

## RESEARCH ARTICLE



# Oral hyoscine butylbromide exerts spasmolytic effects in both gastrointestinal and urogenital tissues in rats

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**Background and Purpose:** Hyoscine butylbromide (HBB) has a low oral (PO) bioavailability. Further, limited data on its activity on non-gastrointestinal (GI) smooth muscle spasms after oral dosing are available, causing its effects beyond the GI tract to be questioned. This pharmacokinetic/pharmacodynamic (PK/PD) study, conducted using female rats, aimed to cover this gap.

**Experimental Approach:** PK study: HBB and atropine (as a comparator agent) were administered PO and IV to rats, and concentrations in plasma and tissues (colon, uterus and urinary bladder; CUB) were measured. PD study 1: concentration–response curves of HBB and atropine ( $10^{-9}$ – $10^{-4}$  M) were obtained for carbachol-induced ( $10^{-5}$  M) pre-contracted tissues; PD study 2: CUB were pre-incubated with HBB and atropine at maximum concentrations ( $C_{max}$ ) from PK studies and carbachol concentration–response curves ( $10^{-9}$ – $10^{-4}$  M) were obtained; PD study 3: HBB and atropine were administered PO and IV to rats as for PK study, CUB tissues were collected at 0.5 h (IV) and 4 h (PO), and carbachol concentration–response curves ( $10^{-9}$ – $10^{-4}$  M) obtained.

**Key Results:** PO HBB showed higher  $C_{max}$  in CUB tissues than in plasma. HBB and atropine reduced, concentration-dependently, carbachol-induced contractions in CUB tissues. PO HBB showed highest spasmolytic activity in colon (40%), followed by uterus (30%) and urinary bladder (10%).

**Conclusion and Implications:** This is the first comparison of PO and IV HBB and atropine in GI and non-GI tissues. Despite low bioavailability, PO HBB accumulated and exerted spasmolytic effects in tissues beyond the GI tract.

## KEYWORDS

colon, PK/PD study, smooth muscle, urinary bladder, uterus

**Abbreviations:** AUC<sub>0–t</sub>, area under the concentration-versus-time curve from time 0 to last measurable concentration; AUC<sub>control</sub>, concentration–response curves obtained in the absence of the drugs; AUC<sub>drugs</sub>, concentration–response curves obtained in the presence of the drugs; AUC<sub>T</sub>, area under the curve of the tracing;  $C_{max}$ , maximum concentrations; CUB, colon, uterus and urinary bladder; F%, absolute oral bioavailability; HBB, hyoscine butylbromide;  $K_{off}$ , small dissociation constant; LC–MS/MS, liquid chromatography–tandem mass spectrometry; PK, pharmacokinetic; PD, pharmacodynamic; PO, oral;  $t_{1/2}$ , terminal elimination half-life;  $T_{max}$ , time to reach maximum concentration.

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# 1 | INTRODUCTION

Abdominal pain is a common symptom of functional gastrointestinal disorders often treated with anti-spasmodic drugs (Brenner & Lacy, 2021). Such conditions affect a significant portion of the global population (>40%) impacting their quality of life and leading to increased healthcare use (Sperber et al., 2021). Hyoscine butylbromide (HBB), an anti-muscarinic drug, shows therapeutic effect through smooth muscle relaxation and is well known to reduce GI spasms (Birdsall et al., 2023; Forbes et al., 2021). The spasmolytic activity of HBB is attributed to its ability to block muscarinic ( $M_2$  and  $M_3$ ) receptors, effectively inhibiting acetylcholine-mediated smooth muscle contractions and providing relief from GI pain (Corsetti et al., 2023). In addition to its applications for GI disorders, HBB is also used in the treatment of genitourinary and bile-duct spasms as well as in the management of dysmenorrhoea (Hasan & Jabbar, 2023; Rathod & Misra, 2008; Sanofi, 2021). The versatility and effectiveness of HBB have led to its widespread use in both oral (PO) and parenteral formulations (Chung et al., 2022; Corsetti et al., 2023).

Following PO administration, HBB exhibited a high polarity owing to its quaternary ammonium structure, resulting in a poor absorption (8%) and a very low systemic bioavailability (<1%). However, an intravenous (IV) administration of HBB led to a rapid distribution into the tissues ( $t_{1/2} = 29$  min) (Samuels, 2009).

As the PO bioavailability of HBB is low, its ability to reach target organs apart from the GI tract was questioned. Therefore, this study was performed to comprehensively investigate the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of PO and IV HBB in GI and non-GI tissues of female Sprague Dawley rats. Atropine, a non-selective muscarinic receptor antagonist, which has an oral bioavailability of 20% in rats, was used as a reference drug (Dorandeu et al., 2023; Şahiner et al., 2020; Tian et al., 2015).

# 2 | METHODS

## 2.1 | Pharmacokinetic study

### 2.1.1 | Animals and environmental conditions

All animal care and experimental procedures at Charles River Ltd., UK complied with the UK Animals (Scientific Procedures) Act 1986 and were carried out under the UK Home Office Project Licence number PP9376768. Animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and the recommendations made by the British Journal of Pharmacology (Lilley et al., 2020).

Female Sprague Dawley rats (RRID:MGI:5651135) weighing 210–320 g (7–8 weeks old) obtained from Charles River Ltd., UK were used. The animals were group housed (except for those used for collection of urine and faeces which were singly housed in meta bowls) under controlled environmental conditions at a temperature of

## What is already known?

- Due to low PO bioavailability, spasmolytic activity of HBB on non-GI tissues has been questioned.
- Limited data exist on the effects of PO HBB on non-GI smooth muscle spasms.

## What does this study add?

- This is the first pharmacokinetic/pharmacodynamic study conducted in rats to address this gap.
- PO HBB results in higher concentrations in non-GI tissues than plasma and exerts spasmolytic effects

## What is the clinical significance?

- Spasmolytic effect of PO HBB in non-GI tissues would support therapeutic efficacy beyond GI tract
- Clinical studies are required to confirm these effects.

21°C–22°C, 12:12 h light: dark cycle, a relative humidity of 45%–65% and a free access to commercial feed pellets (2014 Global Teklad, Envigo) and water ad libitum. They were fasted overnight before dosing and were fed at about 2 h post-dose.

### 2.1.2 | Dosing

A total of 112 rats plus four spares were randomly assigned to HBB and atropine groups ( $n = 56$  each). (1) The HBB group was given a single dose of  $1.8 \text{ mg} \cdot \text{kg}^{-1}$  by either IV or PO route ( $n = 28$ , each). (2) The atropine group received  $0.5 \text{ mg} \cdot \text{kg}^{-1}$  for IV route ( $n = 28$ ) and  $2.5 \text{ mg} \cdot \text{kg}^{-1}$  for PO route ( $n = 28$ ). The IV administration was performed as a slow bolus via the tail vein, whereas the PO administration was via oral gavage. The dose levels selected were based on the maximum dose of the drugs used in the clinic by each route, allometrically adjusted for a rat. No adverse clinical observations were recorded during the study.

For PK analysis, samples of blood, colon, uterus and urinary bladder (CUB) tissues, urine, and faeces were collected from 0–48 h relative to each drug administration, with up to 13 independent collections with 4 animals per group sampled at each time point. For IV administration, blood collection was obtained at 5, 10, 20, 30, 45, 60 min, 1.5, 2, 3, 4, 8, 24 and 48 h. For PO administration, blood collection was obtained at 0, 15, 30, 45, 60 min, 1.5, 2, 3, 4, 8, 24 and 48 h. Drugs in CUB tissues were estimated at 30 min, 1, 2, 4, 8, 24 and 48 h. Accordingly for the three protocols, plasma PK

parameters were determined from 52 ( $4 \times 13$ ) (IV) or 48 ( $4 \times 12$ ) (PO) independent experimental values, and tissue accumulation was determined from 28 ( $4 \times 7$ ) independent experimental values. Drugs in faeces and urine were estimated at 4, 8, 24, 36 and 48 h. In this case the total number of points was 20 independent experimental values. The experimental design was carried out with the minimum number of animals to obtain reliable results.

### 2.1.3 | Tissue collection

To obtain plasma, blood samples (0.2 ml) were collected into K<sub>2</sub>EDTA vacuum tubes at pre-specified sampling time points following drug administration. The blood samples were centrifuged at 1500  $\times$  g for 10 min at 4°C to obtain plasma samples and stored ( $<-65^{\circ}\text{C}$ ) until analysis. At selected periods after dosing, urine and faeces samples were collected, weighed and processed for bioanalysis.

After terminal sample collection, the rats were killed (CO<sub>2</sub> narcosis (minimum of 7 minutes in a CO<sub>2</sub> chamber, followed by exsanguination and cervical dislocation) and CUB tissues were obtained, weighed and stored ( $<-65^{\circ}\text{C}$ ) until processed for bioanalysis.

### 2.1.4 | Sample extraction and analysis

The bioanalysis for both HBB and atropine was conducted using validated bioanalytical methods. Following the appropriate sample preparation, samples were analysed via liquid chromatography–tandem mass spectrometry (LC–MS/MS). Chromatographic separation was achieved using an Ace Generix C18 (2) column ( $50 \times 2.1$  mm). The gradient mobile phase consisted of two components: Mobile Phase A (a mixture of acetonitrile and formic acid in a 100/0.2 v/v ratio) and Mobile Phase B (a mixture of deionised water and formic acid in a 100/0.2 v/v ratio). The detection of compounds was conducted in a positive electrospray ionisation mode, utilising multiple reaction monitor transitions. For HBB, the method demonstrated linearity over a concentration range of 0.001–5.00 ng·mL<sup>-1</sup> (low range) or 0.01–50.00 ng·mL<sup>-1</sup> (high range) for plasma and urine, and 0.01–50.00 ng·g<sup>-1</sup> for uterus, faeces and colon, and 0.03–150.00 ng·g<sup>-1</sup> for urinary bladder. For atropine, the method demonstrated linearity over a concentration range of 0.100–500 ng·mL<sup>-1</sup> for plasma and urine, and 0.050–250 ng·g<sup>-1</sup> for colon and uterus, 0.500–2500 ng·g<sup>-1</sup> for urinary bladder, and 0.167–833 ng·g<sup>-1</sup> for faeces. All analytical data were processed using Sciex Analyst 1.7.2 software.

### 2.1.5 | Non-compartmental pharmacokinetic model

A non-compartmental PK model using composite data was used to evaluate the PK parameters. It included the C<sub>max</sub>, area under the

concentration-versus-time curve from time 0 to last measurable concentration (AUC<sub>0–t</sub>), T<sub>max</sub> and t<sub>1/2</sub>. The absolute oral bioavailability (F %) in plasma was estimated following single IV and PO administrations of atropine or HBB, and the relative levels in the CUB tissues for the two routes were assessed. The C<sub>max</sub> and T<sub>max</sub> were directly obtained from the concentration–time curves, and AUC<sub>0–t</sub> was calculated using the linear trapezoidal method.

## 2.2 | Pharmacodynamic studies

The three distinct PD studies were tailored to address different aspects of the pharmacological effects of the test substances. While these studies differed in certain experimental procedures, there were common steps shared by them as mentioned in Sections 2.2.1 and 2.2.2.

### 2.2.1 | Animals and environmental conditions

The study was conducted in accordance with Directive EU 63/2010 and the Spanish National Legislation RD 53/2013 and was conducted at Universitat Autònoma de Barcelona (UAB), Spain. The UAB Ethics Committee granted approval for the PD studies 1 and 2, with the reference number EUT-MJ001, and for the PD study 3, with the reference number CEEAH 6002. Female Sprague Dawley rats (RRID: MGI:5651135) obtained from Charles River Ltd. (France) were used in the study. They were housed under controlled environmental conditions maintained at 21°C–22°C, 12:12 h light:dark cycle and a relative humidity of 45%–65% and a free access to food (Global Diet 2014C, Teklad, Envigo) and water ad libitum. Rats were killed by decapitation.

### 2.2.2 | Mechanical studies

Transmural muscle strips from CUB tissues were collected and cut ( $3 \times 10$  mm) in a circular direction for the colon and in a longitudinal direction for the uterus and urinary bladder. A muscle bath setup was employed to measure the contractile response of the tissue samples. Strips were attached to a silk thread at both ends and immersed in 10-ml organ baths with Krebs's solution (37 °C, bubbled with a mixture of 5% CO<sub>2</sub>/95% O<sub>2</sub>, pH = 7.4). Thereafter, strips were stretched to a pre-determined force, 1 g for the colon and urinary bladder, 0.5 g for the uterus and allowed to equilibrate (1 h for Protocol 1 and 2, and 15 min for Protocol 3). Thereafter, the mechanical activity was measured using an isometric force transducer (Harvard VF-1) connected to a computer through an amplifier. Digitalised data at a frequency of 25 Hz were collected using DATAWIN1 software (Panlab, Barcelona, Spain), coupled with an ISC-16 analogue-to-digital card installed on a computer.

## 2.3 | Details of Pharmacodynamic study 1

### 2.3.1 | Animals and mechanical activity

A total of seven Female Sprague Dawley rats (RRID:MGI:5651135) (including one spare) weighing 196–258 g (7–8 weeks old) were used for all the protocols. The CUB tissues obtained and prepared as described in Section 2.2.2 were incubated for 15–20 min with carbachol ( $10^{-5}$  M) to induce an increase in the contractile activity. After stabilisation, concentration–response curves of HBB and atropine ( $10^{-9}$ – $10^{-4}$  M) were obtained to estimate the decrease in contractility of the drugs. Tissues were randomly distributed to cover all the protocols, and the tracing analysis was performed blinded.

The area under the curve of the tracing ( $AUC_T$ ) (g·min) of contractions was measured from the baseline, after the incubation of each concentration of HBB and atropine. The following formula was used to measure the percentage mechanical activity after drug incubation (Equation (1)):

$$\text{Basal } AUC \text{ in } \% = \left( \frac{AUC_T \text{ after HBB or atropine incubation}}{\text{Basal } AUC_T \text{ after CCH } 10^{-5} \mu\text{M addition}} \times 100 \right) \times 100 \quad (1)$$

Zero percent represents the complete cessation of spontaneous motility and 100% represents no change compared with the mechanical activity stimulated with carbachol.

To estimate the residual contractility for each concentration of atropine and HBB, the experimental data were fitted to a classical concentration–response curve using the Equation (2):

$$Y = \text{Bottom} + \frac{(\text{Top}100 - \text{Bottom})}{1 + 10^{[(\text{LogEC}50 - X) \times (\text{Hill } s\text{Slope})]}} \quad (2)$$

where Y represents the residual contractility and X is the logarithm of the concentration. In this protocol Top was constrained to 100.

### 2.3.2 | Spasmolytic activity (Study 1)

A PK/PD model (Figure S1) was established to evaluate the pharmacological effect of drug concentrations in the tissues by correlating data from PK study with that of the PD 1 study. In this model, the concentrations obtained for each time in each tissue were transformed into molar. Subsequently, the model was applied using the data obtained from the tissue-specific concentration–response curve evaluation.

The percentage of spasmolytic activity was determined based on concentration response curve (by subtracting 100 from the residual contractility value). Thus, for example, low drug concentrations would cause little inhibition of contractility, which would mean little spasmolytic capacity. At the other extreme, high drug concentrations would cause a high reduction in contractility, which would be associated

with a high spasmolytic capacity. Thus, the value obtained ranged from 0 (no spasmolytic capacity) to 100 (maximum spasmolytic capacity). Prediction 1 corresponds to the potential spasmolytic activity estimated using the model run with the mean of the concentrations at each time point for each tissue.

## 2.4 | Details of Pharmacodynamic study 2

### 2.4.1 | Animals and mechanical activity

Female Sprague Dawley rats (RRID:MGI:5651135) (n = 20, including 3 spares) weighing 183–296 g (7–13 weeks old) were used.

The CUB tissues were obtained and prepared as described earlier in Section 2.2.2. Subsequently, they were incubated for 15 min with a single concentration of HBB or atropine, determined based on the  $C_{\max}$  obtained in the PK study. A control group was incubated with the vehicle (distilled water at the same volume for each concentration). Thereafter, concentration–response curves of carbachol ( $10^{-9}$ – $10^{-4}$  M) were obtained. Tissues were randomly distributed to cover all the protocols, and the tracing analysis was performed blinded.

To estimate the contractile response to carbachol, the  $AUC_T$  (g·min) of contractions from the baseline was measured. Data normalisation was achieved by expressing drug responses as a percentage of the response to KCl (120 mM) added at the end of the experiment. KCl-induced contraction is independent of muscarinic receptors and served as a reference for determining the maximum contractile capacity of the tissues.

The experimental data were fitted to a classical concentration–response curve in Equation (2). In this case the “Bottom” parameter was constrained to zero, where Y represents the carbachol response and X is the logarithm of the concentration’.

## 2.5 | Details of Pharmacodynamic study 3

### 2.5.1 | Animals and drug administration

Thirty-six female Sprague Dawley rats (RRID:MGI:5651135) weighing 189–312 g (approximately 8–12 weeks old) were used in this study. Both HBB and atropine were administered following the same dosage and method of administration as used in the PK study (see Section 2.1.2). A control group was also administered the vehicle (0.9% NaCl solution) PO or IV in the same volume ( $5 \text{ ml} \cdot \text{kg}^{-1}$ ). The animals were randomly assigned to each of the groups. No adverse clinical observations were recorded during the study.

### 2.5.2 | Mechanical activity

The CUB tissues were obtained at 0.5 h after the IV administration and 4 h after the PO administration, as these time points corresponded to the average time needed to achieve  $C_{\max}$  values in

the PK study. The CUB tissues were obtained and prepared as described earlier in Section 2.2.2. However, in this PD study, all tissues were equilibrated for 15 min only to avoid a possible drug dilution. Concentration–response curves were generated as outlined in Section 2.4.2, with tissues from animals administered with the vehicle acting as the control.

## 2.6 | Measurement of spasmolytic activity (PD 2 and 3)

The potential spasmolytic activities of the drugs (HBB and atropine) were determined using the AUC of the concentration–response curves obtained in the absence ( $AUC_{\text{control}}$ ) and in the presence of drugs ( $AUC_{\text{drug}}$ ) using the following formula (Equation (3)):

$$\text{Spasmolytic activity (\%)} = 100 - \left( \frac{AUC_{\text{drug}}}{AUC_{\text{control}}} \times 100 \right) \quad (3)$$

Accordingly, the three possible scenarios were as follows:

1. If the  $AUC_{\text{drug}}$  matched the  $AUC_{\text{control}}$ , the estimated spasmolytic activity was 0, indicating no pharmacological effect.
2. If the  $AUC_{\text{drug}}$  was 0, the spasmolytic activity was considered 100%, indicating complete inhibition of the contractile response induced by carbachol.
3. If the  $AUC_{\text{drug}}$  fell between the above two scenarios, it represented a partial spasmolytic effect, reflecting a partial reduction in the contractile response compared with the control.

To calculate the AUC, two distinct predictions (Predictions 2 and 3) were made.

Prediction 2: Based on the experimental data, the contractile responses for each concentration of carbachol (from  $10^{-9}$ – $10^{-4}$  M) were summed to obtain the total AUC.  $AUC_{\text{control}}$  is the response to carbachol without drugs, whereas  $AUC_{\text{drug}}$  is the response to carbachol in the presence of atropine or HBB.

Prediction 3: Based on the concentration–response curve model, a non-linear regression was performed using GraphPad software. In this case, the  $AUC_{\text{control}}$  and  $AUC_{\text{drug}}$  were calculated by summing the values obtained from the model in the absence ( $AUC_{\text{control}}$ ) and presence ( $AUC_{\text{drug}}$ ) of HBB and atropine. This prediction 3 is equivalent to the integral of the model.

## 2.7 | Data and statistical analysis

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2022). A range of 1–3 strips (n) per animal was used and data are expressed as the total number of animals (N) used in each experimental group. For all PD studies, the number of animals (N) in all experimental groups was six but occasionally groups of seven or eight animals were

used. Parameters analysed in each PD study were as follows: the concentration needed to reach 50% of the  $E_{\text{max}}$  ( $EC_{50}$ ) (PD studies 1, 2 and 3), the slope of the concentration–response curve (hill slope) (PD studies 1, 2 and 3), bottom of the curve (PD study 1), Top (fixed with the control curve) (PD studies 2 and 3), goodness of the fit of the curve (R square) (PD studies 1, 2 and 3) and the degrees of freedom (PD studies 1, 2 and 3).

It is important to note that for all three PD studies, a value of 100 represents the maximum spasmolytic capacity of the drug, whereas a value of 0 represents no spasmolytic capacity. To determine whether the spasmolytic capacity was different from 0, a *t* test was applied for each experimental group. The statistical analysis was carried out with values obtained from different animals (N; in all cases  $n > 5$ ). When repetitions were performed (n values) data were averaged. *P* was considered significant when  $P < 0.05$ .

## 2.8 | Materials

Atropine (CAS No. 51-55-8) and carbachol (CAS No. 51-83-2) were from Merck Life Science S.L.U. (Madrid, Spain) and HBB (CAS No. 149-64-4), was provided by Sanofi (Paris, France).

## 2.9 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org> and are permanently archived in the Concise Guide to PHARMACOLOGY 2023/24 (Alexander, Christopoulos et al., 2023; Alexander, Mathie et al., 2023).

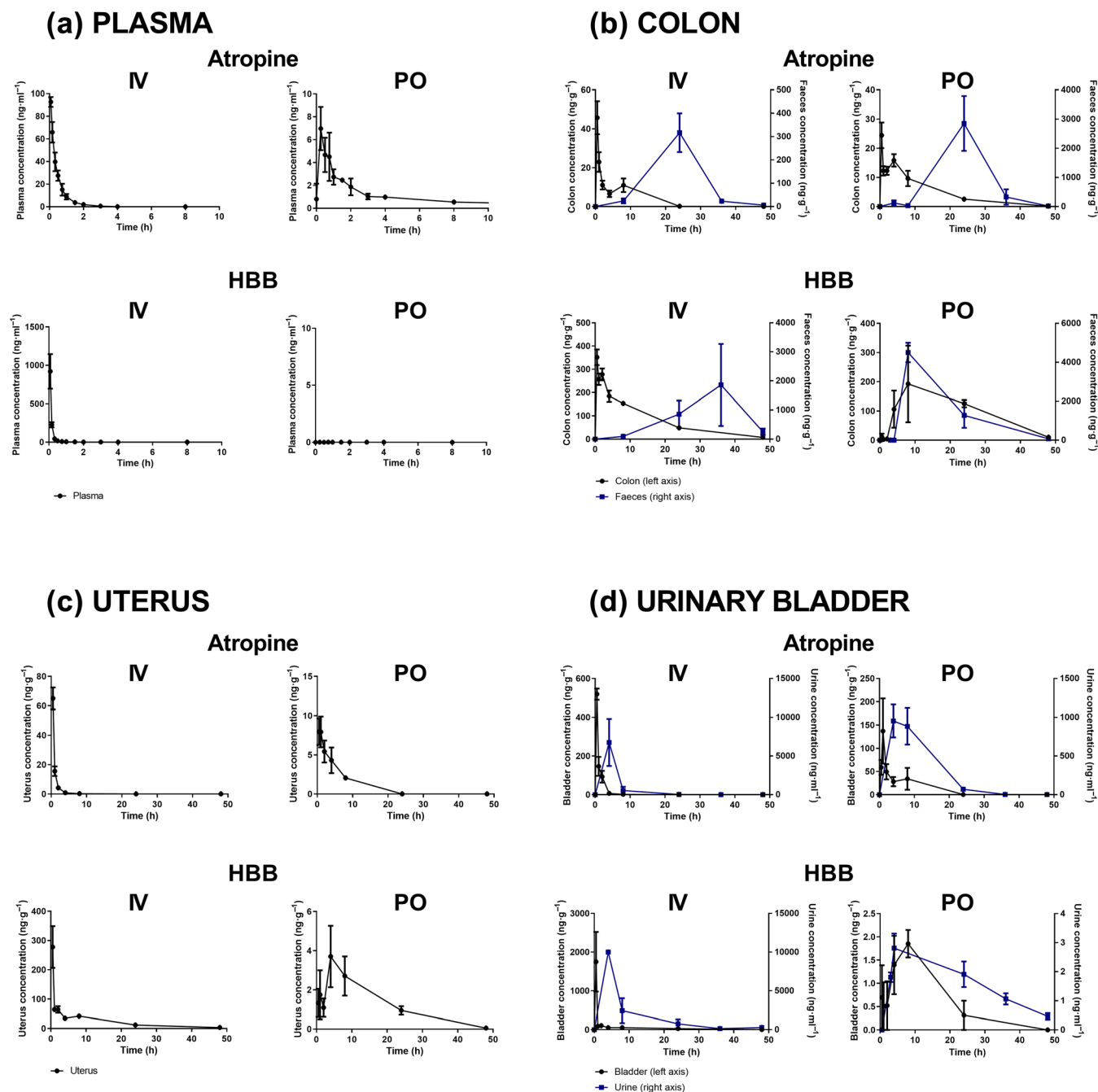
## 3 | RESULTS

### 3.1 | PK study: Is HBB detectable in the CUB tissues of the rat after IV and PO administration?

The concentration–time profiles for HBB and atropine in plasma and CUB tissues following IV and PO administrations are presented in Figure 1. Note that the profiles for faecal and urinary content are shown with the profiles for colon and bladder tissue respectively. The calculated PK parameters ( $C_{\text{max}}$ ,  $T_{\text{max}}$ ,  $AUC_{0-t}$  and absolute PO bioavailability, shown as *F*) for plasma and the CUB tissues, corresponding to both PO and IV routes are summarised in Table 1.

For HBB after the PO administration, the  $C_{\text{max}}$  levels and  $AUC_{0-t}$  in the uterus and urinary bladder as well as colon were much higher than that in plasma. For atropine, the concentration in the tissues after PO administration was broadly of the same order for plasma, colon and uterus but was higher in the urinary bladder, most likely due to notable excretion of unchanged drug in the urinary bladder. For HBB, as would be expected because of the very low PO bioavailability, the concentration in all CUB tissues was





**FIGURE 1** Concentration-time profiles of atropine and HBB after PO and IV administrations in rat (a) plasma, (b) colon, (c) uterus and (d) urinary bladder. Data shown are means  $\pm$  SEM ( $n = 28$  animals, 4 at each time point). HBB, hyoscyne butylbromide; IV, intravenous; PO, oral.

much higher after the IV administration than the PO administration, except in the colon, where concentration was similar for both routes. For atropine, the concentration was around 2- to 13-fold higher in all tissues after the IV administration than after the PO administration, except in the colon, where the concentration as measured by  $AUC_{0-t}$  was similar for both routes. For both drugs and dosing routes,  $T_{max}$  in all tissues was in a narrow range of 0.083–1 h after administration, except for HBB in the CUB after a PO dosing, when  $T_{max}$  was delayed at 4 h–8 h.

Additionally, excretion of HBB and atropine as percentage of dose was estimated over a 48 h period in urine and faeces after IV and PO administrations (Table S1 and Figure 1). Following the PO administration, excretion of both unchanged drugs in urine was minimal (HBB: 0.03% of dose) or low (atropine: 3.09% of dose) compared with that found in faeces (10.9% for HBB, 13.4% for atropine). After the IV administration of HBB and atropine, excretion of unchanged drug in urine (2.50% and 12.8% of dose, respectively) was higher than that in the faeces (1.05% and 1.64% of dose, respectively). For

**TABLE 1** Analysis of PK parameters of atropine and HBB after PO and IV administration in rat plasma, colon, uterus and urinary bladder.

Pharmacokinetic parameters	Atropine		HBB	
	PO	IV	PO	IV
<b>Plasma</b>				
$C_{\max}$ (ng·ml <sup>-1</sup> ) <sup>a</sup>	6.97 ± 0.94	92.68 ± 2.16	0.008 ± 0.005	921.0 ± 112.70
$T_{\max}$ (h)	0.25	0.083	0.25	0.083
AUC <sub>0-t</sub> (ng·h·ml <sup>-1</sup> ) <sup>b</sup>	12.2	44.7	0.0449	286
$F$ (%)	5.46	-	0.01	-
<b>Colon</b>				
$C_{\max}$ (ng·g <sup>-1</sup> ) <sup>a</sup>	24.38 ± 4.41	45.73 ± 8.52	192.5 ± 131.0	351.3 ± 34.04
$T_{\max}$ (h)	0.5	0.5	8	0.5
AUC <sub>0-t</sub> (ng·h·g <sup>-1</sup> ) <sup>b</sup>	236	210	5,050	4,050
$F$ (%)	22.6	-	100	-
<b>Uterus</b>				
$C_{\max}$ (ng·g <sup>-1</sup> ) <sup>a</sup>	7.96 ± 1.69	65.05 ± 7.51	3.70 ± 1.57	277.8 ± 71.47
$T_{\max}$ (h)	0.5	0.5	4	0.5
AUC <sub>0-t</sub> (ng·h·g <sup>-1</sup> ) <sup>b</sup>	35.1	122	61.4	1,380
$F$ (%)	5.75	-	4.45	-
<b>Urinary bladder</b>				
$C_{\max}$ (ng·g <sup>-1</sup> ) <sup>a</sup>	137.3 ± 70.0	519.3 ± 28.93	1.85 ± 0.30	1750 ± 771.8
$T_{\max}$ (h)	1	0.5	8	0.5
AUC <sub>0-t</sub> (ng·h·g <sup>-1</sup> ) <sup>b</sup>	637	992	26.7	11,100
$F$ (%)	12.8	-	0.36	-

Note: AUC<sub>0-t</sub>, area under the concentration–time curve from  $t=0$  to the last determination;  $C_{\max}$ , maximum plasma concentration of drug; HBB, hyoscine butylbromide; IV, intravenous; PO, oral;  $T_{\max}$ , time to reach maximum concentration.

<sup>a</sup>The values shown are means ± SEM of four different values.  $F$  refers to the bioavailability and is the ratio between PO and IV routes of administration.

<sup>b</sup>These values were calculated using composite data.

HBB, the comparison of the unchanged drug excreted in urine after PO and IV administrations suggests a very low PO bioavailability for HBB.

## 3.2 | Pharmacodynamic studies

### 3.2.1 | PD study 1: Does HBB reduce the contractile response of the CUB smooth muscles of rats pre-stimulated with carbachol?

The effects of atropine and HBB on carbachol-induced ( $10^{-5}$  M) pre-contracted CUB tissues were investigated (Figure 2). Incubation with increasing concentrations of atropine and HBB ( $10^{-9}$ – $10^{-4}$  M) showed a progressive reduction in the contraction of CUB tissues in a concentration-dependent manner. The potency of HBB (EC<sub>50</sub>) was greater in the uterus, followed by the colon and then the urinary bladder. Atropine was more potent than HBB in all CUB tissues with the same order of potency as HBB (Table S2).

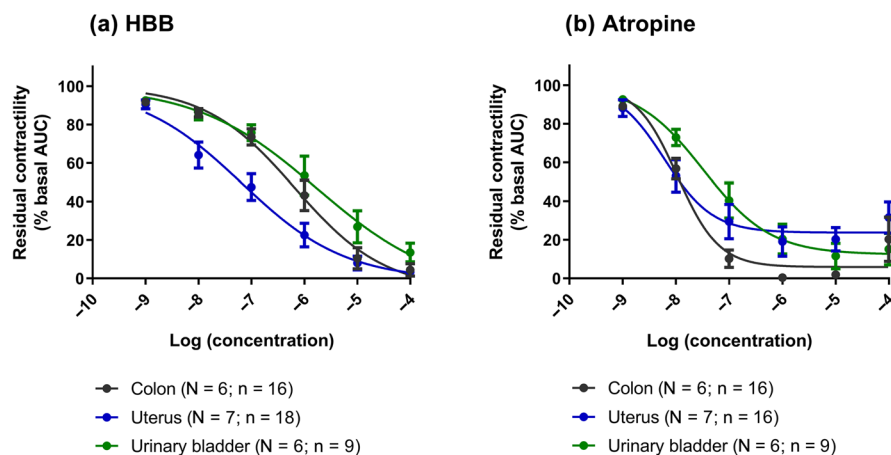
The PK/PD model was developed to assess the spasmolytic effects of atropine and HBB in rat CUB tissues (in vitro) by combining

PK and PD values. The spasmolytic activity was estimated based on the measured drug concentrations at each time point (PK study) and the reduction in contractions induced by carbachol, with each concentration of HBB and atropine tested (PD study 1). The results are shown in Figure 3 and Table 2 (PD study 1: Prediction 1).

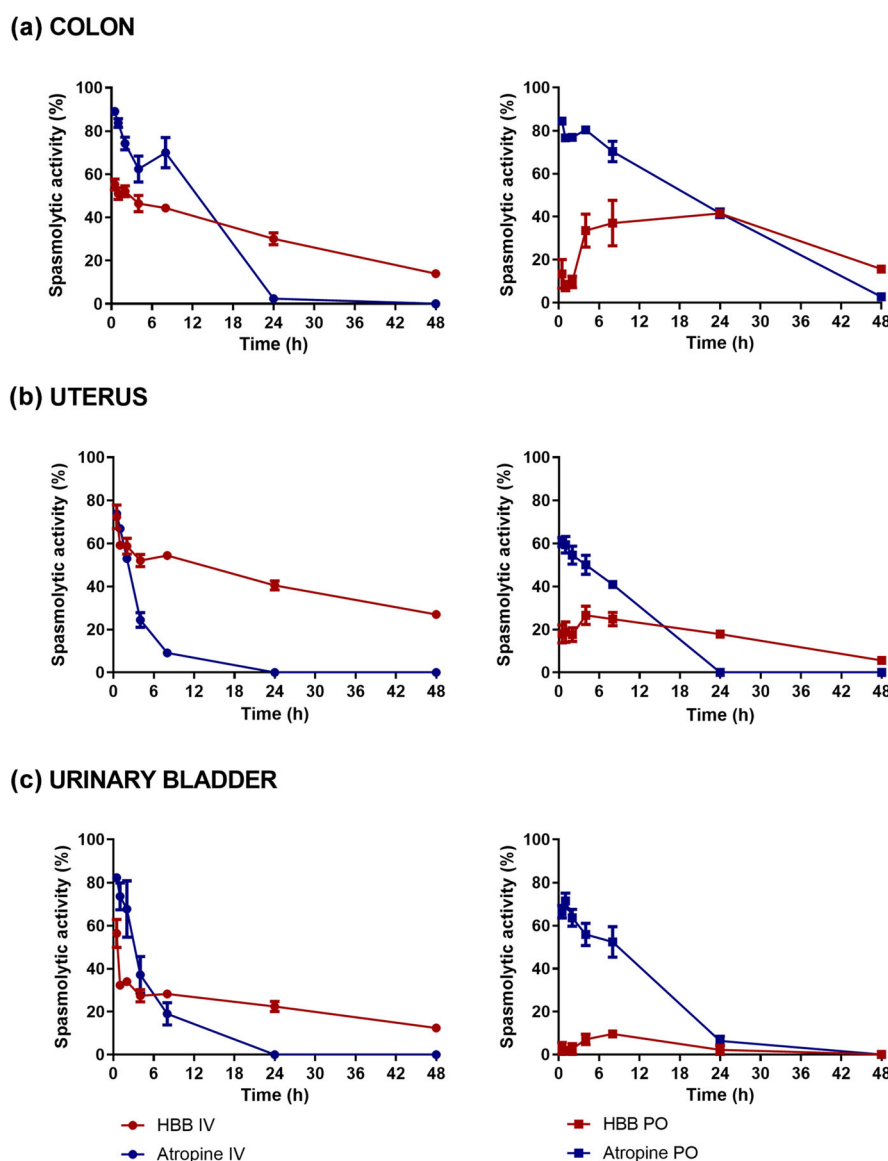
As shown in Figure 3a, in the colon the maximum spasmolytic activity was observed at 24 h following PO HBB, after which it decreased at 48 h (~20%). Atropine, after PO administration, demonstrated a faster and greater spasmolytic effect than HBB. The maximum spasmolytic effect by atropine IV was observed at 0.5 h which decreased at 24 h (~40%), and no effect was estimated at 48 h.

HBB, following PO administration, also showed a spasmolytic effect in the uterus. The maximum spasmolytic activity was observed at 4 h, after which it decreased at 48 h (~5%). Atropine, after PO administration, resulted in a faster and greater spasmolytic effect than HBB, with the maximum effect observed at 0.5 h. The effect decreased gradually over time, with no effect apparent at 24 h (Figure 3b).

In the urinary bladder samples, PO administration of HBB resulted in a slow but gradual increase in spasmolytic effect, with the maximum effect observed at 8 h, followed by a gradual decrease up to



**FIGURE 2** Concentration–response curves of (a) HBB and (b) atropine in the CUB tissues pre-contracted with carbachol (CCH;  $10^{-5}$  M). Data are expressed as means  $\pm$  SEM. N values are shown in the figure. Each point represents the mean of experimental data using Equation (1) and the non-linear regression was calculated with Equation (2). AUC, area under the curve; HBB, hyoscine butylbromide; N, number of animals; n, number of tissue replicates.



**FIGURE 3** PK/PD model showing the spasmolytic effect of atropine and HBB in (a) colon, (b) uterus and (c) urinary bladder. In each time point, the concentration detected in tissues (PK study) was correlated with the reduction of contractility induced by carbachol (CCH;  $10^{-5}$  M; PD study 1). The spasmolytic activity was then estimated by measuring the reduction of contractility in each time point. 0 represents no spasmolytic activity and 100 represents the maximum spasmolytic activity. Data are expressed as means  $\pm$  SEM. For each tissue, the data on the left side of the graph represents data for the IV administration and the right side represents data for the PO administration for both the drugs. HBB, hyoscine butylbromide; IV, intravenous; PD, pharmacodynamic; PK, pharmacokinetic; PO, oral.



**TABLE 2** Spasmodic activity of atropine and HBB in the CUB: comparison between different PD models (PD study 1 vs. PD study 2 vs. PD study 3).

	PD study 1	PD study 2		PD study 3 <sup>d</sup>	
	Prediction 1 <sup>a</sup>	Prediction 2 <sup>b</sup>	Prediction 3 <sup>c</sup> (R <sup>2</sup> )	Prediction 2 <sup>b</sup>	Prediction 3 <sup>c</sup> (R <sup>2</sup> )
<b>Colon</b>					
Atropine IV	89 ± 1	89 ± 2	90 (0.84)	55 ± 5	48 (0.82)
Atropine PO	85 ± 2	87 ± 2	87 (0.79)	50 ± 6	42 (0.79)
HBB IV	55 ± 1	55 ± 3	53 (0.92)	27 ± 8	18 (0.83)
HBB PO	42 ± 1	46 ± 6	45 (0.88)	35 ± 7	22 (0.77)
<b>Uterus</b>					
Atropine IV	74 ± 0.3	98 ± 2	100 (n.d.)	78 ± 13	56 (0.54)
Atropine PO	61 ± 2	88 ± 3	97 (0.63)	51 ± 17	36 (0.69)
HBB IV	72 ± 3	70 ± 5	70 (0.82)	31 ± 25 <sup>e</sup>	30 (0.63)
HBB PO	27 ± 4	37 ± 8	35 (0.87)	8 ± 23 <sup>e</sup>	26 (0.73)
<b>Urinary bladder</b>					
Atropine IV	82 ± 0.2	100 ± 1	100 (n.d.)	76 ± 6	77 (0.78)
Atropine PO	72 ± 4	97 ± 1	96 (0.54)	49 ± 6	45 (0.85)
HBB IV	56 ± 6	78 ± 2	81 (0.95)	33 ± 5	31 (0.91)
HBB PO	10 ± 1	10 ± 10 <sup>e</sup>	4 (0.85)	16 ± 3	12 (0.96)

Note: Prediction 3 (PD studies 2 and 3) has no variability because it is a Prediction based on the linear regression obtained from the whole experimental data of the group. n.d. = not determined: in this case the model did not converge as the data were close to 0 and therefore the estimate of spasmodic activity was considered 100. CUB, colon, uterus and urinary bladder; HBB, hyoscine butylbromide; IV, intravenous; PD, pharmacodynamic; PO, oral; SEM, standard error of mean.

<sup>a</sup>Prediction 1: Data from PD study 1.

<sup>b</sup>Prediction 2: Based on experimental data.

<sup>c</sup>Prediction 3: Based on the concentration–response curve model (R<sup>2</sup> is shown in brackets).

<sup>d</sup>IV: 0.5 h, PO: 4 h. Data represent the prediction of the spasmodic activity as a percentage of the reduction in contractility. 0 is no effect and 100 is a total reduction of contractility. Data are expressed as mean ± SEM.

<sup>e</sup>All values were significantly different from 0 ( $P < 0.05$ ), except these values.

24 h (Figure 3c). Atropine, after PO administration, showed a rapid and greater spasmodic effect, with the maximum effect observed at 1 h, followed by a rapid reduction at 24 h (≈10%), and with the effect abolished at 48 h.

Overall, the IV administration of atropine resulted in a rapid and shorter spasmodic effect than HBB in all CUB tissues.

### 3.2.2 | PD study 2: Do the CUB smooth muscles from rats, pre-incubated with HBB at the same concentration found in the PK study, present a reduced contractile response to carbachol?

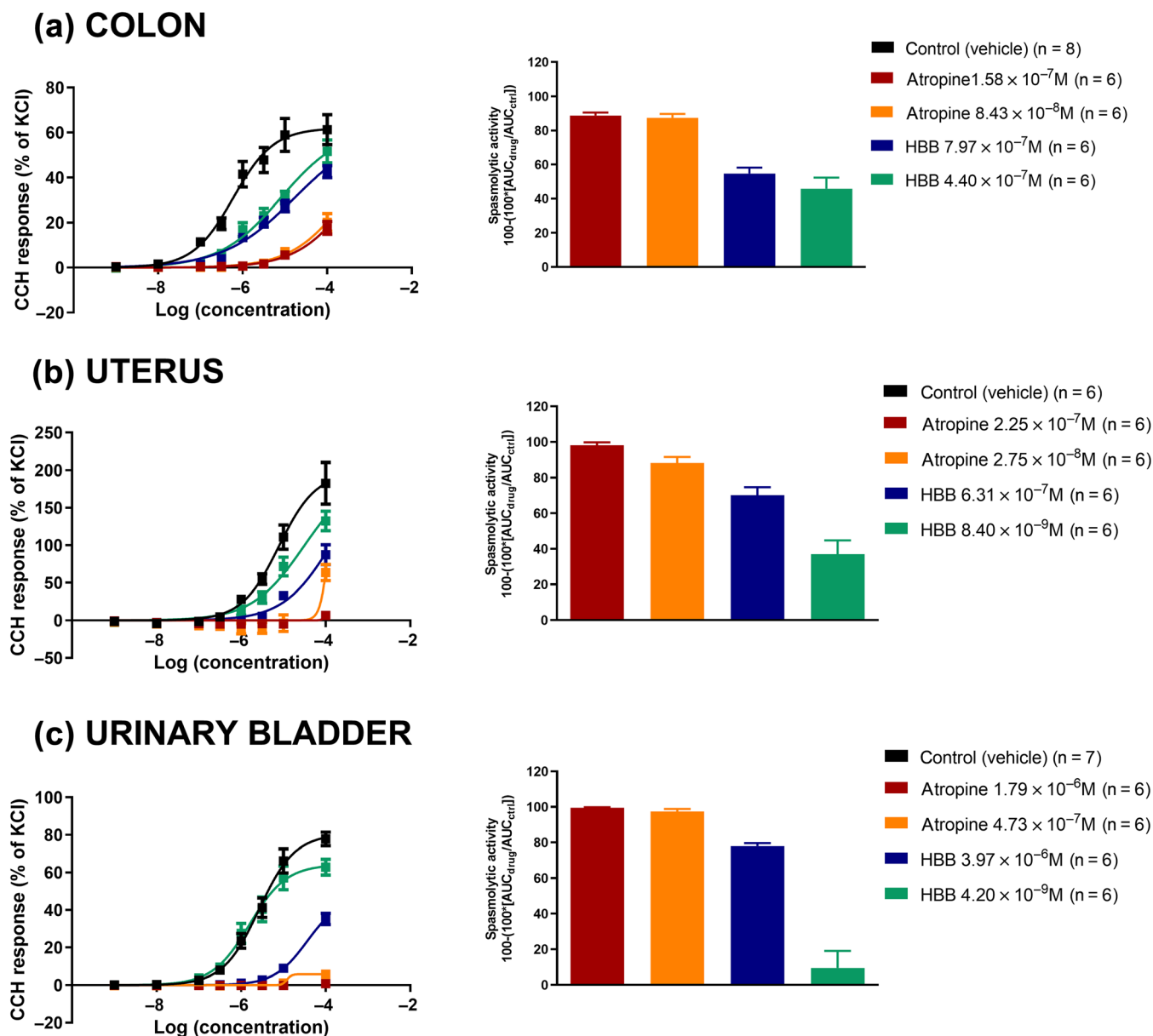
The spasmodic activity of atropine and HBB in CUB tissues was measured by incubating both drugs at  $C_{max}$  obtained in the PK study (Table 1). The results are shown in Figure 4 and Table 2 (PD study 2: Predictions 2 and 3). The spasmodic effect of atropine was greater than 80% in all the tissues after either PO or IV administration. HBB caused a greater spasmodic activity after IV administration than after PO administration in the uterus and urinary bladder. In the colon, the effect of HBB was similar regardless of the administration route. Comparing the rank of order of spasmodic activity in the CUB tissues, PO

HBB was more effective in the colon than in the uterus and the urinary bladder. This is consistent with a local effect of PO HBB in the GI tract.

### 3.2.3 | PD study-3: Do the CUB smooth muscles from rats administered HBB by IV or PO routes present a reduced contractile response to carbachol?

In this study, the rats were administered HBB and atropine at the same dose levels as those used in the PK study, while the control animals received a saline solution. The contractile capacity and spasmodic activity of atropine and HBB were evaluated in rat tissues (0.5 h after IV administration and 4 h after PO administration). The results are shown in Figure 5 and Table 2 (PD study 3: Predictions 2 and 3).

In the muscle bath, exposure to carbachol ( $10^{-9}$ – $10^{-4}$  M) led to a significant concentration-dependent tissue contraction. Animals treated with HBB and atropine showed a rightwards shift in the carbachol-induced concentration–response curve compared with the control (saline), indicating the spasmodic effect of these drugs. Animals treated with atropine had a greater spasmodic effect than those treated with HBB in all CUB tissues, after both PO and IV



**FIGURE 4** Concentration–response curves of carbachol in the presence and absence of drugs (*left panel*) and spasmolytic activity (*right panel*) in (a) colon, (b) uterus and (c) urinary bladder. In this protocol, each tissue was incubated with the  $C_{max}$  of each drug obtained in the PK study (Table 1), and a concentration–response curve with carbachol (CCH) was obtained. The spasmolytic activity was then evaluated by measuring the shift to the right of the concentration–response curve (Equation 3). 0 represents no spasmolytic activity and 100 represents the maximum spasmolytic activity. Data are expressed as mean  $\pm$  SEM.  $N$  values are shown in the figure. For each tissue, the data on the left side of the graph represents data for the IV administration and the right side represents data for the PO administration for both the drugs. AUC, area under the curve; ctrl, control; HBB, hyoscine butylbromide; PK, pharmacokinetic.

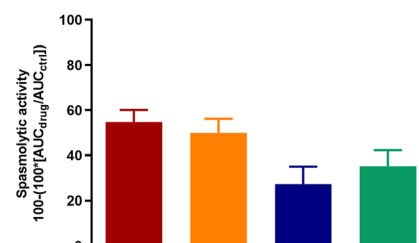
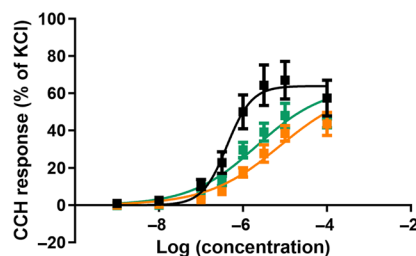
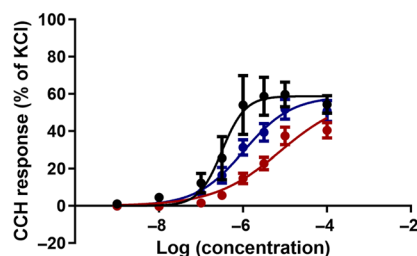
administrations. The spasmolytic effect of HBB after the PO administration was about 22%–35% in the colon, 8%–26% in the uterus and 12%–16% in the urinary bladder.

Table 2 presents a comparative analysis of the spasmolytic activity of atropine and HBB, administered IV and PO, in rat CUB tissues, across the different PD models (PD studies 1, 2 and 3). It can be observed that the spasmolytic activity was quite similar between PD studies 1 and 2. The estimated spasmolytic activity was lower in the PD study 3.

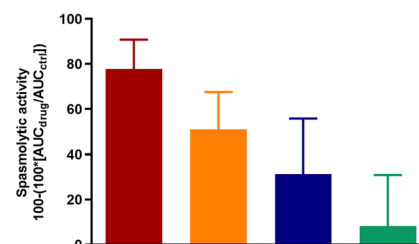
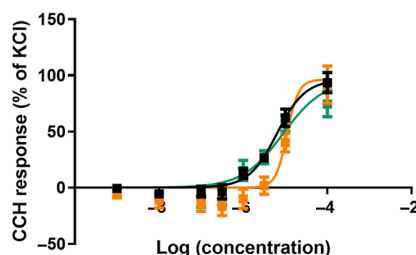
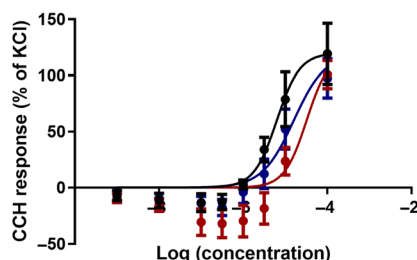
## 4 | DISCUSSION

This is the first PK/PD study that provides insight on the comparative spasmolytic activity of atropine and HBB in GI (colon) and non-GI (uterus and urinary bladder) smooth muscles of rats, following IV and PO administration. The PK study confirmed the low oral bioavailability of HBB but revealed greater concentrations of HBB in CUB tissues than in plasma, while the PD study demonstrated spasmolytic activity both in GI and non-GI tissues.

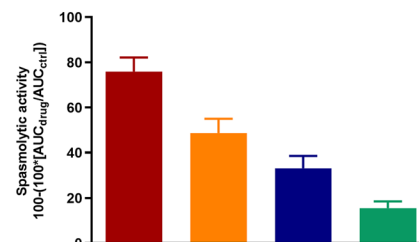
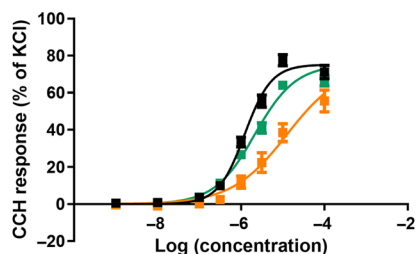
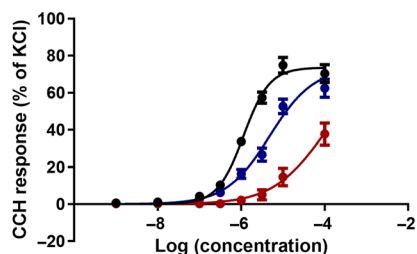
## (a) COLON



## (b) UTERUS



## (c) URINARY BLADDER



● Control (saline IV)  
● Atropine 0.5 mg·kg<sup>-1</sup> IV  
● HBB 1.8 mg·kg<sup>-1</sup> IV

■ Control (saline PO)  
■ Atropine 2.5 mg·kg<sup>-1</sup> PO  
■ HBB 1.8 mg·kg<sup>-1</sup> PO

**FIGURE 5** Concentration–response curves of carbachol in the (A) colon, (B) uterus and (C) urinary bladder after IV (*left panel*) and PO administrations (*middle panel*) of HBB and atropine, and spasmolytic activity of drugs in each rat tissue (*right panel*). Notice that in this PD study, drugs were administered at the same doses as used in the PK study, and a concentration–response curve with carbachol (CCH;  $10^{-9}$ – $10^{-4}$  M) was obtained. The spasmolytic activity was then evaluated by measuring the shift to the right of the concentration–response curve (Equation (3)). 0 represents no spasmolytic activity and 100 represents the maximum spasmolytic activity. Data are expressed as means  $\pm$  SEM. N = 6 each group. For each tissue, the data on the left side of the graph represents data for IV administration and the right side represents data for PO administration, for both the drugs. AUC, area under the curve; ctrl, control; HBB, hyoscine butylbromide; IV, intravenous; PD, pharmacodynamic; PK, pharmacokinetic; PO, oral.

### 4.1 | Pharmacokinetic analysis

HBB, after PO administration, showed a low absorption in plasma (<1%), compared with that following IV administration. This is attributed to the highly polar quaternary ammonium element in its structure (Tytgat, 2007). Moreover, HBB undergoes an extensive hepatic metabolism and is eliminated mainly by biliary excretion (Pentikäinen et al., 1973). The results of the present study confirm those of

previous studies which have reported a poor absorption (8%) and a low systemic bioavailability with PO HBB, making it difficult to detect blood levels at therapeutic doses of 30–60 mg (Outhoff, 2015; Samuels, 2009; Tytgat, 2007).

In this study, PO HBB showed a low bioavailability (0.01%) compared with atropine (5.46%) in plasma. These data and the  $C_{max}$  and  $T_{max}$  values for HBB observed in this study aligned with those reported in other studies (Sanofi, 2021; Corsetti et al., 2023). In

human participants, PO HBB demonstrated a rapid onset (15 min) and effectiveness and tolerability, with limited systemic side effects (Lacy et al., 2013; Samuels, 2009; Storr et al., 2022). Further, PO HBB (500 mg) yielded a maximum plasma concentration ( $C_{\max}$ ) of  $5 \text{ ng ml}^{-1}$  (11 nM) at a time to reach maximum concentration ( $T_{\max}$ ) of 0.25–1.5 h, with only 0.16% of the dose eliminated in urine. Following the IV administration of HBB (100 mg), the plasma  $C_{\max}$  decreased rapidly, and the terminal elimination half-life ( $t_{1/2}$ ) was 1–5 h (Tytgat, 2007). Other studies have reported even longer  $t_{1/2}$  values ranging from 6.2–10.6 h, following the PO administration of HBB at 100–400 mg doses (Corsetti et al., 2023; Sanofi, 2021), indicating a relatively longer duration of action, contributing to its efficacy in managing abdominal cramps and pain. Therefore, the PO form of HBB is preferred as it remains effective in the GI tract and limits systemic side effects due to poor absorption (Samuels, 2009). On the other hand, the IV form of HBB is used to treat acute spasm and colic when a fast relief is required in acute clinical settings (Sanofi, 2021).

Results of this study demonstrate that despite the very limited PO bioavailability of HBB, higher HBB levels were found in the CUB tissues than in plasma, suggesting a high affinity of HBB for these tissues. The study also showed that HBB was detectable for longer periods in CUB tissues than in plasma, especially after a PO administration, ranging from 24–48 h. Similarly, other reports also confirm that despite transient measurable low blood levels, HBB and/or its metabolites have been identified at the sites of activity (Samuels, 2009).

Findings of this study also showed that after the PO administration of HBB, only a minimal amount (0.03% of the dose) of unchanged drug was detected in urine over a 48-h period, while a notably higher percentage of the dose (10.9%) was present in faeces. After the IV administration of HBB, the values were 2.50% and 1.05% of the dose in urine and faeces, respectively. For atropine, in a similar trend over a 48-h period, the percentages of unchanged drug found in urine and faeces were 3.09% and 13.4%, after the PO administration, and 12.8% and 1.64%, after the IV administration, respectively. The comparison of the total amounts of unchanged drug excreted in urine for HBB after PO and IV administrations confirms the low oral bioavailability calculated from plasma data.

Similarly, in an earlier study in rats, after 24 h of the PO administration of radiolabelled HBB, about 6% radioactivity was found in the bile and 2% in the urine. In humans, 90% of radiolabelled HBB gets eliminated through faeces and 2% through urine (Hellström et al., 1970; Sanofi, 2021), which is in general agreement with the findings in the rat studies. This suggests that rats are a suitable model to determine the absorption and distribution of HBB in different tissues (Pentikäinen et al., 1973).

## 4.2 | Pharmacodynamic analysis

As high concentrations of HBB observed in non-GI tissues compared with that in plasma after PO might have PD relevance, it was necessary to establish a relationship between HBB tissue concentrations

and PD effects. Therefore, three different PD studies were conducted. As expected, this study showed that atropine was more potent than HBB and resulted in a greater spasmolytic activity than HBB in all CUB tissues. After PO and IV administration, the spasmolytic activity of atropine was greater than 80% in all tissues. In contrast, HBB showed greater spasmolytic activity through an IV administration than PO in the uterus and urinary bladder but not in the colon where it was similar between the two routes. These results are in line with previous studies. A previous in-vitro study suggested that the PO HBB accumulates in high concentrations in the human gut wall, near epithelial and smooth muscle and thus resulted in an anti-muscarinic action (Krueger et al., 2013). In particular HBB is known to have a high affinity towards the  $M_2$  and  $M_3$  muscarinic receptors on smooth muscles of the GI tract (Tobin et al., 2009; Zhang et al., 2016). In rats, after 24 h of PO administration, about 20% of the radiolabelled HBB was accumulated in the intestinal tissue exhibiting anti-cholinergic effects (Pentikäinen et al., 1973). Earlier studies have reported that the affinity of HBB to mucus and mucosal tissue, results in a slow return of responses after washing out HBB from the lumen of isolated intestinal segments (Krueger et al., 2013). Moreover, IV administration of HBB resulted in a faster relief of strong visceral spasms and pain, when compared to that after PO administration (Krueger et al., 2013). All these data could explain the prolonged action of HBB after PO administration compared with IV administration (Pennefather et al., 1968; Pomeroy & Rand, 1969).

Despite a low oral absorption, the spasmolytic effect of HBB in the colon reached up to 40%, in uterus it reached about 30% and that in the urinary bladder it reached 10%. Evidence from human in vivo studies showed reduced effectiveness in the uterus compared with the colon. HBB after IV administration resulted in a weaker and shorter inhibition of uterine contractions in contrast to the sustained suppression in intestinal movements (Daido et al., 2013). The efficacy of HBB in the rat uterus is due to the involvement of  $M_2/M_3$  receptors in myometrial contractions (Munns & Pennefather, 1998; Stjernquist & Owman, 1985; Xiao et al., 2009). Likewise, in the urinary bladder of rats and humans,  $M_3$  receptors are the primary mediators of contractions, despite the greater presence of  $M_2$  receptors (Abrams et al., 2006; Tong et al., 1997; Wang et al., 1995). This is similar to that observed in the colon where blockade of  $M_3$  receptors is associated with the pharmacological effects of HBB (Traserra et al., 2024). Other possible pharmacological targets are **nicotinic** receptors (Krueger et al., 2013). However, the blockade of nicotinic receptors only occurs at concentrations above  $10 \mu\text{M}$  and, in our experimental conditions, this concentration was never reached in tissues even after IV administration.

Probably because of natural hormonal fluctuations, a higher variability in data was observed in the uterus for both IV and PO administrations of HBB than in the other CUB tissues studied. Ovarian hormones have been implicated in influencing the expression of  $M_2$  and  $M_3$  receptors in the myometrium (Yasuda et al., 2014), and oestrogens, especially, have been shown to enhance the responsiveness to muscarinic agonists (Abdalla et al., 2004). Moreover, differences in the response to carbachol-induced contraction have been observed

between the oestrus and dioestrus phases of the reproductive cycle (Houdeau et al., 2003).

Previous reports confirm the effectiveness of anti-muscarinic agents for overactive bladder (Ito et al., 2009; Kay et al., 2005), although high doses of anti-muscarinic drugs can reduce contractility and eventually lead to urinary retention (Andersson, 2004). In the present study, a bladder filled with urine was consistently observed in the rats treated with atropine, whereas such occurrences were not observed in animals treated with HBB. This observation suggests that the more pronounced antispasmodic effect of atropine may exhibit more side effects than HBB, potentially affecting the normal functioning of the bladder. Certainly, earlier studies have showed urinary retention as a potential side effect associated with atropine (Dreijer et al., 2011).

### 4.3 | Pharmacokinetic/pharmacodynamic modelling

Based on the effectiveness of employing PK/PD model for spasmolytic drugs for optimising outcomes (Heetla et al., 2016), mechanism-based models were used that can pinpoint factors influencing drug effects over time, integrating in vitro and animal data to predict the efficacy and adverse responses of drugs in humans (Goto et al., 2019; Mager et al., 2009; Wong et al., 2017). For this reason, it was decided to add a PK/PD model study. Despite of the well-defined aims, the PK/PD study also had some limitations as it incorporates tissue homogenates composed of mixed cell types and fluids that do not represent the true drug dispersion within the tissue. Though the study assumes that the concentrations used in vitro are the same as those detected in vivo, there could be a part of the drug, which might not be available for pharmacological effects due, for instance, to protein binding (Mouton et al., 2008). Therefore, this could be the possible explanation as to why the data estimated in PD 1 and PD 2 studies (Table 2) are higher than those in PD 3. However, in the PD 3 study, the concentrations were not directly estimated in tissues, as the PD effect was estimated after drug administration. Moreover, the major limitation of the PD study 3 is that the PD effect is measured in tissues from treated animals, which could potentially result in drug dissociation from receptors and dilution when the tissues were placed in the bath. Therefore, to minimise this risk, the stabilisation time was shortened to 15 min in this protocol. This may also explain why spasmolytic activity in the PD study 3 is slightly lower than in the PD 1 and 2 study.

Another important issue is the potential time delay between receptor binding and pharmacological effect. In our model we use the tissue concentration as the reference value and therefore the potential delay between plasma concentration and receptor binding in tissue is already implicit in the model. On the other hand, the drugs have an immediate action at least in vitro, so we think the delay should be small. Finally, a small dissociation constant ( $K_{off}$ ) may be a factor that increases the duration of the pharmacological effect (Ren et al., 2022). For example, the muscarinic antagonist **tiotropium bromide** has a very

low  $K_{off}$  and causes the pharmacological effect to be maintained after washout (Barnes et al., 1995). This has been confirmed in binding studies in CHO cells expressing human  $M_3$  receptors, where the  $K_{off}$  of tiotropium was 0.0015/min, while that of atropine was 0.27/min and that of **N-methyl scopolamine** (0.017/min) (Dowling & Charlton, 2006). Unfortunately, HBB was not tested in this study, but in our experience in studies in vitro, HBB is extremely washable which is not the case for atropine and therefore a high  $K_{off}$ , indicative of a short time delay is expected for HBB.

Earlier clinical studies indicated the effectiveness of HBB in reducing the spasm outside the gastro-urinary/GI tract (del Valle-Laisequilla et al., 2012; Kemp, 1972) and post-operative pain (Sabetian et al., 2017). However, concerns were raised on the pharmacological plausibility of its effect on smooth muscles outside the GI tract, given the low oral bioavailability of HBB (Papadopoulos et al., 2014). The present study showed that HBB given PO can reach tissues outside the GI tract and exert a spasmolytic effect which may explain the efficacy of HBB in treating pain and spasm in non-GI organs.

The strengths of this study are as follows: (1) it is the first of its kind to investigate the PK and PD of HBB in GI and non-GI tissues (uterus and urinary bladder), (2) it is vehicle controlled and (3) it has a rigorous approach as the spasmolytic effect has been tested with three different PD studies. However, there is little information related to the mechanism responsible for the accumulation of the drugs in the tissues, which is a limitation of this study.

In conclusion, the PO administration of HBB in rats resulted in elevated concentrations in CUB tissues, relative to plasma, demonstrating varying spasmolytic potency across tissues, being most potent in the colon (40%), followed by the uterus (30%) and then the urinary bladder (10%). Furthermore, HBB exhibited sustained spasmolytic effects lasting approximately 24–48 h, depending on the tissue, suggesting potentially a prolonged therapeutic effect. The study confirms that there is a high tissue affinity and spasmolytic activity in non-GI smooth muscles, which further explains the therapeutic effect of HBB for spasm and related pain outside the GI tract.

### AUTHOR CONTRIBUTIONS

**S. Trasserra** – Equal: conceptualisation, data curation, formal analysis, methodology, supervision, validation, visualisation, writing—original, review and editing; Supporting: funding acquisition, project administration, resources and software; Lead: investigation. **T. Appelqvist** – Equal: project administration, supervision and writing—review and editing; Supporting: conceptualisation, data curation, formal analysis, funding acquisition, investigation, methodology, resources, writing—original draft. **R. Lange** – Supporting: All steps except writing—review and editing. **M. Corsetti** – Equal: conceptualisation, methodology, funding acquisition, supervision, writing—original, review and editing. **M. Jimenez** – Equal: All steps except supervision; Lead: Supervision.

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### CONFLICT OF INTEREST STATEMENT

Sara Traserra received salary from the Universitat Autònoma de Barcelona, which is funded by a grant from Sanofi. Terence Appelqvist and Robert Lange are currently employed by Sanofi and may hold shares and/or stock options in the company. Maura Corsetti is a consultant for Sanofi and co-chief investigator of a Sanofi-sponsored research grant. Marcel Jimenez received a research grant from Sanofi.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

### DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the BJP guidelines for [Design and Analysis](#), and [Animal Experimentation](#), and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

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## SUPPORTING INFORMATION

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