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Dog model with implanted pump to test boosters for antiretroviral medication

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Abstract

A dog model was developed to test the capacity of boosters for antiretroviral medication. Two dogs were implanted with a modified constant-flow Codman 3000® infusion pump, adapted to release viscous solutions of darunavir (TMC114) at a constant rate of 25 mg/dog/day in the venous blood stream. Booster candidates were given by oral gavage for at least 4 days up to maximum 7 days in cross-over fashion, separated by a wash-out period of minimum 1 week. The booster candidates were tested at doses of 20 and/or 40 mg/kg/day: blood sampling for determination of the boosting effect was performed on the last day of booster administration. The model allowed to (1) compare the boosting ratio of these booster candidates based on the exposure (determination of the area under the curve (AUC) of darunavir in presence versus absence of the booster candidate), (2) detect delay in boosting activity by evaluation of the shift of C_{max} of darunavir following booster administration versus the C_{max} of the booster candidate) and (3) calculate the intrinsic booster capacity, by correcting for the systemic exposure of booster candidate by normalizing the booster ratio for the booster's AUC. The latter parameter (intrinsic booster capacity) allows to determine the booster's metabolic contribution in inhibiting the metabolism of antiretroviral medication (most likely via inhibition of CYP3A4), minimizing the impact of potential effects of the booster at the level of the gastro-intestinal tract.

Keywords: Infusion pump; HIV; Protease inhibitor; Darunavir; Booster; Experimental

1. Introduction

Adherence to HIV treatment is crucial to viral suppression and outcome of HIV infection and a cause of drug resistance (Clotet, 2004; DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents, 2006; Sethi et al., 2003). Yet, according to current recommendations, several anti-HIV medications with a different activity profile are optimally combined for treatment—often referred to as highly active antiretroviral therapy (HAART) in order to maximally and durably suppress the viral load. Hence, patients are often subject to a considerable pill-burden, as well as complex dose regimen—some of the medications to be taken with, others without food, which may further enhance the problem of non-adherence. Not surprisingly, HIV viral suppression, reduced rates of resistance and improved survival have been correlated with high rates of adherence (Sethi et al., 2003; Wood et al., 2003; Bangsberg et al., 2004). The role of medication adherence has also been well documented by the improved outcome following adjustment of adherence (Wood et al., 2003; Rathbun et al., 2005).

In order to eliminate compliance problems, we recently developed an implantable constant-flow infusion pump for

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sustained delivery of highly viscous antiretroviral solutions in the venous blood stream (Baert et al., 2008). In a pilot study in dogs, we showed that – when filled with a viscous solution of protease inhibitor – this delivery system allows to achieve sustained steady-state plasma concentration levels within 1 day. We also demonstrated that during continuous infusion with this device, reproducible plasma concentration levels were obtained for 1 month or longer, also after refill of the pump's bellows reservoir through the large, domed, single-septum access of the pump. The pump design allows thus secure needle retention for bolus injections, thereby minimizing the risk for infections. This is important to the immunocompromised HIV patient who is at high risk of this life-threatening complication. As the pump is fully implantable, it also allows patients to move freely around. The propellant changes from a liquid to a gas at body temperature and exerts a constant pressure on the exterior surface of the reservoir, thereby forcing the drug solution at a constant rate through the filter, flow restrictor and catheter. This type of pump, already applied for pain and other therapies (Codman® Model 3000 Series), can function several years, and therefore, when regularly filled, may translate into considerable benefits in HIV treatment. These include (1) the obvious improvement of adherence, not only by improving direct medication compliance, but also by decrease in the complexity of HAART, (2) a potential decrease in dose and side effects due to less peak-trough variations, and indirectly (3) improvement of outcome of antiretroviral treatment, with (4) a simultaneous decrease in risk for development of viral resistance. The further testing of these pumps is thus highly desirable for human application in the future.

Protease inhibitors are often metabolized by *iso*-enzyme Cytochrome P-450 3A4 (CYP3A4). Inhibition of such an enzymatic metabolisation would improve the exposure and therefore the pharmacokinetic profiles of these compounds. Co-administration of ritonavir (used at subtherapeutical doses) is a common practice in HIV therapies due to the very potent CYP3A4 inhibition properties of this molecule (Clotet, 2004).

The objective of this experimental study was to investigate the effect of various oral booster candidates on the pharmacokinetic profile of darunavir (TMC114) given as a continuous infusion through a modified Codman® 3000 pump implanted in dogs. Darunavir is a newer HIV-1 protease inhibitor, which has recently been developed for co-administration with low-dose ritonavir, registered in a number of countries including the USA (Tibotec, 2006). Darunavir was designed to be active against both wild-type and multi-resistant virus (Koh et al., 2003). In clinical studies in treatment-experienced patients with HIV-1 strains resistant to more than one protease inhibitor, it has been shown to result in substantial greater virological and immunological benefits compared with the standard of care (POWER 1 and 2 study groups, 2007; POWER 2 Study Group, 2007; Katlama et al., 2007). Several new booster candidates in development by Tibotec were tested. An additional objective was to discriminate between their contribution via systemic exposure and their intrinsic metabolic capacity.

2. Methods

2.1. Adapted Codman® 3000 pump

Indwelling central venous catheters are commonly used in patients with neoplastic disease to administer chemotherapy, blood products, antibiotics, and parenteral nutrition. They however entail a high risk for the HIV patients, due to potentially life-threatening infections entering via the external access. An alternative for administering medication is the fully implantable pump. In the case of anti-HIV medications, these pumps should allow to contain large volumes, to release high viscous solutions in view of the difficult dissolution properties of many of these agents in aqueous solutions, and to release these at a constant rate to guarantee minimally effective concentrations for inhibition of the virus. We therefore adapted a Codman 3000® pump (commercially available from Codman & Shurtleff of Raynham, Massachusetts, USA): this pump is normally targeted to release aqueous solutions with viscosities close to that of water (0.7 mPa s or cp) (Baert et al., 2008). This implantable constant-flow infusion pump is provided with a bolus safety valve and a bellows reservoir pump, surrounded by chamber with propellant: the original pump, used for spinal delivery of pain medication (Codman® Model 3000 Series), was adapted in internal diameter of the capillary flow restrictor (increased from 0.05 to 0.1 mm) and in length of catheter, in order to allow a preset constant release rate of 0.5 mL/day of viscous solutions (24 mPa s) at 39 °C (= dog's body temperature) in the venous bloodstream (vena cava).

2.2. Anti-HIV medications and booster candidates

Darunavir, the antiretroviral lead compound TMC114 (Tibotec), is a protease inhibitor, which was in late phase III development stage at time of testing and has recently been approved for oral administration in combination with low-dose ritonavir in several countries, including the USA (Tibotec, 2006). Darunavir was dissolved in 56 mL of polyethylene glycol (PEG 400) (α-Pharma, Belgium) under stirring overnight, resulting in a mixture of 70/30 (w/w) PEG 400/water for injection after adding 24 mL of sterile water (Braun, Belgium) under stirring. The solution was filtered over a 7 μm surfactant-free cellulose acetate (SFCA) membrane filter (Sartorius, Belgium) and 30 mL aliquots of the final solution were transferred into sterile vials (Medipac, Germany) for steam-sterilization (20 min at 120 °C) (Auto Koch Steam Sterilizer, PBI International, Italy) and administration. The final concentration in solution was equal to 50 mg/mL, to allow a daily administration of 25 mg/dog/day at a targeted outflow rate of 0.5 mL/day from the pump. This solution had been found stable over an 8 weeks period at 37 °C.

The booster candidates were all protease inhibitors in research development with Tibotec: TMC00183241 and TMC00372975 are the isomers of TMC41629.

Booster candidates were prepared in bulk solution, each at a concentration of 20 mg/mL: they were each dissolved in PEG 400 under stirring overnight, and butylhydroxytoluene (Merck, Darmstadt, Germany) was added as antioxidant (free radical

scavenger); the solution was held in a closed container. Afterwards, the solution was filtered with an 8 μm filter and stored in amber glass bottles. They were administered to the dogs by oral gavage, thereby adapting the fill weight to the dose mentioned in the specified protocol.

2.3. Design of the in vivo studies

In order to minimally expose animals to experimental procedures, in line with European Council Directives (1986) and European Commission's Protocol on the protection and welfare of animals used for experimental and other scientific purposes (2007 online), a series of small studies were performed in two male Beagle dogs with implanted pumps, exposing them to the least number of blood samples possible in each of the experiments (dog 1 (18,792) and dog 2 (20,024)). The animals were housed in cages without restrictions in food or drinking.

In order to explore the utility of the adapted Codman® 3000 pump for continuous anti-HIV drug delivery, the pumps had been previously implanted subcutaneously on the right flank and fixed intermuscularly to provide stability (Baert et al., 2008). The indwelling catheter of the pump was placed in the caudal vena cava via the femoral vein.

The dogs received continuous infusion with darunavir solution at a dose of 25 mg/dog/day (corresponding to approximately 1.7 mg/kg/day) or placebo (70% PEG 400/30% water for injection). The pumps were refilled every 4–5 weeks up to 16 mL of darunavir or placebo, by direct injection through the septum in the pump's reservoir. Placebo was used during the surgical implantation or experiment-free intervals, in order to avoid the pumps of running dry. An initial utility study (Baert et al., 2008) showed reproducibility of the sustained darunavir plasma levels after refill of the pump.

A further pilot experiment had shown that 10 mg/kg/day of TMC41629 could be detected, but the boosting activity of this dose was fairly low. Therefore, three subsequent experiments assessed the effect of the oral booster candidates at doses of 20 and/or 40 mg/kg/day, while the two dogs were maintained on constant darunavir infusion, further referred to as studies 1, 2 and 3. The booster candidates were always administered on the same time of the day, at 0 h of the darunavir baseline. This time point coincided with the first blood sample taken just after the refill of the pump with darunavir at the start of an experiment. Timing of other blood samples was set per experiment, each time allowing for the minimum number of 2 mL blood samples as needed and indicated per study below.

2.3.1. Study 1

After refilling the pumps with darunavir, a baseline period of 7 days followed before initiation of oral booster for another 7 days: blood samples were drawn every 24 h at time 0. TMC41629 was tested at a dose of 40 mg/kg/day. It was opted to study the booster effects after repeated administration. Therefore, following administration of the booster, repetitive blood samples were taken on the first and last day of the 7-day booster administration: blood plasma concentrations were determined at 1, 2, 4, 6 and 8 h on days 1 and 7. Additional blood samples were taken at 8 and 24 h on days 2–4, at 24 h during the weekend (days 5 and 6), and at 24 h after the last dose. During the booster administration, both the darunavir and booster plasma concentration levels were determined. Based on this study, a final booster model was developed with booster sessions in cross-over fashion, usually lasting 4 days and up to maximum 7 days in the case of intervening weekends. Further, to exclude any carry-over effects due to enzyme induction or metabolic inhibition between the booster sessions, the sequence of the booster candidates was alternated between the two dogs and the booster sessions were separated by a booster wash-out period lasting minimum 1-week. This approach was applied in studies 2 and 3.

2.3.2. Study 2

In order to assess a new booster candidate TMC00183241, an isomer of TMC41629, each dog received two 4-day booster sessions in cross-over fashion, one with TMC00183241 and one with the parent racemic mixture TMC41629, both dosed at 20 mg/kg/day, by daily oral gavage (alternating the sequence of administration between the dogs). The latter dose was selected to obtain additional information of TMC41629's boosting activity at this intermediate dose, its effect already assessed at 10 mg/kg/day in an initial pilot study and at 40 mg/kg/day in study 1.

On Day A, the pumps of both dogs were refilled each with 16 mL of darunavir solution resulting in a dose of 25 mg/dog/day at an outflow of 0.5 mL/day. After 8 days of continuous infusion of darunavir in order to set the baseline, one dog received TMC41629, the other dog TMC00183241 for 4 consecutive days. After the wash-out period of 10 days, the dogs were crossed-over to the other booster candidate. Pharmacokinetic profiles of darunavir and the booster candidates were determined as in study 1, but daily sampling was reduced to one sample at 0 h, except for the last day during darunavir baseline (the day before booster administration) and for day 4 of the booster session, where sampling occurred at 0, 1, 2, 4 and 8 h.

2.3.3. Study 3

The aim of this study was to compare the pharmacokinetics of darunavir after repeated administration of two new booster candidates, TMC00427473 and TMC00372975, administered at a dose of 20 mg/kg/day by oral gavage to the two dogs. The experimental set-up was the same as in study 2, with 4 days of booster exposure and 10 days of wash-out.

2.4. Determination of drug concentrations

The concentrations of darunavir and booster candidates in the solutions were determined using standardized HPLC-analyses. Their plasma concentrations were determined using standardized qualified research LC–MS/MS methods. Blood samples were collected in a vacutainer on EDTA as coagulator (EDTA Vacuette Greiner, Cat No. 454087). Samples were placed immediately on melting ice and plasma was obtained following centrifugation at 4 °C.

Table 1
Studies of the effect of oral booster candidates given to dogs ($n = 2$) during steady state (SS) by continuous infusion with the protease inhibitor darunavir (25 mg/dog/day equalling ~1.7 mg/kg/day) via an implanted constant-rate infusion pump

Study design and duration of booster administration[a]	Booster (oral protease inhibitor)	Daily dose of booster (mg/kg/day)	PK-parameters of darunavir in two dogs[a], individual values: [*Left*] dog 1 – [*Right*] dog 2					Booster ratio[b], Dog 1–2
			Before booster administration		After booster administration			
			C_{ss} (ng/mL)	$AUC_{0-24\,h}$ (ng·h/mL)	C_{max} (ng/mL)	t_{max}[c] (h)	$AUC_{0-24\,h}$ (ng·h/mL)	
Study 1 (open)								
- Baseline: 7 days								
- 7 days booster	TMC41629	40	66–40	1574–950	340–179	4–4	5180–2884	3.3–3.0
Study 2 (open, cross-over)								
- Baseline: 8 days								
- 4 days each booster	TMC41629	20	60–30	1450–710	316–145	6–2	6029–2109	4.2–3.0
- Wash-out: 10 days	TMC00183241	20	60–39	1450–926	318–84	8–8	4553–1447	3.1–1.6
Study 3 (open, cross-over)								
- Baseline: 8 days								
- 4 days each booster	TMC00427473	20	97–50	2316–1210	320–124	6–6	6325–2151	2.7–1.8
- Wash-out: 10 days	TMC00372975	20	85–60	2047–1457	313–101	6–24	5605–2053	2.7–1.4

[a] In cross-over studies, the sequence of administration of the two boosters was reversed in the two dogs, separated by at least 1-week wash-out period per protocol (10 days in the actual experiments): the value on the left hand side is the value obtained in dog 1 (18,792) and the value on the right hand side is from dog 2 (20,024).
[b] Booster ratio is the ratio between the AUC of darunavir (TMC114) with booster divided by the AUC of darunavir without booster co-administration.
[c] For delay in boosting effect versus t_{max} of the booster, see Table 2.

2.5. Data analysis

Peak plasma concentrations (C_{max}), the corresponding peak times (t_{max}) and area under the curve (AUC) values were determined for darunavir and the booster candidates. These pharmacokinetic parameters were determined for darunavir, (1) without the booster candidate, using the plasma concentration values of darunavir obtained during continuous infusion prior to its administration, and (2) on the last day of booster administration (days 4–7, see Section 2.3). The pharmacokinetic parameters of the booster candidate were simultaneously determined with the latter blood samples.

Individual plasma concentration versus time profiles were subjected to a non-compartmental pharmacokinetic analysis using validated WinNonlin software v4.0.1a (WinNonlin 4.0.1a Enterprise, Pharsight Corporation, Mountain View, California, USA). The area under the plasma concentration-time curve from time 0 to time 24 ($AUC_{0-24\,h}$) was calculated using the linear up/log down trapezoidal method.

As two dogs were used per experiment, the individual pharmacokinetic parameters of darunavir and the booster used are given per experiment.

2.6. Calculation of booster ratio and intrinsic booster capacity

The booster ratio was defined as the $AUC_{0-24\,h}$ of darunavir following the last intake of the oral booster candidate, divided by the baseline $AUC_{0-24\,h}$ of darunavir during constant-flow infusion before administration of the booster candidate.

To calculate the intrinsic booster activity or capacity, this boosting ratio was corrected for the systemic exposure ($AUC_{0-24\,h}$) of the booster candidate at the last intake, by dividing the booster ratio as defined above by the $AUC_{0-24\,h}$ of the

Table 2
PK-characteristics of the booster candidates (dosed at 20 or 40 mg/kg/day) in presence of darunavir (25 mg/dog/day equalling ~1.7 mg/kg/day) on the last day of their administration: given in cross-over fashion with ~1 week of wash-out between both study periods

Booster (protease inhibitor) dosed at 20 mg/kg/day	Dose of booster treatment (mg/kg/day)	PK-characteristics in presence of darunavir (10 mg/kg/day), last day of booster[a]					
		Dog 1 (18,792)			Dog 2 (20,024)		
		C_{max} (ng/mL)	t_{max} (h)	$AUC_{0-24\,h}$ (ng·h/mL)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0-24\,h}$ (ng·h/mL)
Study 1							
TMC41629	40	11,000	2	41,889	5,520	1	23,301
Study 2							
TMC41629	20	9,950	2	40,650	5,150	1	14,224
TMC00183241	20	15,600	2	117,181	7,680	2	45,366
Study 3							
TMC00427473	20	7,740	4	114,937	3,690	2	22,050
TMC00372975	20	16,200	2	159,570	10,900	2	88,125

[a] Day 7 of booster administration in study 1, days 4 in studies 2 and 3.

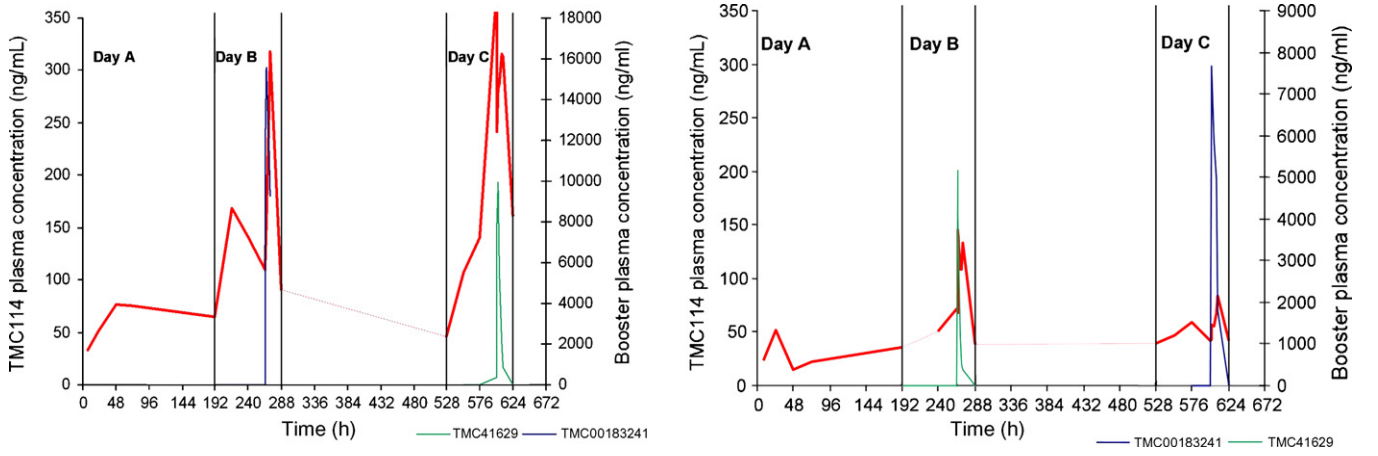

Fig. 1. Pharmacokinetic (PK) profiles of darunavir (TMC114—red bold line)[1] in two dogs, receiving a continuous infusion of darunavir via implanted constant-flow pump during baseline (Day A till Day B) and two oral booster sessions started on Days B and C in cross-over fashion, separated by a 10-day wash-out period. The sequence was alternated between the two dogs: [*Left*]: dog 1: sequence TMC00183241–TMC41629; [*Right*] dog 2: sequence TMC41629–TMC00183241.[2]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

respective candidate: values were expressed at the minus fifth exponential level ($\times 10^{-5}$). Results are further presented per booster, using the mean value of the booster ratios obtained in the two implanted dogs.

3. Results

The results of the booster potential found in the three studies are tabulated in Table 1. The pharmacokinetic parameters of the booster candidates relevant to systemic exposure are given in Table 2. The results obtained with 20 mg/kg/day of the booster candidates are illustrated in Figs. 1 and 2.

3.1. Study 1

Following oral administration of TMC41629 40 mg/kg/day, systemic exposure of darunavir was increased, with plasma darunavir AUCs increasing by about three-fold and the maximum plasma values increasing five-fold. During the steady-state baseline, mean plasma concentrations of darunavir were 66 and 40 ng/mL in dog 1 and 2, respectively; $AUC_{0–24\,h}$ values were 1574 and 950 ng·h/mL, respectively (Table 1). After administration of the booster, C_{max} values were 340 and 179 ng/mL, respectively, and the AUC values determined up to 24 h after the last dose ($AUC_{0–24\,h}$) were 5180 and 2884 ng·h/mL, respectively.

Following single-dose administration, the peak plasma concentrations of TMC41629 itself were observed at 2 h post-dose, indicating rapid absorption of the booster and thereby reaching 8860 ng/mL to 11,400 ng/mL (sequence of writing: dog 2–dog 1), with $AUC_{0–8\,h}$ values ranging from 35,767 to 59,712 ng·h/mL, respectively. Yet, at steady state (day 7), peak plasma concentrations were 5520 and 11,000 ng/mL, and the $AUC_{0–8\,h}$ values 20,810 and 40,490 ng·h/mL, respectively, indicating a 30% decrease versus day 1 of the booster and suggesting metabolic induction. The $AUC_{0–24\,h}$ values were 23,301 and 41,889 ng·h/mL, respectively (Table 2).

3.2. Study 2

In this study, the boosting effect of TMC41629 and its isomer TMC00183241 were confirmed at 20 mg/kg/day. Table 1 gives the C_{max} and the $AUC_{0–24\,h}$ values. TMC41629 boosted the systemic exposure of darunavir by a factor of 3.0 (given 1st in dog 1) to 4.2 (given as 2nd booster in dog 2) at 20 mg/kg/day. There was some delay in boosting effect, as C_{max} values were achieved at 2–1 h for TMC41629 versus 6–2 h for darunavir (Table 1). The low t_{max} value indicated fast absorption of the booster. The other booster candidate TMC00183241 had a lower boosting capacity, enhancing the systemic exposure by 3.1–1.6 only in both dogs. This isomer was similarly fast absorbed (achieving its C_{max} at 2 h in both dogs), and had a higher $AUC_{0–24\,h}$, suggesting delayed boosting activity and considerably lower intrinsic capacity to inhibit the metabolism of darunavir (Table 2).

3.3. Study 3

After monitoring the baseline darunavir levels for 8 days following pump refilling with no evidence of significant variability, both protease inhibitors tested led to similar booster ratios in the two dogs, independently of their sequence of administration (Fig. 3; Table 1). Administration of TMC00427473 resulted in an increased systemic exposure of darunavir by a factor of 2.7

[1] *Day A*: Pumps were re-filled with darunavir, followed by plasma concentration determination on days 2, 3, 4 and 8 to determine the baseline TMC 114 concentration (without co-administration of booster) after filling the pump. *Day B*: Dogs started the first 4-day booster session (TMC00183241 in dog 1, TMC41629 in dog 2). *Day C*: Dogs started the second 4-day booster session (TMC41629 in dog 1, TMC00183241 in dog 2).

[2] Plasma concentrations of the individual boosters on the last day of the 4-day booster sessions (green line: TMC41629; blue line: TMC00183241), assessed at 0 h on days 1–3 and at 0, 2, 4, 6, 8 and 24 h of day 4.

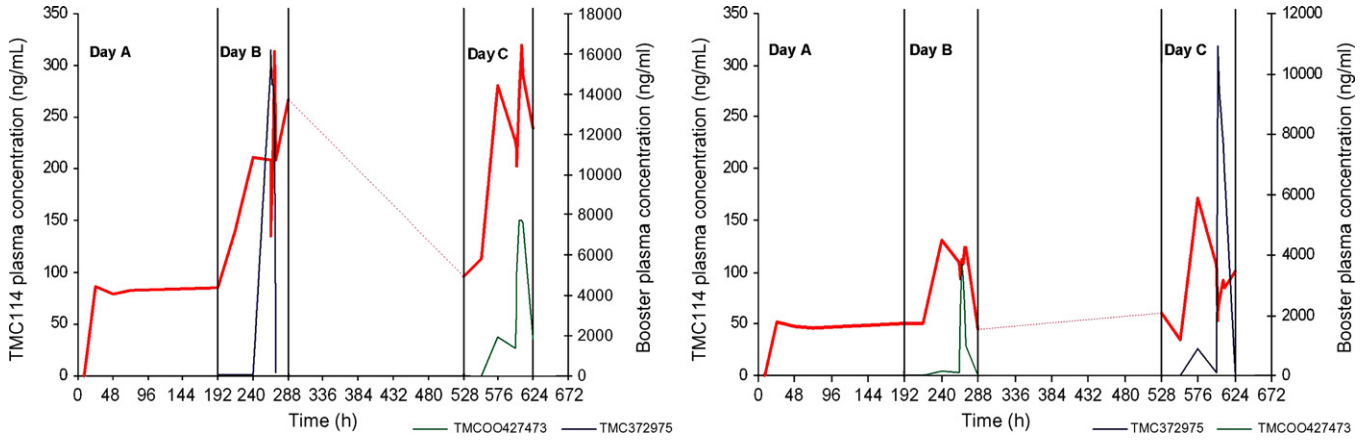

Fig. 2. Pharmacokinetic profiles of darunavir (TMC114—red bold line)[3] in two dogs, receiving a continuous infusion of darunavir via implanted constant-flow pump during baseline (Day A till Day B) and two oral booster sessions started on Days B and C in cross-over fashion, separated by a 10-day wash-out period. The sequence was alternated between the two dogs: [*Left*]: dog 1: sequence TMC00372975–TMC00427473; [*Right*] dog 2: dog 2: sequence TMC00427473–TMC00372975.[4]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.).

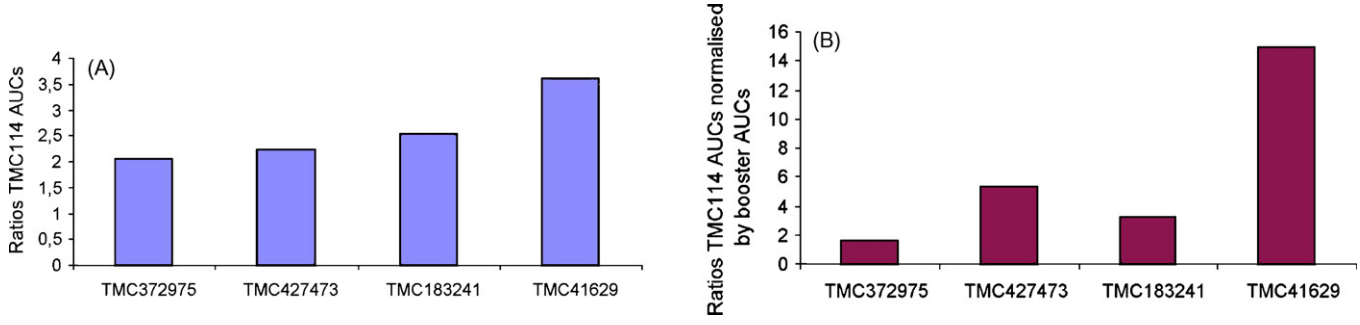

Fig. 3. Boosting capacity of different protease inhibitors administered by oral gavage to dogs ($n = 2$) receiving a continuous venous infusion of the protease inhibitor darunavir (TMC114) 25 mg/dog/day via an implanted adapted Codman® 3000 pump[5]: (A) unweighted for systemic exposure,[6] (B) corrected for the systemic exposure of the booster candidate[7]: "intrinsic boosting activity".

(dog 1, booster 1st in sequence) to 1.8 (dog 2, booster 2nd in sequence) (Table 1), while administration of the booster candidate TMC00372975 led to a similar booster ratios, being 2.7 (dog 1, booster 2nd in sequence) and 1.4 (dog 2, booster 1st in sequence), respectively. Yet, as can be deduced from Table 2, which lists the p*K*-values of the boosters, the systemic exposure, expressed as $AUC_{0–24\,h}$, was found to be lower for TMC00427473 than for the other booster candidate. Calculation of the intrinsic booster activity indicated that TMC00427473 had a better intrinsic ability to inhibit CYP 3A4 metabolism than TMC00372975.

In addition, the differences between t_{max} values for darunavir (>6 h) compared with the boosters (2–4 h) indicated a delayed boosting effect.

3.4. Intrinsic boosting capacity

Fig. 3 shows the boosting capacity of the boosters before and after correction for their systemic exposure. In terms of boosting activity, TMC41629 appeared clearly to be the best compound to improve the darunavir pharmacokinetic parameters, followed closely by the three other compounds. However, after correction for the booster's exposure, TMC41629 showed to have the highest 'intrinsic boosting activity' or intrinsic capacity in improving darunavir plasma concentrations.

4. Discussion

This experimental model using sustained infusions of antiretroviral medication via an implanted pump in dogs, thereby

[3] *Day A*: Pumps were re-filled with darunavir, followed by plasma concentration determination on days 2, 3, 4 and 8 to determine the baseline TMC concentration (without co-administration of booster) after filling the pump. *Day B*: Dogs started the first 4-day booster session (TMC00372975 in dog 1, TMC00427473 in dog 2). *Day C*: Dogs started the second 4-day booster session (TMC00427473 in dog 1, TMC00372975 in dog 2).

[4] Plasma concentrations of the individual boosters on the last day of the 4-day booster sessions (green line: TMC00427473; blue line: TMC00372975), assessed at 0 h on days 1–3 and at 0, 2, 4, 6, 8 and 24 h of day 4.

[5] Mean values of two dogs.

[6] Boosting capacity expressed by the ratio of the baseline darunavir plasma $AUC_{0–24\,h}$ with co-administration of the orally given booster candidate over the darunavir $AUC_{0–24\,h}$ without co-administration of the booster candidate.

[7] Intrinsic boosting capacity: AUC-normalized boosting activity = boosting capacity as expressed above, divided by the AUC value of the booster, thereby giving a better indication of the intrinsic metabolic inhibition capability of the booster (expressed as 10^{-5}).

using the protease inhibitor darunavir in this case, proved to be a useful model to detect booster activity of other orally administered booster candidates. The model proved to be sensitive for:

(1) detection of booster activity of antiretroviral medication, also allowing to detect dose-dependent features and delayed booster effects: this is possible because the constant flow of the pump allows to determine easily and reliably the impact of plasma levels of the booster on the sustained plasma levels of the pump-released medication, this with a minimal number of animals and blood samples;
(2) interactions between boosters and the anti-HIV agent due to hepatic metabolic effects, as the steady state of the darunavir baseline plasma concentration was reached within the first 2 days after pump refill and booster effects can be studied subsequently in an effective, reliable way;
(3) discrimination of the intrinsic booster efficacy by elimination of the impact of systemic exposure via correction for the AUC of the booster.

As was shown by the AUC-values obtained after administration of booster candidates (20 mg/kg/day), darunavir was boosted mostly by TMC41629, followed by TMC00183241, TMC00427473. However, as also shown by Figs. 1 and 2, the systemic exposure (the resultant from absorption and/or metabolic turnover of the booster) seemed to be quite different between the booster candidates. The correction of the booster capacity by taking the $AUC_{0-24\,h}$ of the respective booster candidates into account showed that the intrinsic boosting activity was lower for the latter three boosters.

As the effects are fully consistent between the two dogs for all boosters tested, the implanted adapted constant-flow Codman® 3000 pump in dogs may prove to be a reliable test system for screening boosters of anti-HIV medication, while minimizing the animals' exposure to handling for dosing and painful procedures. In view of the importance of boosters in anti-HIV treatment to combat resistance of the ever mutating virus—with HAART currently recommended as standard approach (Clotet, 2004; DHHS Panel, 2006), this experimental set-up may likely become an interesting research tool in the search for effective treatment to combat HIV in human.

Although the model allows to assess predominantly the hepatic contribution of the booster (minimizing the intestinal contribution), the model does not allow to distinguish the mechanisms responsible for boosting or delayed boosting. The delayed boosting effect can be due to the intrinsic pharmacokinetics of the booster candidates itself and/or the intrinsic mechanisms of inhibition. Moreover, as can be expected from *in vivo* experiments, the exact hepatic mechanism of inhibition – most likely to occur at the level of CYP – cannot be elucidated, *in vitro* assessments being the usual approach.

The dog model also differs from the standard model that we have used for studying booster candidates for darunavir in our laboratories, using the New Zealand rabbit. This model was chosen because – as in human – the impact of ritonavir on the darunavir exposure is very high in the rabbit when compared with other species and because the metabolic pathways in the rabbit are close to human. The dog model differs in many ways:

(1) In the rabbit model, both darunavir and the booster candidate were administered orally. In the dog model, the anti-HIV agent was given by continuous infusion, while the booster candidate was given orally. Hence, the dog model allows to minimize the impact of interactions of booster and anti-HIV agent at the level of the gastro-intestinal tract, such as effects on absorption or metabolic effects on gastro-intestinal CYP3A4 following oral intake of both drugs. Although gastro-intestinal effects cannot be fully excluded because darunavir undergoes enterohepatic cycling, this impact is minimized: in the rat, 54% of an oral radioactive darunavir load undergoes enterohepatic circulation during 24 h, but only 1% of the fraction is unchanged darunavir (Tibotec, data on file; no data in rabbits and dogs). The dog model thus allows to estimate mainly the booster's effect on the hepatic metabolism of the infused anti-HIV agent.
(2) On oral co-administration of the anti-HIV agent and a booster, plasma levels fluctuate between peak and trough levels, being the result of the fluctuations in plasma profile of both the anti-HIV agent and the booster. Moreover, plasma concentrations of the anti-HIV agent may possibly be higher due to their impact on the intestinal barrier. In the dog model, sustained plasma levels of the anti-HIV agent were achieved via continuous infusion and the rise in darunavir plasma level was caused by the booster only. This model may therefore also prove relevant when developing the application of the pump for anti-HIV treatment in human. If the plasma concentration level is kept stable above minimum inhibitory levels below which the virus is able to replicate or mutate, the risk for rebound of the infection or induction of resistance may be reduced. There is ample evidence that trough plasma levels of anti-HIV agents are predictive to response; maintaining them above certain plasma levels has proven to improve virologic response and to reduce rebound of the infection (PENTA 5 Study Group, 2004; Duong et al., 2005; Gatti et al., 2002; Torti et al., 2005; Wu et al., 2006).
(3) In the rabbit model, we usually perform single dose experiments – one without and one with co-administration – for ranking of the booster candidates. The dog model evaluated the effect of repeated administration of the booster candidates during a continuous infusion with the anti-HIV agent—darunavir in this case. As the dog is receiving continuous delivery of the anti-HIV agent and as many anti-HIV agents, such as darunavir, affect the liver's metabolic capacity due to direct effects on enzymes (Lachau-Durand et al., 2005), this model allows to eliminate potential acute effects of the anti-HIV agent, as well as to take into account the impact of metabolic induction during chronic administration. The model is thus also closer to human use in the respect that booster effects are studied during chronic administration.
(4) Last but not least, species may differ among each other with respect to anatomy of the gastro-intestinal tract, drug

absorption and metabolism (de Zwart et al., 1999). As a result, pharmacokinetics of anti-HIV agents during booster co-administration may differ. This has been shown in a preclinical study, studying the effect of oral chronic co-administration of ritonavir (different doses) and darunavir on the pharmacokinetics of darunavir in the rabbit, dog and other species (Lachau-Durand et al., 2005). Systemic exposure of darunavir during ritonavir co-administration was not increased but inconsistent in dogs, while increased similarly in human and rabbits. The effect was modest in mice and rats. Moreover, autoinduction effects of darunavir were particularly high but prevented by co-administration of ritonavir in rats.

(5) The ranking of the booster candidates may therefore differ between the models. The dog model thereby allows to better estimate the booster ratio and intrinsic booster capacity of the booster candidate via its metabolic contribution.

In conclusion, we developed a dog model using continuous infusion of an anti-HIV agent which allows to test the intrinsic booster capacity of booster candidates, thereby determining primarily their metabolic contribution and allowing to correct for the impact of their systemic exposure.

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