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Structural determinants of nervous system exposure of adibelivir (IM-250) and related herpes helicase-primase inhibitors across animal species

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ABSTRACT

The high incidence and prevalence of herpes infections pose a significant health burden worldwide. Herpes simplex virus infections are the cause of herpes labialis, genital herpes or herpes keratitis and in rare cases life-threatening herpes encephalitis, meningitis or disseminated disease. After primary infection, herpes simplex viruses (HSVes) establish latency in the trigeminal and sacral ganglia and at least 30 % of patients experience clinically manifestant recurrences for life. For effective treatment of these neurotrophic HSVes, adequate drug exposure in the nervous system is essential.

Here we report the post administration exposure of structurally different helicase-primase inhibitors (HPIs) in plasma, blood, organs and, in particular, the nervous system of animals by HPLC/MS. In diverse animal species, after single or multiple doses of helicase-primase drugs by oral or intravenous administration, only adibelivir (IM-250) achieved concentrations in the nervous system in the range of plasma or blood levels (ratio 0.5 to 4 nervous system/plasma), while other helicase-primase inhibitors with distinct structures, including amenamevir, pritelivir or ABI-5366, showed a low brain/plasma ratio of less than 0.1. The efficient passage of helicase-primase drugs through the blood-brain and blood-nerve barrier is based on their distinct structure and chemical properties. In preclinical studies published so far, adibelivir was efficacious in the herpes encephalitis and neonatal animal model and reduced the reactivation competence of the neuronal latent herpes viral reservoir. Ongoing clinical trials with HPIs will show whether sufficient drug exposure in brain and ganglia will translate into more effective herpes therapies for patients.

1. Introduction

Herpesviruses coevolved with the Animalia kingdom. The quiet pandemic of herpesviruses causes a significant health burden worldwide. The majority of people are infected with at least one strain of the herpes-virus family (HHV-1 to HHV-8). The alpha herpesviruses (HHV-1 to HHV-3), herpes simplex type-1 (HSV-1 or HHV-1) and −2 (HSV-2 or HHV-2) and varicella zoster virus (VZV or HHV-3), are neurotrophic. In the general population, the prevalence of HSV-1 exceeds 60 %, and infection rates of HSV-2 are in the range of 15–30 % (Howley and Knipe, 2023). HSV infections cause a variety of diseases such as genital herpes, herpes labialis (cold sores), sight-impairing keratitis, and, less frequently, potentially life-threatening disease (HSV encephalitis or generalized disseminated viremia including hepatitis and pneumonia) mainly in immunocompromised patient populations, e.g. transplant recipients, patients with an inherited immunodeficiency and newborns.

During primary infection, neurons of the trigeminal or dorsal root sensory ganglia are infected and episomal HSV DNA persists for life in a latent state in nuclei of the infected nerve cells. At least 30 % of patients experience periodic reactivation in response to diverse stimuli leading to shedding of contagious infectious virus and recurrent disease (Howley and Knipe, 2023). Idoxuridine and trifluridine were the first drugs used in antiviral therapy (De Clercq and Li, 2016). These nucleosides were launched in the 1960s and 1980s for the topical treatment of herpes keratitis but posed safety concerns. Vidarabine was approved for systemic therapy of herpes encephalitis. The milestone in antiviral therapy, acyclovir (ACV), has a remarkable safety profile but moderate efficacy (De Clercq and Li, 2016; Kleymann, 2005). Systemic administration of ACV has been the gold standard for herpes simplex and varicella zoster therapy since its approval in 1981. Prodrugs of ACV and its congener penciclovir (PCV), namely, valacyclovir (VACV) and famciclovir (FCV), were designed to improve systemic exposure after oral administration. These prodrugs were introduced in the 1990s and allow for more convenient dosing of once or twice daily. ACV in comparison requires

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administration three to five times a day. In cases of herpes encephalitis or neonatal herpes, intravenous (i.v.) ACV must be administered to ensure adequate cerebral exposure. However, neither oral nor i.v. formulations of the nucleosides have an impact on the neuronal latent viral reservoir.

At the beginning of the 21st century, novel herpes-virus helicase-primase inhibitors (HPIs) with a different mechanism of action entered clinical trials, namely amenamevir (Kawashima et al., 2017), pritelivir (Kleymann et al., 2002; Kleymann, 2004; Betz et al., 2002; Wald et al., 2014; Wald et al., 2016), adibelivir/IM-250 (Gege et al., 2021; Bernstein et al., 2023), HN0037 (Hou et al., 2022; Gege and Kleymann, 2022, 2024a), ABI-1179 (Cho et al., 2024; Gege and Kleymann, 2024b; Assembly Biosciences, 2025; Narayanan et al., 2025) and ABI-5366 (Pajouhesh et al., 2024; Assembly Biosciences, 2025; Gane et al., 2025; Shen et al., 2025; Gege and Kleymann, 2025) (Fig. 1). To date, only amenamevir is approved in Japan to treat varicella zoster since 2017 and herpes simplex infections since 2023. However, due to its low distribution in nervous tissues, the use of amenamevir to treat herpes disease involving the ganglia or central nervous system has raised concerns (Tada et al., 2024). In contrast, IM-250 reduces the reactivation competence of HSV-1 and HSV-2 in preclinical animal models (Gege, 2021; Bernstein et al., 2023).

In general, effective treatment of neurotropic herpesviruses requires sufficient exposure of the active compound at the neuronal target site, in particular, ganglia and brain. However, it proved difficult to achieve sufficient concentrations in neuronal tissues with nucleoside analogs such as ACV or distinct HPIs to attenuate chronically persistent HSV infections (Whitley and Baines, 2018).

Here, we provide an analysis of the blood-brain barrier (BBB) and blood-nerve barrier (BNB) penetration (Hitchcock and Pennington, 2006; Fridén et al., 2009) of diverse helicase-primase inhibitors and correlate these in a structure-nervous system exposure relationship.

2. Results

A variety of structurally diverse helicase-primase inhibitors (Table 1) have been administered to animals at varying doses and frequency and the pharmacokinetic (PK) profile analyzed with respect to exposure in plasma, blood, organ and nervous tissue at discrete times points post administration.

The blood, plasma and brain concentrations of diverse HPIs were measured after a single administration of the respective HPI in mice at 10 mg/kg (Table 1).

Surprisingly, a structural relationship of HPIs with respect to brain exposure ranking from low to high was observed (Table 1, ABI-5366 < amenamevir < IM-201 < IM-253 < IM-205 < pritelivir < IM-315 < IM-204 and < adibelivir (IM-250)) and the corresponding brain/blood or plasma ratio increases in the following order (Table 1 and Fig. 2, amenamevir < ABI-5366 < pritelivir < IM-201 < IM-253 < IM-205 < IM-315 < IM-204 and < adibelivir (IM-250)). A low brain/plasma ratio of <0.1 was observed for amenamevir, ABI-5366 and pritelivir (30–50 fold less than adibelivir), a medium ratio of 0.2–0.5 was calculated for IM-201, IM-253 and IM-205 and a high ratio of brain/plasma exposure of 0.5–1.5 is shown for IM-315, IM-204 and adibelivir. Adibelivir showed the highest brain/plasma ratio and the highest drug concentration in the brain. The brain/plasma ratio of adibelivir (range 1.1–1.5) and IM-253 (range 0.2–0.3) is relatively constant with respect to the dose and frequency administered in the range of 10–300 mg/kg as summarized in Table 1. The ratios of higher brain exposure follow a trend or function along increasing lipophilicity (calculated AlogP) and decreasing topological polar surface areas (tPSA) as depicted in Table 1. Structurally, for achieving brain exposure, the methylsulfonimidoyl moiety in adibelivir and IM-204 (Uhlig, 2021) is preferred over the primary sulfonamide in IM-315 and the aminosulfonimidoyl group in IM-205. Additionally, the 4-(2,5-difluorophenyl(phenyl)) substituent is advantageous compared to the solubility-conferring 4-(2-pyridyl(phenyl)) structural element. The presence of a cyclic urea moiety in ABI-5366 appears to interfere with the structure–brain exposure relationship, as evidenced by comparison with the structurally matched analog IM-315. Whether this is attributable to the nitrogen atom in the urea group, the cyclic nature of the moiety, conformational changes, or a combination of these factors remains to be elucidated.

Finally, the blood/plasma ratio of adibelivir in mice is 1.0 (at 10 and 100 mg/kg per os (orally) at 24 h post administration).

In another study we analyzed the brain/plasma ratio of adibelivir in diverse species and gender at defined time points ranging from 4 to 24 h after administering diverse doses of 0.5–300 mg/kg orally or intravenously either as single (SD) or multiple doses (MD) for several to 28 days (Fig. 3).

The brain/plasma ratio is constant in the range of 0.4–1.5 in mice, rats and dogs after giving single and multiple doses of adibelivir analyzed at diverse time points post administration. In addition, there is a slight trend for lower brain/plasma ratios within this range after multiple dosing (MD).

The ratio is higher for guinea pigs and rabbits with a range of 2.4–3.4 and 2.9–4.1, respectively. The i.v. administration of 30 mg/kg to rats formulated in HP-ß-CD shows slightly higher ratios in the range of 2.2–2.7 as compared to oral administration. The brain/plasma ratio appears to be constant independent of the gender of species treated, e.g. for male and female rats (Fig. 3).

Next, the permeability of adibelivir was analyzed with respect to the tissue distribution as well as blood-brain and blood-nerve barrier in

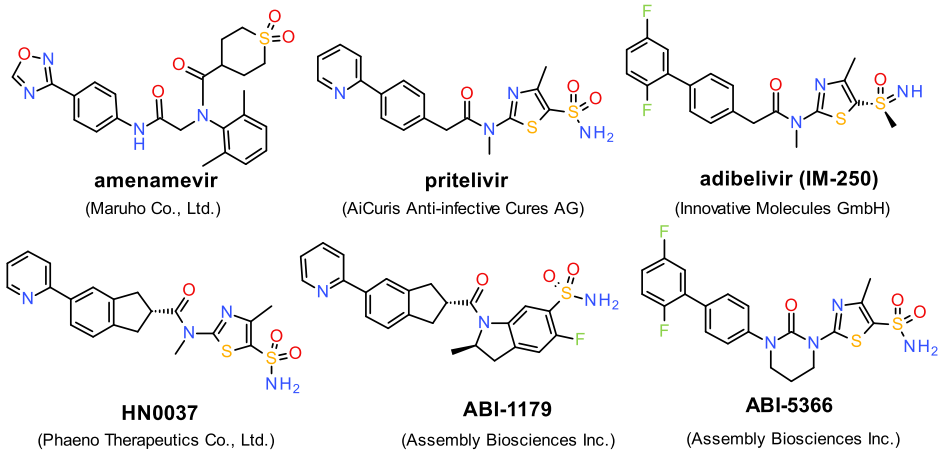
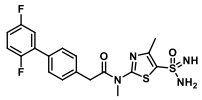
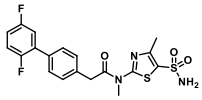
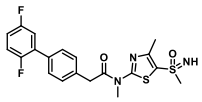
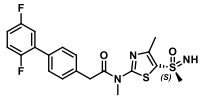
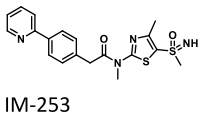
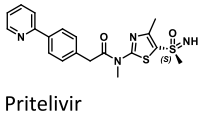
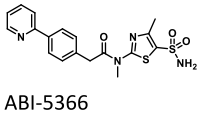
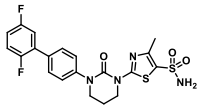
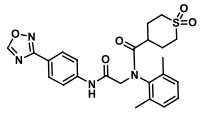


Fig. 1. Structures of helicase-primase inhibitors (HPIs) on the market and in clinical trials: amenamevir (ASP-2151), pritelivir (AIC-316, BAY 57–1293), adibelivir (IM-250), HN0037, ABI-1179 (structure proposal) and ABI-5366.

Table 1
Mean exposure of HPIs in blood or plasma and brain of mice (CV 2–42 %; n = 3–4) at discrete time points (2–24 h) after oral administration of single (SD) or multiple doses (MD) of 10–300 mg/kg in 0.5 % HPMC/PBS pH 7.4.

Compound	tPSA [Å^2] AlogP [a]	Dose (mg/kg) per os	C_{max} [μM]	$AUC_{0\text{-}24}$ (ng·h/ml)	$T_{1/2}$ (h)	Plasma or Blood (μM)	Brain (μmolal) [h]	Ratio brain/plasma
IM-205 	100 3.2	**10 SD**	**13.9**	**37600**	**3.4**	**5.6** Blood	**2.3 [6h]**	**0.4**
IM-315 	93 2.9	**10 SD**	**9.3**	**75613**	**~20**	**8.6**	**7.7 [4h]**	**0.9**
IM-204 	74 4.1	**10 SD**	**15**	**59997**	**6.9**	**11.1** Blood	**14 [4h]**	**1.3**
IM-250 (adibelivir) 	74 4.1	**10 SD** 30 SD 100 SD 300 SD 100 SD 100 MD Day 7[b]	**19**	**85634**	**6–7**	**11.4** 18 54 56 64 95	**17 [4h]** 25 [4h] 81 [4h] 64 [4h] 96 [4h] 146 [4h]	**1.5** 1.4 1.5 1.1 1.5 1.5
IM-201 	86 2.9	**10 SD**	**11**	**22464**	**2.3**	**5** Blood	**0.8 [4h]**	**0.2**
IM-253 	86 2.9	**10 SD** 300 MD Day 4[b]	**17**	**38023**	**1.9**	**5.8** 106	**1.4 [4h]** 29 [2h]	**0.2** 0.3
Pritelivir 	105 1.8	**10 SD**	**37**	**186000**	**6**	**55**	**2.8 [4h]**	**0.05**
ABI-5366 	96 2.9	**10 SD**	**7.3**	**61806**	**~20**	**3.1** 3.2	**0.1 [4h]** 0.2 [24h]	**0.05** 0.06
Amenamevir 	118 2.2	**10 SD**	**5.8**	**23500**	**2.4**	**7.2** 7	**0.2 [4h]** 0.3 [2h]	**0.03** 0.05

[a] tPSA values were calculated with ChemDraw 22.0 and AlogP (Ghose-Crippen-Viswanadhan-Octanol-Water-distribution coefficient) with BIOVIA Draw 2022.
[b] Days treated and day on which analysis has been performed.

dogs. The overall tissue distribution of adibelivir in beagle dogs was analyzed after multiple once daily doses of 100 mg/kg for 8 consecutive days at 24 h post last administration (Fig. 4). Adibelivir is equally distributed over the brain, ganglia, spinal cord, bone marrow, spleen and skin within a tissue/plasma ratio of 0.5–1.2 at a terminal plasma concentration of 18.2 μM. Liver samples show a higher and the cerebrospinal fluid (CSF) a lower exposure than this narrow range of 0.5–1.2. Adibelivir is mainly excreted via the bile (Innovative Molecules, 2025); this may explain the higher ratio of 2 for liver tissue. The CSF is low in protein content (Fautsch et al., 2024). Since adibelivir is highly protein-bound (approximately 99 %, (Gege, 2021)), the CSF to plasma ratio is low (approximately 0.01, Fig. 4), thus reflects the free (unbound) fraction of the drug, which is about 100 nM (Fig. 4). In contrast, the total concentrations in other tissues range from approximately 8 to 22 μM. Other parameters such as different lipids, type and concentration, may contribute to the CSF/plasma ratio of HPIs.

Finally, the permeability of adibelivir and pritelivir was analyzed with respect to the blood-brain and blood-nerve barrier in rodents (rats, n = 4–5). Single doses of 3 mg/kg of adibelivir and pritelivir were formulated for oral administration and intravenous injections and administered orally or via slow bolus injection to rats. 4 h post administration the drug concentrations were analyzed in plasma, CSF, spinal cord, brain and trigeminal and sacral ganglia (Fig. 5). Irrespective of the formulation or administration route, the exposure of adibelivir and pritelivir in plasma is in the range of 3.5–4.6 and 5.2–7.3 μM, respectively. While adibelivir shows high exposure in the spinal cord, brain, and trigeminal and sacral ganglia exceeding the IM-250 *in vivo* IC_{50} of approx. 1 μM (accounting for the protein binding) at least 10 times, tissue concentrations of pritelivir, in contrast, reach only a low fraction of the plasma exposure (<0.05).

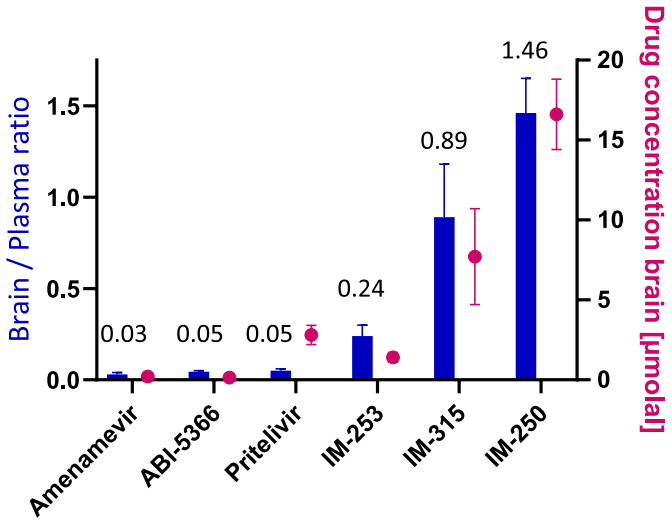


Fig. 2. Drug concentrations of HPIs in the brain of mice and the corresponding brain/plasma ratios.
A single oral dose (10 mg/kg) of the HPIs amenamevir, ABI-5366, pritelivir, IM-253, IM-315 and IM-250 (adibelivir) was administered to mice (n = 3) and 4 h post infection the plasma and brain concentrations were measured. IM-250 showed the highest brain/plasma ratio (left y axis in blue bars) and the highest drug concentration in the brain (right y axis in red dots, mean drug concentration with standard deviation). The brain/plasma ratio increased in the order amenamevir < ABI-5366 = pritelivir < IM-253 < IM-315 and < IM-250 (adibelivir). The determined brain concentration increased in the following order ABI-5366 < amenamevir < IM-253 < pritelivir < IM-315 and < IM-250 (adibelivir).

Again, while the brain/plasma ratio was 0.05 and 0.08 for pritelivir, the ratio of adibelivir was 2.1 after oral gavage and up to 2.6 after slow bolus iv injection, respectively. As observed also in non-rodents (beagle dogs), in rodents, the concentration of adibelivir (82–250 nM) and pritelivir (40–53 nM) in the CSF is low due to the low protein content in the CSF thereby representing the free fraction of the drug.

3. Discussion

In the absence of a prophylactic vaccine, herpes simplex viruses have plagued humanity worldwide since ancient times causing herpes labialis, genital herpes, herpes keratitis or severe life-threatening infections of generalized viremia such as herpes encephalitis, hepatitis or pneumonia especially in the immunocompromised patient population (Howley and Knipe, 2023). Disease is currently mainly treated orally with standard of care nucleosides (such as acyclovir, valacyclovir and famciclovir) (De Clercq and Li, 2016; Kleymann, 2005). Severe disease (e.g. herpes encephalitis and neonatal herpes) is treated with intravenous acyclovir (Whitley and Baines, 2018) and in addition topical therapy is available for herpes keratitis and skin lesions (Kleymann, 2005; Whitley and Baines, 2018). Therapy is moderately active, reducing the course of the disease by one day in case of herpes labialis and genital herpes (Valtrex (valacyclovir). US package insert. GSK; revised 2022) and reduces mortality of herpes encephalitis from 70 to 15 % with high-dose intravenous acyclovir (Whitley and Baines, 2018). The pitfall of the current treatment options is that therapy has no impact on the key feature of herpes simplex viruses, namely efficacy in reducing recurrent disease from the latent viral reservoir in ganglia of the nervous system that has been established for life during primary infection in the infected host. This raises the question of whether drugs with sufficient exposure in the nervous system might demonstrate greater efficacy in the treatment of herpes encephalitis, neonatal herpes or, if proven,

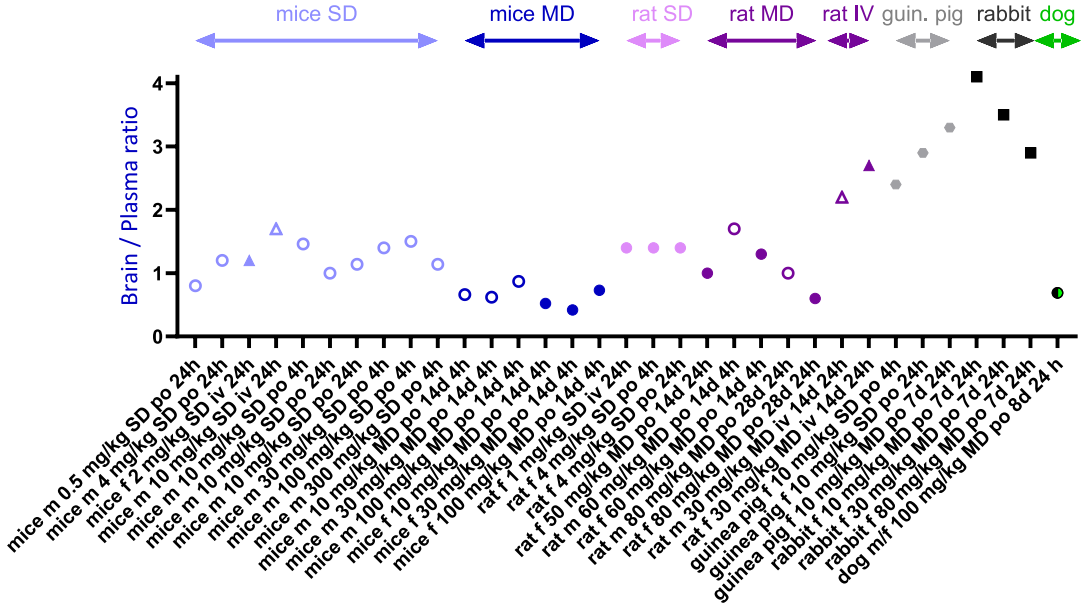


Fig. 3. Brain/plasma ratios of adibelivir in diverse species and gender at defined time points after administering diverse doses orally or intravenously either as single (SD) or multiple doses (MD) over several days.
The mean brain/plasma ratios (CV range 1–40 %) of adibelivir in diverse species (mouse (n = 3 SD and n = 10 MD), rat (n = 3 SD and iv, n = 5 MD), guinea pig (n = 4 SD and n = 6 MD), rabbit (n = 3) and dog (n = 1m+1f)) and gender (m/f) at defined time points ranging from 4 to 24 h after administering diverse doses of 0.5–300 mg/kg orally (in 0.5 % HPMC/PBS pH 7-4) or intravenously (in serum (mice) or HP-ß-CD (rats)) either as single or multiple doses (once daily across all species) for 7 up to 28 days.
The brain/plasma ratio is constant in the range of 0.4–1.5 in mice, rats and dogs after giving single and multiple doses of adibelivir analyzed at diverse time points post administration. The ratio is higher for guinea pigs and rabbits with a range of 2.4–3.4 and 2.9–4.1, respectively. The iv administration of 30 mg/kg to rats formulated in HP-ß-CD shows slightly higher ratios in the range of 2.2–2.7 when compared to oral administration. The brain/plasma ratio appears to be constant independent of the gender of the species treated as shown e.g. for male and female rats. Abbreviations: po = per os, iv = intravenous (triangle), m = male (open sympol), f = female (closed symbol), SD = single dose, MD = multiple doses.

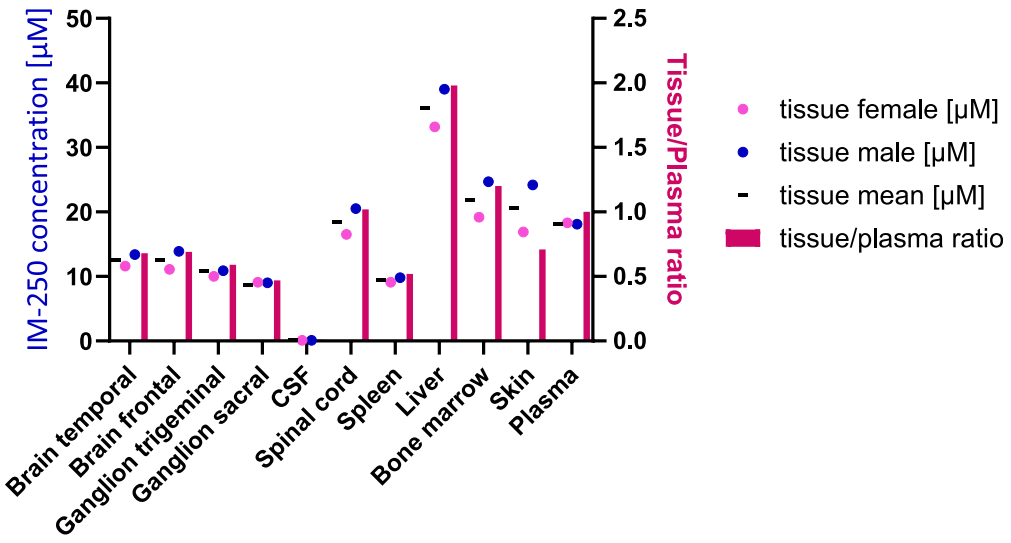


Fig. 4. Tissue distribution of adibelivir in beagle dogs after multiple dosing of 100 mg/kg once daily for 8 consecutive days.
A male and female beagle dog were treated orally for 8 days once daily with 100 mg/kg micronized adibelivir formulated in 0.5 % HPMC/PBS pH 7.4. The respective tissues were analyzed for adibelivir exposure 24 h post last administration. Adibelivir is equally distributed over the brain, ganglia, spinal cord, bone marrow, spleen and skin within a tissue/plasma ratio of 0.5–1.2. With respect to this range, the concentration of adibelivir is higher in the liver (ratio of 2) and lower in the CSF (ratio of 0.01 mimicking the free fraction).

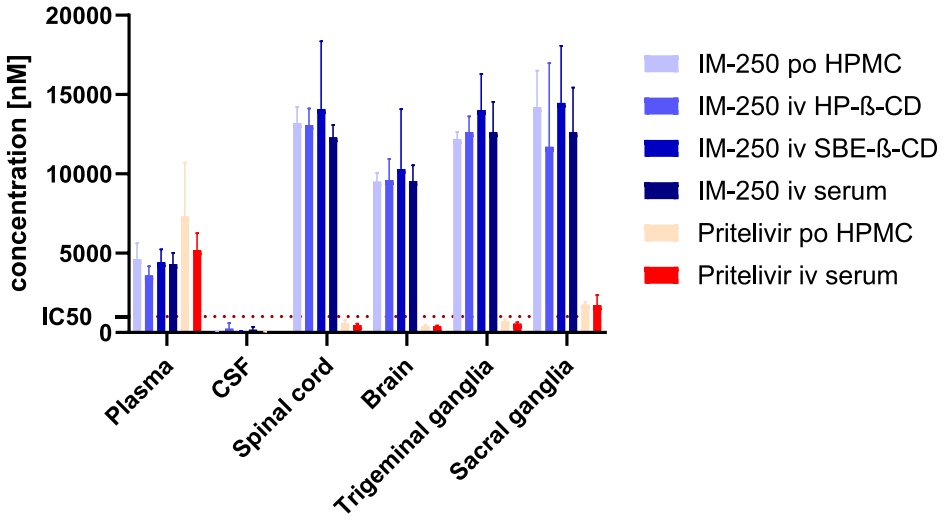


Fig. 5. Exposure of adibelivir and pritelivir in nervous tissues of rats 4 h after a single dose of 3 mg/kg.
Single doses of 3 mg/kg of adibelivir (IM-250) and pritelivir were formulated for oral administration in 0.5 % HPMC/PBS pH 7.4 and intravenous injections in HP-ß-CD, SBE-ß-CD or serum and administered orally or via tail vein slow bolus injection to rats (n = 4). 4 h post administration the drug concentrations (mean values with standard deviation are depicted with a CV range 8–34 %) were analyzed in plasma, CSF, spinal cord, brain and trigeminal and sacral ganglia. Irrespective of the formulation or administration route the exposure of adibelivir and pritelivir in plasma is in the range of 3.5–4.6 and 5.2–7.3 µM, respectively. While adibelivir shows high exposure in the spinal cord, brain, and trigeminal and sacral ganglia exceeding the IM-250 *in vivo* IC_{50} at least 10 times (dotted line), pritelivir in contrast reaches only a fraction of the plasma concentration in the respective tissues including the nervous tissue.

HSV-triggered Alzheimer's disease and have an impact on the reactivation capacity of the nervous latent viral reservoir in the ganglia. In this context, valacyclovir may not be the best treatment option to test the antiviral hypothesis in Alzheimer's disease (NCT03282916), as recently reported during the Alzheimer's Association International Conference (Devanand, 2025).

The moderate efficacy of the standard of care therapy with acyclovir and valacyclovir in severe herpes simplex infections may be explained by the short half-life and low exposure in the nervous tissue. The drug levels of intravenous acyclovir and oral valacyclovir reach an exposure of approximately 88 µM in plasma and 17 µM in the CSF at 3 × 10 mg/kg = 30 mg/kg/day (Whitley et al., 1982) and 28.1 µM in plasma and 3.5 µM in the CSF at a 3000 mg dose/day of valacyclovir (Pouplin, 2011), respectively, whereby the CSF concentration is about 10–20 % of the plasma concentrations. The reported concentrations are above the *in vitro* IC_{50} of 0.5–3.9 µM for acyclovir, however, below the *in vivo* IC_{50} of approx. 15 µM (Fig. S1 in (Gege, 2021)) in case of oral valacyclovir and in the range of the *in vivo* IC_{50} in case of acyclovir. The *in vivo* IC_{50} for acyclovir during disseminated herpes simplex infections is even higher when adjusted for the multiplicity of infection (Fig. S1 in (Gege, 2021)), which correlates with higher mortality rates for patients with disseminated disease in the clinic.

Here we show that adibelivir has exposure in brain and ganglia in the range of plasma concentration by crossing the blood-brain barrier (BBB) and penetrating the blood-nerve barrier (BNB), whereas other HPIs such as amenamevir, pritelivir, ABI-1179 and ABI-5366 show only an

exposure of less than 10 % in the nervous system compared to plasma levels. Low exposure of ABI-5366 and pritelivir in brain and ganglia has been reported in rats at an oral dose of 15 mg/kg (Shen et al., 2025) and independently confirmed in this study. The brain/plasma ratio of adibelivir shows little variation (within the limits and parameter tested) over time and is almost independent of factors such as dose, formulation, dosing frequency, route of administration, species and gender. In mice the brain/plasma ratio (1.1–1.5) is fairly constant with respect to changes in dosing (Table 1). In diverse species e.g. mice, rats and dogs the brain/plasma ratio ranges from 0.4 to 1.5. The ratio is higher for guinea pigs and rabbits (ratio 2.4–3.4 and 2.9–4.1), respectively (Fig. 3). We attribute these differences to species, gender, diet, ADME (absorption, distribution, metabolism and excretion), lipid and protein content and concentrations with varying binding affinities and subtle differences in the BBB and BNB.

The outcome of therapy of herpes simplex infections with helicase-primase drugs in the clinic (amenamevir) and ongoing clinical trials (adibelivir, pritelivir, ABI-5366 and ABI-1179) will show whether HPIs with sufficient neuronal exposure can efficiently treat herpes disease including herpes encephalitis and neonatal herpes and reduce latency and the frequency of recurrences by affecting the reactivation competence of the latent neuronal reservoir of HSVes as demonstrated preclinically in animal models for adibelivir (Gege et al., 2021; Bernstein et al., 2023). Interestingly, ABI-1179 and adibelivir were evaluated in the HSV-2 guinea pig model of genital herpes. While treatment over 49 days with adibelivir, a HPI with high nervous system exposure, fully silenced recurrences after 7 treatment cycles (Bernstein et al., 2023), treatment with ABI-1179 for 120 days did not silence recurrences (Cho et al., 2024; Cho et al., 2025) indicating low exposure in the ganglia probably in the range of ABI-5366.

First-in-human clinical trials for amenamevir (Kusawake, 2017), HN0037 (Hou, 2022), pritelivir (Kropeit, 2023), ABI-1179 (Assembly Biosciences, 2025), adibelivir (Blank et al., 2025) and ABI-5366 (Assembly Biosciences, 2025; Gane et al., 2025) show a half-life of 0.3, 2, 3, 4, 5 and ~20 days and a C_{max} of 0.5, 3.8, 1.6, 5.0, 2.3, 1.4 μg/ml, and an AUC_{inf} of 5.8, 293, 109, 555, 332, 805 μg h/ml at a 100 mg dose, respectively. Applying the brain/plasma ratio assuming approximately constant ratios across species, this will lead to an approximate concentration of 0.015, 0.08, 3.5 and 0.07 μg/ml for amenamevir, pritelivir, adibelivir and ABI-5366 in the human brain, respectively. Adibelivir has therefore the potential to achieve sufficient exposure in the nervous target tissue exceeding the *in vivo* IC_{50} of 0.44 μg/ml (1 μM) and IC_{90} of 1 μg/ml (2.3 μM) assuming similar exposure as observed in diverse animals. Currently the low exposure of amenamevir in the nervous system makes it a questionable treatment choice for CNS infections with HSV in the clinic (Tada et al., 2024). Ongoing clinical trials with pritelivir, HN0037, adibelivir, ABI-5366 and ABI-1179 will show whether sufficient exposure in the nervous system and or improvement of potency of HPIs will outperform the standard of care with nucleosidic drugs by exceeding the target IC_{50} or IC_{90} values with the free fraction of the respective drugs in the neuronal target tissues. Interim results of a Phase 1b study with a weekly dose of 350 mg ABI-5366 at a C_{max} of 2.9 μg/ml (6.4 μM) show significantly reduced viral shedding and lesions versus placebo in HSV-2 infected patients (Assembly Biosciences, 2025).

In conclusion, the efficient passage of helicase-primase drugs through the blood-brain and blood-nerve barrier is based on their distinct structure and chemical properties; in particular, based on the methylsulfonimidoyl moiety combined with the 4-(2,5-difluorophenyl (phenyl)) substituent of thiazolyl-acetamides. Regarding the chemical properties, the ratios of higher exposure in the nervous system follow a trend or function along increasing lipophilicity (calculated AlogP) and decreasing topological polar surface areas (tPSA). Exposure of the helicase-primase drugs in the nervous system will help to predict their usefulness in treating simple mucocutaneous HSV diseases such as herpes labialis or genital herpes, or all HSV infections in general, including herpes encephalitis, neonatal herpes and ultimately reducing the latent virus pool of reactivatable neurons altering a long-held paradigm that this was not possible.

4. Methods

4.1. Chemicals and drug substances

IM-201 (WO2017/174640), IM-204 (WO2017/174640), IM-205 (WO2017/174640), IM-250 (WO2019/068817), IM-253 (WO2019/068817), IM-250HCl (WO2023/135303), IM-315 (WO2001/047904) and ABI-5366 (WO2024/049760) were synthesized based on the protocols of publications and patents indicated in brackets. Amenamevir (WO2005/014559) and pritelivir (WO2001/047904) were purchased from MedChemExpress. 0.9 % NaCl for injection, 2-hydroxypropyl-β-cyclodextrin (HP-β-CD, MS = 0.4–1.5), sulfobutylether-ß-cyclodextrin (SBE-β-CD sodium salt) and hydroxypropyl methylcellulose (HPMC; H9262, CAS 9004-65-3) were purchased from B. Braun Germany, Cyclolab and Sigma-Aldrich, respectively.

4.2. Animals and welfare

Beagle dogs were delivered at an age of 29 months from Marshall BioResources, USA. The dogs were housed individually in kennels divided in an inside and outside yard with a total floor space of total 9 m^2. Each kennel had a temperature controlled inside yard maintained at a temperature of 22 °C ± 3 °C (maximum range). Commercial ssniff Hd-H V3234 (ssniff-Spezialdiäten GmbH, Germany) served as food. 40 g/kg body weight of this food were offered to each dog daily for 2 h. Tap water of drinking water quality was offered ad libitum.

SD rats and C57BL/6 mice were delivered from Janvier, Europe at an age of 6–8 weeks and housed in fibre cages (Bedding grade 5, plastic houses cages (3–5 animals per cage)) at 22–24 °C with a relative humidity of 50–60 % at an alternately lit and darkened in a 12-h dark/12-h light cycle. Tap water and food (ssniff Pri V1534-000 mouse and rat maintenance diet) were provided at libitum.

NZW female rabbits were delivered from Charles River, Germany with an age of 12 weeks and kept in barn housing at 22–24 °C with a relative humidity of 50–60 % at an alternately lit and darkened in a 12-h dark/12-h light cycle. Tap water and food (ssniff V2333-000 rabbit maintenance diet) were provided at libitum.

4.3. Formulation and dosing

The respective compounds were dissolved in DMSO at a 20x stock solution, diluted prior to dosing 1:20 in 0.5 % HPMC, PBS pH 7.4 and 5 ml/kg of the formulated drug were administered using an oral feeding tube at an application volume of 2–5 ml/kg; e.g. for a 10 mg/kg dose a 40 mg/ml stock solution was diluted to 2 mg/ml in 0.5 % HPMC, PBS pH 7.4 and dosed at 5 ml/kg. Micronized drug ($d_{90} < 10$ μm) incl. IM-250 HCl salt was directly suspended in 0.5 % HPMC, PBS 7.4. IV formulations were prepared in serum (0.6 mg/ml HPIs final DMSO 5 %), HP-ß-CD (1 mg/ml HPIs, hydroxylpropyl-ß-cyclodextrin 15 mg/ml in 0.9 % NaCl for injection) or SBE-ß-CD (1 mg/ml HPIs, sulfobutylether-ß-cyclodextrin 13,3 mg/ml in 0.9 % NaCl for injection) and administered by slow bolus via the tail vein.

4.4. Pharmacokinetic

Animals (n = 3–10) were dosed at 10 mg/kg or as indicated in the range of 0.5–300 mg/kg. A pharmacokinetic (PK) profile was analyzed by taking blood samples at the time points indicated, e.g. at 30, 60, 120, 240, 480, 720 and 1440 min post administration, by bleeding from a tail vein or by cardiac puncture at 1440 min. Blood samples were harvested in Na-heparinized or EDTA containing tubes on ice. Blood samples were analyzed or, subsequently, tubes were centrifuged at 6800 rcf for 8 min

at 4 °C to generate plasma. Plasma samples were stored at −20 °C until analysis.

To determine the drug concentration in the respective blood, plasma or organ samples at terminal time points, animals were euthanized/sacrificed at 2, 4, 6 or 24 h post administration. Samples were stored frozen at −20° (for weeks) to −80 °C (for months) until analysis. The pharmacokinetic analysis was performed by applying a non-compartment model using the current Kinetica software (Thermo Scientific, Waltham, USA). All given parameters were obtained by trapezoid area calculation.

4.5. Preparation of blood, plasma and organ samples for HPLC/MS analysis

2-6 vol of acetonitrile were added to the blood and plasma samples. The samples were homogenized by vortexing for 5–10 s and were then centrifuged for 10 min at 6000–14000 rpm. Organ samples were digested by adding one volume of proteinase K solution (0.5 mg/ml in 20 mmol/L sodium phosphate buffer) followed by incubation for 60 min at 50 °C. Homogenates were generated by FastPrep™ tissue homogenization 2x times at 6 m/s for 20 s. Proteins were precipitated by adding 6 vol of acetonitrile (+ internal standard) to the homogenate followed by another homogenization step at 6 m/s for 20 s. Samples were centrifuged for 10 min at 14000 rpm. Some of the respective supernatants were diluted 1:1 with water before injection.

4.6. Determination of drug concentration in animal samples by HPLC-MS/MS measurement

Drug concentrations were measured in blood, plasma and in organ samples with an Agilent 1260 Infinity system coupled to a triple quadrupole Sciex API 4500 LC/MS/MS detector or Q Exactive Plus (Orbitrap) accurate mass spectrometer equipped with a heated electrospray (H-ESI) interface (positive full scan mode). A Dr. Maisch Reprosil-pur Phenyl column (50 × 2 mm, 3 μm) or Kinetex Phenyl-Hexyl column (50 × 2 mm, 2.6 μm) was used for separation. The mobile phase was composed of water containing 0.1 % formic acid (eluent A) and acetonitrile containing 0.1 % formic acid (eluent B). Gradient used was: 90 % A and 10 % B for 1 min, to 100 % B in 1 min, 100 % B for 3 min, to 90 % A and 10 % B in 1 min and 90 % A for 2 min. The standard curve of analyte (5–100000 nmol/L) and the quality controls (100, 1000 and 10000 nmol/L) were prepared by serial dilution in plasma. Precipitation of proteins was achieved by adding 6 vol of acetonitrile. The samples were homogenized by vortexing for 5–10 s and were then centrifuged for 10 min at 6000–14000 rpm. Lower limit of quantification (LLOQ) of drugs was 10–50 nmol/L and upper limit of quantification (ULOQ) was 100000 nmol/L.

4.7. Ethics

The pharmacokinetic studies were performed at LTP Germany (licensed dog study 37268) or under a license obtained by Synovo GmbH or Pharmacelsus GmbH for the testing of drug candidates in the rodent pharmacokinetic model (approval number SYN 06/20 or 2.4.2.2/24–2015 or GB 3-2.4.2.2.-05/2018, respectively).

CRediT authorship contribution statement

Christian Gege: Writing – review & editing, Visualization, Validation, Methodology, Investigation, Data curation. **Thomas Hoffmann:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization. **Gerald Kleymann:** Writing – original draft, Validation, Supervision, Project administration, Investigation, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: CG and GK are inventors of patent families WO2017/174640 (Title: Aminothiazole derivatives useful as antiviral agents) and WO2019/068817 (Title: Enantiomers of substituted thiazoles as antiviral compounds). CG is a consultant to Innovative Molecules GmbH. GK and TH are shareholders of Innovative Molecules GmbH.

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Data availability

Data will be made available on request.

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