	106
Ibuprofen	94	116	96	106	95	101	93	100
Flurbiprofen	93	93	103	95	96	106	96	101
Ketoprofen	111	98	118	100	118	94	109	98
Meloxicam	105	97	100	110	101	99	85	127
Piroxicam	101	86	100	98	96	98	75	125
Indomethacin	93	129	93	128	84	94	75	105
Naproxen	109	94	101	103	97	98	127	88
Warfarin	-	-	-	-	93	101	-	-
Considering experimental errors, 70-130% stability and 70-130%recovery is acceptable.								

To check the percent unbound values are similar or significantly different between the evaluated four species, a two tailed t-test was conducted comparing dog with human, minipig with human and monkey with human. The p-values were calculated using GraphPad Prism® software.

The statical analysis data for comparison of plasma protein binding in different species are presented in Table 3.

Table 3. Statistical Analysis of Percent Unbound Values and Summary of p-values Comparing Dog, Minipig and Monkey to Human Species

* p-Value by two tailed t-test			
Compound (NSAID) name	**Dog vs Human**	**Minipig vs Human**	**Monkey vs Human**
Celecoxib	0.017 (S)	0.5542 (NS)	0.1318 (NS)
Diclofenac	0.036 (S)	0.1064 (NS)	0.2036 (NS)
Ibuprofen	0.2716 (NS)	0.0743 (NS)	0.6754 (NS)
Flurbiprofen	0.0189 (S)	0.4028 (NS)	0.3833 (NS)
Ketoprofen	0.0231 (S)	0.1208 (NS)	<0.0001 (S)
Meloxicam	<000.1 (S)	0.2085 (NS)	0.0051 (S)
Piroxicam	<000.1 (S)	0.009 (S)	0.0149 (S)
Indomethacin	0.0417 (S)	0.0771 (NS)	0.5261 (NS)
Naproxen	0.0469 (S)	0.6416 (NS)	0.3288 (NS)
* p-Values were calculated by comparing the six replicates of percent unbound values between human and dog, human and minipig, human and monkey. * p-value <0.05 means significantly different (S) while >0.05 means not significantly different (NS).			

Metabolic Stability in Liver Microsomes: In vitro metabolic stability assay results of these NSAIDs in dog, minipig, monkey and human liver microsomes are presented below:

After 30 min of incubation in pH 7.4 buffer and at 37 °C, it was observed that diclofenac and ibuprofen were metabolized by minipig and human liver microsomes however was stable in dog and monkey liver microsomes at 37 °C.

Intrinsic clearance values of piroxicam were similar in minipig and human liver microsomes and piroxicam was stable in monkey and human liver microsomes with low intrinsic clearance.

Indomethacin was unstable in minipig liver microsomes and stable in dog, monkey and human liver microsomes, Meloxicam was moderately stable in dog and minipig but was stable in monkey and human liver microsomes.

The positive control verapamil that run along with the NSAIDs was unstable (half-life was 2.3-6.5 min) in dog, minipig, monkey and human liver microsomes with high intrinsic clearance values (>200 µL/min/mg protein) indicating that the liver microsomes used in this study were metabolically active [36].

The percent metabolism, half-life ($t_{1/2}$) and CL_{int} values of the NSAIDs in dog, minipig, monkey and human liver microsomes are summarized in Table 4 and Table 5.

Table 4. Percent Metabolism of the NSAIDs at 30 min in Dog, Minipig, Monkey and Human Liver Microsomes

% Metabolism at 30 min				
Compound (NSAIDs) Name	**Dog**	**Minipig**	**Human**	**Monkey**
Celecoxib	56	74	38	42
Diclofenac	0	28	88	0
Ibuprofen	0	62	18	16
Ketoprofen	0	0	0	0
Flurbiprofen	14	100	64	0
Meloxicam	23	48	0	0
Piroxicam	0	8	1	0
Indomethacin	0	25	0	11
Naproxen	0	21	0	6
Verapamil (Positive Control)	89	99	96	97

Table 5. Half-life ($t_{1/2}$) and CL_{int} Values of the NSAIDs in Dog, Minipig, Monkey and Human Liver Microsomes

Half life ($t_{1/2}$) and CL_{int} Values								
Compound (NSAIDs) Name	**Dog**		**Minipig**		**Human**		**Monkey**	
	Half Life (min)	**CL_{int} (µL/min/mg protein)**	**Half Life (min)**	**CL_{int} (µL/min/mg protein)**	**Half Life (min)**	**CL_{int} (µL/min/mg protein)**	**Half Life (min)**	**CL_{int} (µL/min/mg protein)**
Celecoxib	24	61	15	92	>30	33	26	54
Diclofenac	>30	7	>30	22	10	145	0	0
Ibuprofen	>30	2	23	59	>30	21	>30	12
Ketoprofen	>30	0	>30	0	>30	0	>30	0
Flurbiprofen	>30	7	9	155	23	60	0	0
Meloxicam	>30	19	>30	44	>30	0	12	0
Piroxicam	>30	0	>30	9	>30	9	>30	0
Indomethacin	>30	0	>30	21	>30	0	>30	9
Naproxen	>30	0	>30	18	>30	0	>30	2
Verapamil (Positive Control)	6.5	214	2.3	611	6	226	3.1	446

DISCUSSION

The present study was conducted using nine NSAIDs to evaluate if minipig was a better model to predict human safety and efficacy relative to the conventional dog species. There are drawbacks in using dog species in preclinical studies such as its dissimilarities with human, potential for emesis, and certain social constraints. [1, 37] The current investigation is based on the similarity in anatomy and physiology between minipig and human species. Two in vitro ADME characteristics, plasma protein binding and metabolic stability in liver microsomes were compared in dog, minipig, monkey and human species and it was shown that minipigs are a good alternate during drug development.

Plasma protein binding is an important parameter to comprehend the efficacy of a drug in different pre-clinical species. The fraction of unbound drug in conjunction with other in vitro ADME parameters is used to understand in vivo properties of the compound including its availability at the target of interest. The fraction unbound is also used to calculate the dose to be administered. Binding of the drugs to the proteins of different animals and humans may not be similar hence it can affect the efficacy and safety of a compound [19-24].

If the drug is highly and tightly bound (slow dissociation) then it may result in restricted distribution of drug into target tissue (reduced Vd), decreased metabolism, clearance and prolonged half-life, retention of drug in plasma compartment resulting in limited brain penetration, and higher requirement of loading doses but lower maintenance doses [22].

The current in vitro plasma protein binding assay for nine reported NSAIDs was conducted in dog, minipig, monkey and human plasma by equilibrium dialysis method using a HT Dialysis® apparatus [21] to compare the binding in three non-rodent model (dog, minipig and monkey) with human. pH of the plasma was adjusted to 7.4 before the study because the plasma protein binding is pH dependent [23-24]. To keep the plasma pH constant, CO_2 incubator was used, and 5% CO_2 was supplied throughout the incubation time of 6 h.

For evaluation of plasma protein binding by the equilibrium dialysis method, the compound must be stable for 6 h and soluble in plasma. If a compound has poor solubility, instability in plasma, and/or high nonspecific binding, then alternate methods such as ultracentrifugation and ultrafiltration need to be employed. However, in this study all the 9 compounds (NSAIDs) along with the

positive control warfarin were soluble and stable up to 6 h in dog, minipig, monkey and human plasma. The percent stability of the NSAIDs were in the range of 75-127%. The percent recovery for the NSAIDs were 86-129% hence there were no non-specific binding of the tested NSAIDs to the dialysis membranes or HT Dialysis® apparatus.

The plasma protein binding results showed correspondence between minipig and human statistically. Eight out of nine evaluated NSAIDs showed significantly similar binding in minipig and human, vs. only one case for dog and human. In many cases, monkey was also closer to human than dog (six compounds out of nine compounds showed similarity). However, there are more constraints and challenges in conducting monkey studies [13] therefore minipig may be a better non-rodent model for pre-clinical studies for NSAIDs.

Similarly, the four species, dog, minipig, monkey and human, were compared in metabolic stability study in liver microsomes. The positive control verapamil that run along with the NSAIDs was unstable (half-life was 2.3-6.5 min, CL_{int} >200 µL/min/mg protein) in dog, minipig, monkey and human liver microsomes indicating that the liver microsomes used in this study were metabolically active [32,36].

Diclofenac and ibuprofen were metabolized more by minipig and human liver microsomes but were stable in dog and monkey liver microsomes at 30 min. This result can be explained by understanding the expression of various CYP isozymes in the different species. Diclofenac and ibuprofen are reported substrates for cytochrome P450 (CYP) 2C9 [36,37], CYP 2C9 like CYPs are not expressed in dog but expressed in minipig and human liver microsomes hence metabolism of the diclofenac and ibuprofen was observed in minipig and human but not in dog.

Piroxicam showed similar intrinsic clearance in minipig and human liver microsomes and no intrinsic clearance in monkey and human. Indomethacin was stable in dog, monkey and human but was unstable in minipig liver microsomes. Flurbiprofen was more metabolized by minipig and human liver microsomes compared to dog and monkey liver microsomes. Meloxicam was metabolized in dog and minipig but was stable in monkey and human liver microsomes.

To conclude, the drugs which are metabolized by CYP 2C9, CYP 2C9 like CYPs, aldehyde oxidase (AO), NAT and OAT3 showed similar metabolism in minipig and human. If a drug candidate is known to be metabolized by these enzymes-minipig may be the best choice for preclinical studies.

CYP content in liver microsomes (nM/mg of protein) is also an important factor to be considered in drug metabolism. This CYP content could be the reason for high metabolism of indomethacin and meloxicam in minipig compared to human. CYP content present in liver microsomes differs in the different species [39], human CYP content - 0.307±0.16 nM/mg of protein (n=18), minipig CYP content - 0.81±0.15 (n=9), monkey CYP content 1.03±0.11 (n=5), and dog CYP content 0.39±0.04 (n=6) [38,39].

The recent research in oligonucleotides showed the minipig is a suitable non-rodent model in the safety assessment of single stranded oligonucleotides [41-43] and immunosafety sciences [44-45]. Pharmaceutical industries started using minipigs as pre-clinical non-rodent model recently [46].

The current study showed that the plasma protein binding and hepatic metabolism was similar between minipig, and human compared to dog and monkey in case of NSAIDs. However, the results of the current study are limited to NSAIDs and the plasma protein binding and hepatic metabolism for other drug classes may show differences between minipig and human species. Hence different classes of drugs should be investigated and compared across species to determine the best or most suitable pre-clinical non-rodent species for pre-clinical trials.

CONCLUSION

The objective of this in vitro study was to compare the plasma protein binding and metabolism characteristics of nine NSAIDs in different non-rodent preclinical species to find the relatively closest species to human. The results of

the current investigation corroborated the anatomical and physiological similarities of minipig with human and showed that the minipig results were comparable to human than dog and monkey for both the ADME assays with these NSAIDs. Hence minipig may prove to be a better preclinical non-rodent model to predict human safety and efficacy for studying NSAIDs drug class. The same ADME studies can be extended to other drug classes to understand if such a correspondence exists in those cases. Before conducting in vivo toxicological studies for new chemical entities (NCEs), these ADME assays might be helpful for selecting the best pre-clinical model.

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تقييم الخنازير المصغرة كنماذج ما قبل السريرية المتفوقة غير الإكلينيكية: رؤى من ارتباط بروتين البلازما والتمثيل الغذائي لمضادات الالتهاب غير الستيروئيدية المسوقة مقارنة بين الأنواع

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ملخص

وفقًا لمتطلبات السلطات التنظيمية، يجب إجراء دراسات ما قبل السريرية على الأقل على نوع واحد من القوارض ونوع واحد غير القوارض. وعادةً ما تُعتبر الكلاب من الأنواع غير القوارض المفضلة للدراسات ما قبل السريرية على الرغم من أن الخنازير الصغيرة والقرود أقرب فسيولوجيًا إلى البشر من الكلاب. وكان الهدف من هذه الدراسة هو إثبات أن الخنازير الصغيرة قد تكون نموذجًا أفضل للدراسات ما قبل السريرية مقارنة بالكلاب لبعض فئات الأدوية. في الدراسة الحالية في المختبر، تم تقييم ارتباط بروتين البلازما والاستقرار الأيضي في ميكروسومات الكبد لتسعة أدوية مضادة للالتهابات غير الستيرويدية (NSAIDs) مسوقة في أنواع الخنازير الصغيرة والكلاب والقرود والبشر. أظهرت ثمانية من أصل تسعة مضادات التهاب غير ستيرويدية تم اختبارها ارتباطًا إحصائيًا مماثلًا لبروتين البلازما في بلازما الخنازير الصغيرة والبشر والذي كان مختلفًا عن بلازما الكلاب والقرود. وبالمثل، أظهرت اختبارات استقلاب الدواء استقلابًا مشابهًا في ميكروسومات الكبد لدى الخنازير الصغيرة والبشر، والذي كان مختلفًا مقارنة بميكروسومات الكبد لدى الكلاب والقرود. أظهرت نتائج كلا الاختبارين تشابهًا أكبر بين الخنازير الصغيرة والبشر، مما يشير إلى استخدام أنواع الخنازير الصغيرة كنموذج غير قوارض أفضل قبل السريرية لمضادات الالتهاب غير الستيرويدية بدلاً من أنواع الكلاب التقليدية. بالإضافة إلى ذلك، قد يساعد استخدام أنواع الخنازير الصغيرة الأكثر سهولة في توفير الوقت والموارد أثناء الدراسات قبل السريرية وقد يساعد في دراسات السلامة لدى البشر أثناء التجارب السريرية في المراحل اللاحقة.

الكلمات الدالة: الخنازير الصغيرة، ربط بروتين البلازما، غسيل الكلى المتوازن، مضادات الالتهاب غير الستيرويدية.

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