PK/PD Assessment of Selective Phosphodiesterase Inhibitors in a Mouse Model of Autoimmune Hepatitis

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ABBREVIATIONS:

AC – Adenylyl cyclase

AIC – Akaike information criterion

AIH – Autoimmune hepatitis

ALT – Alanine transaminase

AP-1 – Activator protein 1

AST – Aspartate transaminase

ConA – Concanavalin A

IL-17 – Interleukin 17

NF- κ B – Nuclear factor kappa-light-chain-enhancer of activated B cells

PDE – Phosphodiesterase

PDEi – Phosphodiesterase inhibitors

PKA – Protein kinase A

PK/PD – Pharmacokinetic/pharmacodynamic

TNF α – Tumor necrosis factor α

ABSTRACT

Autoimmune hepatitis (AIH) is a life-threatening disorder currently treated with non-specific immunosuppressive drugs. It is postulated that phosphodiesterase (PDE) inhibitors, as agents exerting anti-inflammatory and immunomodulatory activities, may constitute a possible treatment for autoimmune disorders. This study develops a PK/PD model to assess the effects of PDE-selective inhibitors, namely, cilostazol (PDE3), rolipram (PDE4), and BRL-50481 (PDE7), in a mouse model of AIH. The pharmacokinetics of the PDE inhibitors (PDEi) were assessed in male BALB/c mice following IP administration. In pharmacodynamic studies, mice received PDEi and AIH was induced in these animals by IV injection of concanavalin A (ConA). Serum drug concentrations, TNF α , IL-17, and aminotransferase activities were quantified. The PK/PD analysis was performed using ADAPT5 software. The PK/PD model assumes inhibition of cAMP hydrolysis in T-cells by PDEi, ConA-triggered formation of TNF α and IL-17, suppression of TNF α and IL-17 production by cAMP, and stimulatory effects of TNF α and IL-17 on the hepatic release of aminotransferases. Selective blockage of PDE4 leads to the highest inhibition of cAMP degradation in T-cells and amelioration of disease outcomes. However, inhibition of both PDE3 and PDE7 also contribute to this effect. The proposed PK/PD model may be used to assess and predict the activities of novel PDEi and their combinations in ConA-induced hepatitis. A balanced suppression of different types of PDE appears to be a promising treatment option for AIH; however, this hypothesis warrants testing in humans based on translation of the PK/PD model into clinical settings.

SIGNIFICANCE STATEMENT

A novel PK/PD model of PDE inhibitor effects in mice with ConA-induced autoimmune hepatitis was developed involving a mechanistic component describing changes in cAMP concentrations in mouse T-cells. According to model predictions, inhibition of PDE4 in T cells causes the highest cAMP elevation in T cells, but suppression of PDE3 and PDE7 also contribute to this effect. A balanced inhibition of PDE3, PDE4 and PDE7 appears to be a promising treatment strategy for AIH.

INTRODUCTION

Autoimmune hepatitis (AIH) is a life-threatening disorder. Along with other autoimmune conditions, AIH is related to a dysregulated immune response directed against self-antigens. Current standard treatments of this disorder were established in 1960's, and they are based on the use of immunosuppressive drugs such as azathioprine in combination with corticosteroids, which usually have to be taken for a lifetime from the time of the diagnosis (Mackay, 2008). Unfortunately, these medications are not always effective and exhibit numerous dose- and time-dependent severe adverse effects (Beretta-Piccoli *et al.*, 2017).

Phosphodiesterase (PDE)3, PDE4, and PDE7 are present in T-lymphocytes and are responsible for degradation (hydrolysis) of the second messenger cyclic adenosine 5'-monophosphate (cAMP) to its inactive form adenosine 5'-monophosphate (AMP) (Giembycz *et al.*, 1996; Smith *et al.*, 2003; Jankowska *et al.*, 2017). In immune cells, cAMP is continuously synthesized from adenosine 5'-triphosphate (ATP) by adenylyl cyclase (AC). While PDE4 and PDE7 selectively hydrolyze cAMP, PDE3, in addition, regulates cyclic guanosine 5'-monophosphate (cGMP) concentrations. Inhibitors of these enzymes have been extensively studied in recent years as possible therapeutics in inflammatory and immune-related disorders (Bielekova *et al.*, 2000; Jankowska *et al.*, 2017; Chen *et al.*, 2018). Increased quantities of cAMP in T-cells activate protein kinase A (PKA) that subsequently causes phosphorylation of transcription factors responsible for regulation of expression of genes coding inflammatory mediators, such as tumor necrosis factor α (TNF α) and interleukin 17 (IL-17) among others (Eigler *et al.*, 1998; Shin *et al.*, 1998). Therefore, immunomodulatory effects of PDE inhibitors (PDEi) may be, at least partially, attributed to the increased intercellular cAMP concentrations, thereby triggering pathways that regulate production and release of inflammatory mediators.

TNF α is one of the key mediators of liver cell damage in human AIH (Czaja, 2014). Therefore, biological therapies targeting this cytokine were successfully used in patients with AIH refractory to conventional treatment (Than *et al.*, 2016; Beretta-Piccoli *et al.*, 2017). Another cytokine engaged in AIH pathogenesis is IL-17 (Kamijo *et al.*, 2011). Elevated concentrations of this signaling molecule were also observed in a concanavalin A (ConA)-induced mouse model of AIH (Yan *et al.*,

2012). Acute liver damage induced by intravenous (IV) injection of ConA in mice is an established and commonly used animal model of AIH (Wang *et al.*, 2012). Similarly to human disease, in this model hepatocyte death is mainly driven by T-cells that infiltrate the liver. Both TNF α and IL-17 have been shown to contribute to hepatocyte death in ConA-induced liver injury. Their mechanism of action is indirect and can be attributed to the increased expression and production of other proinflammatory mediators that participate in the recruitment and activation of immune cells responsible for liver cell death (Yan *et al.*, 2012; Filliol *et al.*, 2016). In addition, TNF α , by interacting with TNF receptors, activates pathways leading to apoptosis of hepatocytes (Leist *et al.*, 1995; Trautwein *et al.*, 1998). It has been shown that neutralization of either IL-17 or TNF α led to significantly reduced liver damage in ConA-induced hepatitis (Gantner *et al.*, 1995; Xu *et al.*, 2011). Our study uses serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities as biomarkers of liver injury, since these enzymes are released into the blood from damaged hepatocytes and, therefore, their serum activities correlate with the extent of liver cell death (Smith *et al.*, 2019). Serum ALT and AST activities have been previously used in pharmacokinetic and pharmacodynamic (PK/PD) modeling as biomarkers of liver injury in studies related to drug-induced hepatotoxicity (Fetterly *et al.*, 2008; Remien *et al.*, 2012; Bhavnani *et al.*, 2019; Li *et al.*, 2021) as well as in animal models of liver damage (Min *et al.*, 2018; Świerczek *et al.*, 2021).

PK/PD modeling allows for better understanding of determinants and mechanisms of drug action and disease progression and facilitates prediction of drug effects with different dosing regimens of therapeutic agents. In our previous work, we provided the first mathematical description of effects of a non-selective PDEi, GRMS-55 in ConA-induced hepatitis in mice (Świerczek *et al.*, 2021). The current study seeks to develop a more mechanistic PK/PD model that describes individual effects of three selective PDEi, namely, a PDE3 inhibitor, cilostazol, a PDE4 inhibitor, rolipram, and a PDE7 inhibitor, BRL-50481, in a mouse model of AIH. Finally, the predictive performance of the proposed PK/PD model was evaluated using data from our previous publication on the investigational PDEi GRMS-55 (Świerczek *et al.*, 2021).

MATERIALS AND METHODS

Chemicals and Reagents

The ConA, DMSO, and PEG 400 were obtained from Sigma-Aldrich (Steinheim, Germany). Cilostazol, BRL-50481, and rolipram were bought from Cayman Chemical (Ann Arbor, MI). All other reagents, chemicals, and solvents were of LCMS or analytical grade and were purchased from Merck (Darmstadt, Germany).

Animals

Male BALB/c mice weighing 21 ± 3 g were obtained from the Animal Facility of the Department of Clinical Immunology and Transplantology at Jagiellonian University Medical College (Kraków, Poland). Animals were housed at 22°C and under 12–12 h light–dark cycle and were given *ad libitum* access to water and food. They were acclimatized for 1 week prior to studies. All procedures involving animals were performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health and approved by the First Local Ethical Committee on Animal Testing in Kraków, Poland.

Pharmacokinetic Study

The PK of cilostazol, rolipram, and BRL-50481 was assessed in male BALB/c mice following intraperitoneal (IP) administration. BRL-50481 was given at a single dose of $50 \text{ mg} \cdot \text{kg}^{-1}$ ($n = 3$), cilostazol at a dose of $20 \text{ mg} \cdot \text{kg}^{-1}$ ($n = 3$), and rolipram at a dose of $10 \text{ mg} \cdot \text{kg}^{-1}$ ($n = 3$). These compounds were dissolved in vehicle composed of 10% (v/v) DMSO and 10% (v/v) PEG 400 in water for injection. The mice were sacrificed by exsanguination at 5, 15, 30, 60, 120, and 240 min after dosing. Blood was collected, allowed to clot at room temperature for 30 min, and then centrifuged at $3000 \times g$ for 10 min to separate the serum, which was stored at -20°C until analysis.

ConA-Induced Hepatitis

The ConA was dissolved in saline for injection at a concentration of $2 \text{ mg} \cdot \text{mL}^{-1}$. The mice were given IP doses of one of the PDEi or the equivalent volume of vehicle for the control group and, 30 minutes later, animals were given an IV injection of ConA at a dose of $20 \text{ mg} \cdot \text{kg}^{-1}$ *via* tail vein. An additional

group of mice was given vehicle alone IP followed by an IV injection of saline. The animals were sacrificed by exsanguination at 1, 2, 4, 8, 16 and 24 h post-ConA dosing (n= 4-5 mice per time point in each study or control group). Blood samples were handled as above.

Analytical methods

Concentrations of TNF α in serum were quantified using a Rat TNF- α Quantikine ELISA Kit (R&D Systems, Minneapolis, MN) according to the protocol provided by the manufacturer.

Serum concentrations of IL-17 were measured using a MilliplexTM MAP Kit (Millipore, Billerica, MA) according to the manufacturer's instructions on a MAGPIX LuminexTM instrument. Data acquisition and analysis was carried out using xPONENT software (Luminex Corporation, Austin, TX).

Activities of ALT and AST in serum were quantified using a chemistry analyzer BS-800 (Shenzhen Mindray Bio-Medical Electronics Corporation, Ltd., Shenzhen, China).

Serum concentrations of rolipram, BRL-50481, and cilostazol were quantified by LC-MS/MS methods. Stock solutions and working solutions of all compounds were prepared in methanol. All analyzed test samples, quality control (QC), and standard samples were prepared according to the following protocol. The standard and QC samples were prepared by adding 5 μ L of working solutions of the drugs to 45 μ L of blank mouse serum to yield the required concentrations of the compounds. One hundred μ L of 0.1% formic acid in acetonitrile was added to 50 μ L of analyzed serum samples, QC samples, or standard samples. Afterward, the samples were vortexed for 10 min (IKA Vibrax VXR, Germany) and centrifuged at $8000 \times g$ for 10 min at room temperature. Five μ L of supernatant was subjected to LC-MS/MS analysis using an Exion LC AC HPLC system (Danaher Corporation, Framingham, MA) coupled with a Sciex QTRAP 4500 triple-quadrupole MS (Danaher Corporation). The autosampler temperature was set at 15°C. Chromatographic separation was performed at 25°C using an XBridgeTM C18 (3 $\times$ 50 mm, 5 μ m) column (Waters, Ireland). The mobile phase composed of 0.1% formic acid in acetonitrile (eluent A) and 0.1% formic acid in water (eluent B) was pumped at a flow rate of 0.3 mL $\cdot$ min⁻¹ in a gradient elution mode. After injection, the percentage of eluent B was

maintained for 2 min at 10%, then increased linearly to 80% during 3 min, and was maintained at 80% for 1 min. Subsequently, it decreased linearly to 10% during 0.1 min. Finally, it was maintained at 10% for 3.9 min until the end of analysis resulting in total run time of 10 min. All detections were performed in a positive ion mode and the MS parameters for each compound were optimized. The MRM transitions for cilostazol were 370.2→288.3 and 370.2→125.0, for BRL-50481 245.1→90.0 and 245.1→136.0, and for rolipram 276.2→208.1 and 276.2→191.1. The first transition was used as a quantifier and the second for the identity verification. The ion source temperature for cilostazol was maintained at 500°C and for rolipram and BRL-50481 at 450°C. The ion spray voltage was set at 5000 V for cilostazol and at 4500 V for rolipram and BRL-50481. The curtain gas was set at 30 psi for rolipram and at 40 psi for BRL-50481 and cilostazol. The collision gas was set at medium for all analyzed compounds. Data acquisition and processing were carried out using the Analyst 1.7 software. The calibration curves were constructed by plotting the ratio of the peak area of the studied PDEi versus its concentration and generated by weighted (1/x) linear regression analysis. The validated quantitation ranges were 0.1 to 20 mg·L⁻¹ for cilostazol, 0.01 to 10 mg·L⁻¹ for BRL-50481, and 0.0005 to 5 mg·L⁻¹ for rolipram. The accuracy of samples was within 15% deviation from the nominal values, and precision was within 15% relative standard deviation. No significant problems related to a matrix effect or stability were observed during the analysis of the samples.

Pharmacokinetics

Serum concentration-time profiles of the investigated PDEi were fitted using linear compartmental models with first-order absorption as shown in **Fig. 1** to obtain PK parameter estimates. The equations and initial conditions for the selected models are:

For BRL-50481 and rolipram:

$$\frac{dA}{dt} = -k_a \times A; \quad A(0) = D \times F, \quad (1)$$

$$V_c \times \frac{dC_p}{dt} = k_a \times A + CL_D \cdot C_t - CL_D \times C_p - CL_T \times C_p; \quad C_p(0) = 0, \quad (2)$$

$$V_t \times \frac{dC_t}{dt} = CL_D \times C_p - CL_D \times C_t; \quad C_t(0) = 0, \quad (3)$$

For cilostazol:

$$\frac{dA}{dt} = -k_a \times A; \quad A(0) = D \times F, \quad (4)$$

$$V_d \times \frac{dC_p}{dt} = k_a \times A - CL_T \times C_p; \quad C_p(0) = 0 \quad (5)$$

where A is the amount of compound at the absorption site, C_p is the serum drug concentration, C_t is the concentration in the peripheral compartment, CL_T is the total clearance, V_d is the volume of distribution, V_c is the volume of the central compartment, V_t is the volume of the peripheral compartment, CL_D is the distribution clearance, k_a is the first-order absorption rate constant, D is the IP dose, and F is the bioavailability following IP administration.

PK/PD Modeling

The PK/PD model illustrated in **Fig. 1** was developed considering the mechanism of action of the investigated compounds and the pathophysiological mechanisms leading to liver damage in ConA-induced hepatitis. In the proposed PK/PD model, a hypothetical compartment represents cAMP concentrations in mouse T-cells. Literature data indicate that these cells are responsible for the production and release of pro-inflammatory mediators such as TNF α and interleukins in response to ConA challenge (Cao *et al.*, 1998; Yan *et al.*, 2012). Additionally, intracellular cAMP in immune cells regulates production of these signaling molecules (Wehbi and Taskén, 2016). This regulatory function of cAMP is associated with its ability to activate PKA that subsequently controls transcription factors responsible for regulation of transcription of inflammatory mediators (Shin *et al.*, 1998) as depicted in **Fig. 2**. The PDEi have the ability to regulate intracellular concentrations of cAMP by inhibiting its degradation. Therefore, the compartment representing cAMP concentrations in T-cells allows integration of the PK of the PDEi with changes in time of their pharmacological activity, which is manifested by inhibition of production of pro-inflammatory cytokines, namely TNF α and IL-17. The following equation describes the turnover of cAMP in T-cells and the influence of the PDE-selective inhibitors:

$$\frac{dC_{cAMP}}{dt} = k_{in_cAMP} - \frac{V_{max_PDE3} \times C_{cAMP}}{K_{m_PDE3} \times (1 + \frac{C_{M_CIL}}{K_{i_CIL}}) + C_{cAMP}} - \frac{V_{max_PDE4} \times C_{cAMP}}{K_{m_PDE4} \times (1 + \frac{C_{M_ROL}}{K_{i_ROL}}) + C_{cAMP}} - \frac{V_{max_PDE7} \times C_{cAMP}}{K_{m_PDE7} \times (1 + \frac{C_{M_BRL}}{K_{i_BRL}}) + C_{cAMP}},$$

$$cAMP(0) = R_{0_cAMP} \quad (6)$$

where C_{M_CIL} , C_{M_ROL} , and C_{M_BRL} are the serum molar concentrations of the compounds, C_{cAMP} is the concentration of cAMP in T-cells, and R_{0_cAMP} is the baseline concentration of cAMP in these cells. The R_{0_cAMP} was fixed to 1 μ M based on the literature data indicating that, in several cell types, basal cAMP concentration equals approximately 1 μ M (Koschinski and Zaccolo, 2017). It is noted that this equation assumes competitive inhibition of PDE by the studied compounds. For simplicity, it was assumed that mouse T-cells constitute a single compartment in which cAMP is evenly distributed in the intracellular fluid. The cAMP is assumed to be constantly produced with the zero-order rate constant (k_{in_cAMP}) in mouse T-cells, which was calculated as:

$$k_{in_cAMP} = \frac{V_{max_PDE3} \times R_{0_cAMP}}{K_{m_PDE3} + R_{0_cAMP}} + \frac{V_{max_PDE4} \times R_{0_cAMP}}{K_{m_PDE4} + R_{0_cAMP}} + \frac{V_{max_PDE7} \times R_{0_cAMP}}{K_{m_PDE7} + R_{0_cAMP}} \quad (7)$$

Based on literature data, it was assumed that in mouse T-cells, cAMP is degraded with the participation of PDE3, PDE4, and PDE7 and this reaction is saturable, since PDE-catalyzed hydrolysis of cAMP is an enzymatic reaction. In addition, each type of PDE contributes differently to the overall hydrolytic activity of PDE in immune cells (Yang et al., 2003). Parameters V_{max_PDE3} , V_{max_PDE4} , and V_{max_PDE7} represent maximum velocity of enzymatic hydrolysis leading to degradation of cAMP by different PDE types in mouse T-cells. The parameter V_{max} , being the sum of maximum velocities for PDE3, PDE4, and PDE7 ($V_{max} = V_{max_PDE3} + V_{max_PDE4} + V_{max_PDE7}$), was calculated based on the literature data (Yang et al., 2003) as:

$$V_{max} = \frac{PDE_{TA}}{V_{if}} = \frac{8600 (\frac{f_{mol\ cAMP\ hydrolysed}}{10^6 (cells) \cdot 1 (h)})}{60 (fL)} = 143 (\frac{\mu M}{h \cdot cell}) \quad (8)$$

where PDE_{TA} is the total PDE activity against cAMP in mouse lymphocytes and V_{if} is the intracellular fluid volume in mouse T-cells (Bobak et al., 2011). This approach assumes that among different PDE types only PDE3, PDE4, and PDE7 contribute to degradation of cAMP in T-cells, which is in line with

previous studies (Yang et al., 2003). Using the PK/PD model, V_{max_PDE3} and V_{max_PDE4} parameters were estimated, and V_{max_PDE7} was calculated as a secondary parameter by subtraction ($V_{max_PDE7} = V_{max} - V_{max_PDE3} - V_{max_PDE4}$). The K_{m_PDE3} , K_{m_PDE4} , and K_{m_PDE7} are Michaelis constants of cAMP against the corresponding PDEs as obtained from the literature (Bender and Beavo, 2006), and K_{i_CIL} , K_{i_ROL} , and K_{i_BRL} are inhibition constants of cilostazol against PDE3 (Zhang *et al.*, 2002), rolipram against PDE4 (Ohsawa *et al.*, 1998), and BRL-50481 against PDE7 (Smith et al., 2004). The K_i is a dissociation constant describing the binding equilibrium between an inhibitor and enzyme. It is an intrinsic measure of the affinity of an inhibitor for a corresponding enzyme calculated as a ratio of dissociation (k_{off}) and association (k_{on}) rate constants.

In the present PK/PD model, the equation describing changes in time of the $P(t)$ variable that accounts for stimulation of TNF α production in response to ConA administration is:

$$\frac{dP}{dt} = \begin{cases} 0 & \text{for } t < 0.5, \\ -P \times k_{deg} & \text{for } t \geq 0.5, \end{cases} \quad P(0) = P_{t0}, \quad (9)$$

where P_{t0} is the initial value of the $P(t)$ variable and k_{deg} is the first-order rate constant for the $P(t)$ variable decay. An indirect response model (Dayneka *et al.*, 1993) was used to describe TNF α turnover:

$$\frac{dC_{TNF\alpha}}{dt} = \begin{cases} 0 & \text{for } t < 0.5, \\ (1 + P) \times \left(1 - \frac{I_{max_TNF\alpha} \times (C_{cAMP} - R_{0_cAMP})}{IC_{50_TNF\alpha} + (C_{cAMP} - R_{0_cAMP})}\right) \times k_{in_TNF\alpha} - k_{out_TNF\alpha} \times C_{TNF\alpha} & \text{for } t \geq 0.5, \end{cases}$$

with $C_{TNF\alpha}(0) = R_{0_TNF\alpha}$, (10)

where $C_{TNF\alpha}$ is the serum TNF α concentration, $k_{out_TNF\alpha}$ is the first-order TNF α degradation rate constant, $k_{in_TNF\alpha}$ is the zero-order TNF α production rate constant, and $k_{in_TNF\alpha} = R_{0_TNF\alpha} \times k_{out_TNF\alpha}$. It was assumed that an increase in cAMP concentration above the baseline level in T-cells leads to the suppression of TNF α and IL-17 production, and if cAMP concentration is high enough, it completely inhibits the production of these inflammatory mediators. Therefore, $I_{max_TNF\alpha}$ is the maximum inhibition of TNF α production by cAMP (fixed to 1) and $IC_{50_TNF\alpha}$ is the increase in

concentration of cAMP in T-cells above baseline that produces 50% of $I_{max_TNF\alpha}$. ConA was administered at 30 min after injection of the PDEi; thus, a 0.5 h delay was reflected in equations 9 and 10. The subsequent equations constitute a series of transit steps accounting for the delayed effects of TNF α :

$$\frac{dT_{1_TNF\alpha}}{dt} = (C_{TNF\alpha} - T_{1_TNF\alpha}) \times \frac{1}{\tau_{TNF\alpha}}; \quad T_{1_TNF\alpha}(0) = R_{0_TNF\alpha}, \quad (11)$$

$$\frac{dT_{n+1_TNF\alpha}}{dt} = (T_{n_TNF\alpha} - T_{n+1_TNF\alpha}) \times \frac{1}{\tau_{TNF\alpha}}; \quad T_{n+1_TNF\alpha}(0) = R_{0_TNF\alpha}, \quad n = 1, 2, 3 \quad (12)$$

$$\frac{dT_{5_TNF\alpha}}{dt} = (T_{4_TNF\alpha} - T_{5_TNF\alpha}) \times \frac{1}{\tau_{TNF\alpha}}; \quad T_{5_TNF\alpha}(0) = R_{0_TNF\alpha} \quad (13)$$

Five transit compartments were optimal based on comparison of Akaike Information Criterion (AIC) for the tested models. The equation describing changes in time of $P'(t)$ variable that accounts for stimulation of IL-17 precursor production is:

$$\frac{dP'}{dt} = \begin{cases} 0 & \text{for } t < 0.5, \\ -P' \times k'_{deg} & \text{for } t \geq 0.5, \end{cases} \quad P'(0) = P'_{t0}, \quad (14)$$

where P'_{t0} is the initial quantity of the $P'(t)$ variable, k'_{deg} is the first-order rate constant of $P'(t)$ variable loss. An indirect response model combined with five transit steps described the turnover of IL-17:

$$\frac{dT_{1_IL17}}{dt} = \begin{cases} 0 & \text{for } t < 0.5, \\ (1 + P') \times \left(1 - \frac{I_{max_IL17} \times (C_{cAMP} - R_{0_cAMP})}{IC_{50_IL17} + (C_{cAMP} - R_{0_cAMP})}\right) \times k_{in_IL17} - k_{out_IL17} \times T_{1_IL17} & \text{for } t \geq 0.5, \end{cases} \quad \text{with} \quad (15)$$

$$T_{1_IL17}(0) = R_{0_IL17},$$

$$\frac{dT_{n+1_IL17}}{dt} = (T_{n_IL17} - T_{n+1_IL17}) \times \frac{1}{\tau_{IL17}}, \quad T_{n+1_IL17}(0) = R_{0_IL17}, \quad n = 1, 2, 3 \quad (16)$$

$$\frac{dC_{IL17}}{dt} = (T_{4_IL17} - C_{IL17}) \times \frac{1}{\tau_{IL17}}, \quad C_{IL17}(0) = R_{0_IL17}, \quad (17)$$

where $T_{1_IL17} - T_{4_IL17}$ are the quantities of precursors of IL-17 in subsequent transit compartments, k_{in_IL17} is the zero-order production rate constants of the first IL-17 precursor, $k_{in_IL17} = R_{0_IL17} \times k_{out_IL17}$, and k_{out_IL17} is the first-order degradation rate constants of the IL-17 precursor. The I_{max_IL17} is the maximum inhibition of the IL-17 precursor production by cAMP, IC_{50_IL17} is the increase in concentration of cAMP above baseline producing 50% of I_{max_IL17} , and C_{IL17} is the serum concentration of IL-17.

The following equations constitute indirect response models accounting for increases in ALT (A_{ALT}) and AST (A_{AST}) serum activities arising from liver cell damage triggered by TNF α and IL-17:

$$\frac{dA_{ALT}}{dt} = (1 + (T_{5_TNF\alpha} - R_{0_TNF\alpha}) \times (C_{IL17} - R_{0_IL17}) \times S_{ALT}) \times k_{in_ALT} - k_{out_ALT} \times A_{ALT},$$

$$A_{ALT}(0) = R_{0_ALT}, \quad (18)$$

$$\frac{dA_{AST}}{dt} = (1 + (T_{5_TNF\alpha} - R_{0_TNF\alpha}) \times (C_{IL17} - R_{0_IL17}) \times S_{AST}) \times k_{in_AST} - k_{out_AST} \times A_{AST},$$

$$A_{AST}(0) = R_{0_AST}, \quad (19)$$

where k_{in_AST} and k_{in_ALT} are the zero-order rate constants of AST and ALT production, k_{out_AST} and k_{out_ALT} are the first-order rate constants of AST and ALT degradation, and R_{0_AST} and R_{0_ALT} are the baseline activities of AST and ALT in healthy mice. The S_{AST} and S_{ALT} are stimulation coefficients accounting for production of AST and ALT in response to increases in IL-17 and TNF α concentrations. The exact mechanism of hepatocyte damage mediated by IL-17 and TNF α in ConA-induced hepatitis is not entirely elucidated; however, TNF α has been shown to induce apoptosis and necrosis of liver cells and to induce expression of other inflammatory mediators participating in liver damage (Leist et al., 1995; Trautwein et al., 1998). Similarly, IL-17 is responsible for increased production of other pro-inflammatory cytokines that may trigger hepatocyte death (Yan et al., 2012). We assumed a combined stimulatory effect of IL-17 and TNF α on aminotransferase activity in mouse serum. Both ALT and AST activities in serum are commonly used biomarkers of liver injury, since they are released into the blood from damaged hepatocytes (Fetterly et al., 2008; Min et al., 2018; Smith et al., 2019).

Simulation

The predictive performance of the PK/PD model was assessed using our published data (Świerczek et al., 2021). The previous study was performed in female BALB/c mice, which were dosed with the investigational PDEi GRMS-55 and subsequently challenged IV with ConA at a dose of 20 mg·kg⁻¹. In that study, GRMS-55 was given IP at two dose levels, 50 and 100 mg·kg⁻¹ at 30 min before ConA challenge. GRMS-55 inhibits human recombinant PDE with *IC*₅₀ values of 13.4 μM for PDE3, 26.9 μM for PDE4, and 7.3 μM for PDE7 (Świerczek et al., 2020). According to the results of our previous investigation involving a molecular modeling approach, we assume that GRMS-55 is a competitive PDE inhibitor (Chłóń-Rzepa *et al.*, 2018). However, this assumption was not experimentally proven. The inhibition constant (*K*_i) values for GRMS-55 against these PDE were calculated. If a *K*_i value for a particular inhibitor against a corresponding enzyme is not available, it can be calculated from an *IC*₅₀ value (Cheng and Prusoff, 1973). For competitive enzymatic inhibitors:

$$K_i = \frac{IC_{50}}{1 + \frac{a}{K_m}} \quad (20)$$

where *a* is the concentration of a substrate (cAMP) in the assay and *K*_m is a Michaelis constant of cAMP against the corresponding PDE. By using these calculated *K*_i values and our PK parameters of GRMS-55, simulations of changes in TNFα serum concentrations and ALT and AST activities in ConA-induced AIH in mice were performed. Since baseline serum TNFα concentration and ALT/AST activities in mouse serum were different in this data set, those values were adjusted in the PK/PD model.

Data Analysis and Computation

ADAPT5 (Biomedical Simulations Resource, University of South California, Los Angeles) was utilized to fit the PK and PK/PD models to the data using a naïve-pooled approach and to perform simulations. The model code is provided (Supp.) The parameters were estimated using the maximum likelihood algorithm. The variance model was a combined additive and proportional error model:

$$V_i = (\sigma_1 + \sigma_2 \times Y_i(t_i))^2 \quad (21)$$

where *V*_{*i*} is the variance of the drug concentration or response at the *i*th time point (*t*_{*i*}), *Y*_{*i*}(*t*_{*i*}) is the model prediction at the *i*th time point, and *σ*₁ and *σ*₂ are additive and proportional error terms. The

goodness of fit of tested PK and PK/PD models was analyzed using visual inspection of model fitting, the AIC, and the coefficients of variation (CV%) of the estimated parameters. First, PK parameters were estimated, which were then fixed to run the PK/PD model. The PK/PD model was fitted simultaneously to all sets of PD data; therefore, a single set of PD parameters was obtained. The area under the intracellular cAMP curve was calculated from model predictions using the linear trapezoidal method. Maximum concentration (C_{max}) and time of maximum concentration (t_{max}) values were obtained from the model predictions. Graphs and illustrations were generated using OriginPro 2021b (OriginLab Corporation, Northampton, MA) and CorelDRAW 2020 (Corel Corporation, Ottawa, Canada).

RESULTS

Pharmacokinetics

The PK of cilostazol, rolipram, and BRL-50481 in male BALB/c mice were assessed following their IP administration. A one-compartment model with first-order absorption and linear elimination was used for cilostazol and a two-compartment model with first-order absorption and linear elimination was used for rolipram and BRL-50481. Profiles of the investigated compounds along with model fittings are presented in **Fig. 3**. We could not provide absolute (i.e. IV dose) values of V or CL of the drugs since we did not administer the drugs intravenously. Therefore, only apparent values of V/F and CL/F could be estimated using the PK models.

All compounds were rapidly absorbed from the peritoneal cavity. For BRL-50481 and rolipram, the first sampling point at 5 min after dosing was the observed t_{max} in serum. Fitted serum concentration-time profiles of the PDEi well captured the experimental data for all compounds.

The rolipram concentrations in serum were above the K_i value for this compound against PDE4 for around 0.9 h. Serum concentrations of BRL-50481 exceeded the K_i value against PDE7 for approximately 2.1 h; for cilostazol they remained above the K_i value against PDE3 for more than 4 h after administration. The estimated values of PK parameters of the compounds are presented in **Table 1**. The serum terminal half-life for rolipram was 12 min, for BRL-50481 it was 20 min, and for

cilostazol it was 47 min. Due to the lack of early sampling points (earlier than 5 min) we were not able to precisely estimate k_a values for rolipram and BRL-5048. Therefore, these values were fixed based on the preliminary results of model fittings.

PK/PD Modeling

A semi-mechanistic PK/PD model was developed that describes anti-inflammatory and hepatoprotective effects of selective PDE3, PDE4, and PDE7 inhibitors in this mouse model of AIH. As it can be seen in **Fig. 4**, the fittings with the model fairly well captured serum concentration- and activity-time profiles of the biomarkers. Concentration-time profiles of TNF α (**Fig. 4A-C**) show that TNF α reached the peak concentration within 1 h following ConA administration. The fitted t_{max} of TNF α was 0.9 h after ConA challenge. Rolipram most strongly suppressed TNF α and reduced the mean observed serum C_{max} of this cytokine by 63%, while cilostazol and BRL-50481 decreased TNF α by 36% and 48% at 1 h following ConA compared to the control group. The model-fitted t_{max} of IL-17 was 3.6 h. The mean concentration of IL-17 measured at 4 h post-ConA was reduced by 76% after rolipram and by 6% for cilostazol and BRL-50481. However, at 8 h post-ConA dosing, IL-17 concentrations were reduced by 64% and 50% for cilostazol and BRL-50481, and by 42% for rolipram. These results indicate a rapid and strong effect of rolipram and a weaker but prolonged anti-inflammatory activity following both cilostazol and BRL-50481.

The model-fitted t_{max} of transaminase activities in serum were 12.7 h for ALT and 11.2 h for AST after ConA (**Fig. 4**). The ALT serum activity, which is a more liver-specific biomarker, was the most strongly reduced (by 57%) by rolipram at 16 h. In the case of the cilostazol treated group, ALT was reduced by 43%, and for BRL-50481 by 41% compared to the control group at 16 h post-ConA. The values of the PD parameters estimated using the PK/PD model are listed in **Table 2**.

The rate of IL-17 production seems to be similarly sensitive to the elevation in intracellular cAMP concentrations as the rate of TNF α production, since the IC_{50} value of cAMP for production of the IL-17 precursor was 33% lower than that of TNF α and this difference was close to the CV% for these parameters. The maximum hydrolytic activity of PDE4 against cAMP was estimated to contribute by 59% to the overall PDE hydrolytic activity against cAMP in mouse T-cells. This value is

higher than those for PDE3 and PDE7, which, based on the model estimation, constitute 21% and 20%, of the total PDE activity against cAMP. These findings are in line with previous studies indicating the highest contribution of PDE4, while there is lower and almost equal participation of PDE3 and PDE7 in the PDE hydrolytic activity against cAMP in mouse lymph node lysates (Yang *et al.*, 2003). The PK/PD model predicted intracellular cAMP concentration-time profiles in mouse effector T-cells as shown in **Fig. 5**.

According to these predictions, administration of rolipram at an IP dose of 10 mg·kg⁻¹ caused the highest and sharpest increase in intracellular cAMP with the C_{max} of 8.7 μM at 0.4 h post-rolipram dosing, while BRL-50481 and cilostazol produced 3.5- and 3.8-fold less at the C_{max} of 0.2 h and 0.4 h. The area under the effective intracellular cAMP concentration-time curve (i.e., calculated above the baseline cAMP concentration of 1 μM) from time 0 to 4 h was 4.6 μM·h for rolipram, 1.3 μM·h for BRL-50481, and 2.7 μM·h for cilostazol. Therefore, the high and sharp increase in intracellular cAMP after rolipram caused the strongest anti-inflammatory and, in consequence, hepatoprotective effect compared to the other compounds. Thus, the predicted concentration-time profiles of cAMP in T-cells are consistent with the rapid and strong anti-inflammatory effect of rolipram and weaker but prolonged activities of cilostazol and BRL-50481.

The elimination half-life of ALT from serum of 12.5 h calculated as $\ln(2)/k_{out_ALT}$ was twice as long as that of AST, while literature data indicate a half-life of 25 h for ALT in mice and 3-10 h in rats (York, 2017; Smith *et al.*, 2019). The stimulation coefficient of ALT production was 12% lower than that for AST production indicating that the rate of AST release into the blood is slightly more sensitive to increases in TNFα and IL-17 than the rate of ALT release.

Predictive Performance of the PK/PD Model

The predictive performance of the newly designed PK/PD model was assessed using the results from our previous publication on the non-selective PDEi GRMS-55 (Świerczek *et al.*, 2021). The observed serum concentration-time profiles of GRMS-55 and the results of PK model predictions are presented in **Fig. 6A**. The calculated values of K_i and PK parameter estimates for GRMS-55 are listed in **Table 3**. These K_i values and PK parameters as well as the PD parameter estimates obtained in the present

study (**Table 2**) were utilized to perform a simulation of the expected anti-inflammatory and hepatoprotective effects of GRMS-55 in the mouse model of AIH. The model-predicted intracellular cAMP concentrations are presented in **Fig. 6B**. The observed time-courses of TNF α , ALT, and AST in mouse serum along with the model predictions are presented in **Fig. 7**. The TNF α concentrations in the vehicle-treated group were reasonably predicted by the PK/PD model, while groups pre-treated with GRMS-55 were moderately overpredicted. The possible reason for this overprediction might be other unknown mechanisms of action of GRMS-55 that are not related to PDE inhibition. This compound is a theophylline derivative, which may interact with adenosine receptors, thereby activating other anti-inflammatory pathways (Boswell-Smith *et al.*, 2006). Also, the K_i values calculated for GRMS-55 using the Cheng and Prusoff equation could have been overestimated leading to underprediction of intracellular cAMP concentrations and anti-inflammatory effects of this compound. In general, the extent of ALT and AST production induced by TNF α and IL-17 was reasonably predicted by the model; however, transaminase activities were underpredicted at early time points in all groups of mice. It is important to note that the onset of AIH in female mice occurred earlier compared to male mice, since transaminase activities increased at 2 h post-ConA dosing in females, while they remained at baseline levels until 4 h in males. This observation indicates that there may exist sex-related differences in disease development, which were also observed in previous studies involving a ConA-induced hepatitis model (Takamoto *et al.*, 2003).

DISCUSSION

The most common treatment strategies of AIH are based on the use of immunosuppressive drugs in combination with corticosteroids (Cropley and Weltman, 2017). Several alternative treatment options of AIH have been tested, such as use of biologics or adoptive transfer of regulatory T-cells, but they display limited efficacy, exhibit rare but severe adverse effects, and are often unavailable to patients due to high costs (Czaja, 2016). The PDEi, especially those inhibiting PDE3, PDE4, and PDE7, are currently being investigated for the treatment of immune-related disorders (Kumar *et al.*, 2013; Jankowska *et al.*, 2017; Ručilová *et al.*, 2021). Cilostazol is a drug registered for treatment of intermittent claudication (Liu *et al.*, 2001). Rolipram has been investigated as a potential anti-inflammatory and antidepressant drug (Kumar *et al.*, 2013). BRL-50481 is an investigational PDE7 inhibitor that displayed anti-inflammatory activity in several animal models of immune-related disorders (Christensen *et al.*, 2012; Martín-Álvarez *et al.*, 2017).

The terminal half-life of cilostazol in our mice was substantially shorter than that found in rats following oral dosing, which was 2.4 h (Yoshioka *et al.*, 2018). Similarly, the rolipram terminal half-life in mice was shorter compared to the values reported for rats, rabbits, monkeys, and humans, which ranged 1-3 h (Krause and Kühne, 1988). To our knowledge, the PK of BRL-50481 has not been reported. Our study demonstrates rapid absorption, biphasic disposition, and relatively fast elimination with a terminal half-life less than 30 min.

ConA-induced hepatitis in mice is characterized by extensive production and release of inflammatory mediators, such as interleukins, TNF α , and IFN- γ (Wang *et al.*, 2012; Yan *et al.*, 2012). TNF α engages in the pathophysiological mechanisms of ConA leading to apoptosis and necrosis of hepatocytes (Gantner *et al.*, 1997; Trautwein *et al.*, 1998; Wolf *et al.*, 2001; Filliol *et al.*, 2016). Similarly to the results of previous investigations in mice, in this study TNF α serum concentrations peaked around 1 h following ConA and were decreased in the presence of PDEi (Gantner *et al.*, 1997; Goto *et al.*, 2009; Świerczek *et al.*, 2020). IL-17 expression and serum concentrations were monitored in several previous studies involving the ConA-induced AIH model in mice (Nagata *et al.*, 2008; Yan *et al.*, 2012). Based on the literature, in ConA-induced hepatitis, TNF α is mainly produced by liver-

associated T helper cells and by peripheral blood T helper cells, while IL-17 is mainly produced by Th17 subset (Cao *et al.*, 1998; Kawaguchi *et al.*, 2004). The present model does not take into consideration different types and subsets of T cells and this issue should be investigated in future studies. In our PK/PD model, we assumed a combined stimulatory effect of TNF α and IL-17 on the release of ALT and AST into blood. This assumption agrees with the literature indicating that inhibition of TNF α as well as IL-17 signaling leads to reduced liver damage in ConA-induced hepatitis (Trautwein *et al.*, 1998; Yan *et al.*, 2012). Thus IL-17 may constitute a potential therapeutic target for the treatment of AIH.

Serum ALT and AST are commonly used markers of liver damage in both humans and animals, but they have some limitations. Serum ALT has greater specificity for liver injury, since only small quantities of this enzyme are present in the skeletal muscle, heart, and kidneys. In turn, AST serum activity, due to its abundance in different tissues, may also increase in several other pathological states, such as myocardial infarction, acute pancreatitis, renal disease, hemolytic anemia, or trauma (Smith *et al.*, 2019). Serum activities of ALT and AST were previously used as biomarkers of liver injury in studies involving PK/PD modeling related to drug-induced hepatotoxicity (Fetterly *et al.*, 2008; Bhavnani *et al.*, 2019) as well as in a rat model of cholestatic liver injury (Min *et al.*, 2018). Recently, a general model was proposed to describe biomarker release after cellular insult (Li *et al.*, 2021). This model, which is an extension of a previously developed mathematical model (Remien *et al.*, 2012), captured plasma ALT changes after paracetamol overdose. Serum ALT and AST activities were used in our previous study involving a ConA-induced hepatitis model (Świerczek *et al.*, 2021). To our knowledge, the latter study involved the first mathematical description of this mouse model of AIH. The PK/PD model presented herein may be considered the second-generation model of PDEi effects in ConA-induced hepatitis, since the previously developed PK/PD model that described the effects of GRMS-55 was composed of more empirical components. Features and strengths of the present model are that the known K_i values for the three drugs were employed (Table 2) and the joint responses to all three drugs were used to describe the turnover parameters for TNF α , IL-17, cAMP,

ALT, and AST. This allowed the totality of the control and drug affected responses to contribute to the estimated model parameters.

Intracellular cAMP concentrations regulate expression and production of inflammatory mediators (Shin *et al.*, 1998; Raker *et al.*, 2016). Plasma and tissue concentrations of cAMP were used as markers of pharmacological response in several previous investigations involving PK/PD modeling (Jin *et al.*, 2004; Świerczek *et al.*, 2017). In this study while intracellular cAMP was not measured, a mechanistic component representing cAMP concentrations in mouse T-cells that describes its changes caused by selective PDEi was employed. This approach enabled linking the PK of the investigated compounds to their anti-inflammatory activity. The model-estimated IC_{50} values of cAMP on production of TNF α and IL-17 were close to measured cAMP intracellular concentrations required to activate PKA in intact cells (Koschinski and Zaccolo, 2017). Since selective PDEi were used in this investigation, it was possible to assess partial contributions of each PDE type to regulation of intracellular cAMP in T-cells. PDE4 has the most prominent impact on the intracellular cAMP concentrations in mouse T-cells, while PDE3 and PDE7 are responsible for approximately 21% and 20% of the total PDE hydrolytic activity against cAMP. This observation is supported by a study showing that PDE4 was mainly responsible for cAMP degradation in mouse lymph node lysates, while both PDE3 and PDE7 displayed lesser and almost equal hydrolytic activity (Yang *et al.*, 2003).

The PK/PD model developed in this study was tested by predicting the anti-inflammatory and hepatoprotective activities of GRMS-55. In general, our PK/PD model reasonably predicted the serum time-courses of TNF α , ALT, and AST. The modest discrepancies may arise from GRMS-55 being a theophylline derivative that may interact with adenosine receptors (Boswell-Smith *et al.*, 2006) and from sex-related differences in the rate and extent of liver damage in ConA-induced hepatitis (Takamoto *et al.*, 2003). Therefore, for simulating PDEi effects in female mice using model parameters obtained in this study, additional experiments may be required to adjust values of some parameters, such as in the pharmacokinetics as well as S_{ALT} and S_{AST} .

The limitations of this study were that cilostazol concentrations were only measured up to 4 h after dosing, and at this time point they were still relatively high. However, according to the model

predictions, intracellular cAMP concentration in T-cells almost returned to baseline at 4 h post-cilostazol dosing. Furthermore, cAMP concentrations in immune cells were not monitored but only inferred based on the available profiles for other biomarkers. Literature data indicate that there exists a compartmentation of cAMP within cells (Johnstone *et al.*, 2018). This model does not accommodate a compartmentation and spatial distribution of cAMP within the intracellular space. Also, this study might have been expanded by adding various doses of PDEi or different schedules including dosing of the compounds after ConA injection. While the IP dosing of the PDEi is convenient and may enhance first-pass exposures of the liver to the drugs, dosing by other routes such as IV and oral would help extend the current version of the PK/PD model. The PK/PD model does not take into consideration the role of protein binding of the investigated compounds and its possible impact on their uptake into T cells and subsequent interaction with PDE. No reliable data regarding serum protein binding of cilostazol and BRL-50481 in mice were available. Nonetheless, the proposed PK/PD model reasonably described the relationships between the PK of the investigated compounds and their comparative anti-inflammatory and hepatoprotective activities. Besides hepatocytes and T cells that appear to be key players in AIH development, stellate cells and Kupffer cells are also important since they participate in the interactions between immune cells and hepatocytes (Wang *et al.*, 2012). Therefore, it would be reasonable to investigate and account for the contributions of these liver cells to disease development in future studies. Elucidating what types of immune cells are crucial in disease development, their location during disease onset, and how the investigated compounds are distributed to these cells to better describe their concentration-effect relationships would be of interest.

The present or extended PK/PD model might be applicable in a clinical study as follows. The pharmacokinetics of the drugs, including protein binding, over a range of doses can be directly obtained in human subjects. Concentrations of cAMP and drug inhibition of ConA-induced cytokine production in various types of lymphocytes in collected blood samples can be assessed in vitro both with drug added in cell cultures and being present in vivo after dosing human subjects to generate *Imax* and *IC50* values. The turnover parameters of cAMP, cytokines and ALT and AST can be adjusted from mice to humans using allometric scaling. Patients with various degrees of AIH could be

assumed to start at a disease-induced steady-state of hepatic damage with elevated serum cytokines and ALT and AST that can be accommodated in the PK/PD model to anticipate and quantitate the effects of the drugs on the expected improvements during a multiple-dosing drug trial. The ex vivo effects of the PDEi on lymphocytes from both mice and such patients could be assessed for possible prognostic use.

In conclusion, an advanced semi-mechanistic PK/PD model was developed that describes interrelations among the PK of PDEi, their inhibitory activity against PDE in immune cells, cAMP concentrations in T-cells, serum TNF α and IL-17 concentrations, and their joint effect on serum transaminase activities. According to the model predictions, PDE4 has the largest contribution to the total PDE hydrolytic activity against cAMP in mouse T-cells but PDE3 and PDE7 also contribute. Balanced inhibition of PDE3, PDE4, and PDE7 leading to the reduction of TNF α and IL-17 production may be a promising alternative treatment strategy for AIH. The developed PK/PD model may be used to design experiments involving ConA-induced hepatitis and to select promising PDEi and their combinations with other drugs for further preclinical investigation.

AUTHORSHIP CONTRIBUTIONS

Participated in research design: A.Š. and E.W.

Conducted experiments: A.Š. and B.P.

Performed data analysis: A.Š.

Wrote or contributed to the writing of the manuscript: A.Š., E.W., and W.J.J.

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Figure Captions

Figure 1. Schematic of the PK/PD model to describe the time courses of the PDEi, concentrations of TNF α and IL-17, and activities of ALT and AST in the serum of male BALB/c mice pre-treated IP with BRL-50481, rolipram, cilostazol, or vehicle and subsequently challenged IV with ConA. The symbols are defined with the equations and listed in **Tables 1** and **2**. Subscripts BRL, CIL, and ROL refer to BRL-50481, cilostazol, and rolipram.

Figure 2. Proposed mechanism of PDEi-mediated regulation of inflammatory cytokine production in T-cells. Key: AC – adenylyl cyclase, PKA – protein kinase A, NF- κ B – nuclear factor kappa-light-chain-enhancer of activated B cells, AP-1 – activator protein 1.

Figure 3. Serum concentration-time profiles of cilostazol (**A**), BRL-50481 (**B**), and rolipram (**C**) administered IP to male BALB/c mice. Symbols represent mean ($\pm$ SD) measured values ($n=3$), solid curves depict concentrations fitted by the pharmacokinetic models, and dashed lines represent the K_i values of the studied compounds against the corresponding PDE.

Figure 4. Serum concentration-time profiles of TNF α (**A-C**), IL-17 (**D-F**), and activity-time profiles of ALT (**G-I**) and AST (**J-L**) following IP administration of BRL-50481, rolipram, cilostazol, or vehicle to male BALB/c mice followed by IV challenge with ConA. Symbols are measured values and solid curves depict fittings with the PK/PD model. Note: Upper curves are measurements after ConA and vehicle control; lower curves are with the indicated PDEi treatments.

Figure 5. PK/PD model-predicted intracellular cAMP concentration-time profiles in T-cells following IP administration of selective PDEi in mice.

Figure 6. (A) Mean ($\pm$ SD) serum concentrations of GRMS-55 following IP administration at doses of 50 or 100 mg $\cdot$ kg $^{-1}$ in female BALB/c mice. Observed quantities are shown as symbols, and concentrations predicted by the PK/PD model are shown as solid curves. The blue dashed line indicates the K_i value for GRMS-55 against PDE7, red dashed line against PDE4, and green dashed line against PDE3. **(B)** PK/PD model-predicted intracellular cAMP concentration-time profiles in T-cells following IP doses of GRMS-55.

Figure 7. Serum concentrations of TNF α (**A-C**) and activities of ALT (**D-F**) and AST (**G-I**) in mice challenged IV with ConA in the absence or presence of GRMS-55 at IP doses of 50 or 100 mg·kg⁻¹. Observed quantities are shown as symbols, PK/PD model simulations are solid lines.

Tables

Table 1.

Pharmacokinetic parameter estimates for BRL-50481, rolipram, and cilostazol following IP administration in male BALB/c mice.

		BRL-50481	Rolipram	Cilostazol
Parameter (Units)	Definition	Final Estimate (CV%)		
V_c/F or V_d/F ($L \cdot kg^{-1}$)	Apparent volume of distribution	2.106 (22.3)	1.179 (14.4)	1.164 (0.7)
V_t/F ($L \cdot kg^{-1}$)	Peripheral volume of distribution	2.775 (39.6)	0.4028 (19.3)	-
k_a (h^{-1})	Absorption rate constant	13.9 (fix ^a)	11.3 (fix ^a)	10.45 (1.8)
CL_T/F ($L \cdot h^{-1} \cdot kg^{-1}$)	Apparent total clearance	18.65 (7.9)	13.99 (5.6)	1.219 (0.4)
CL_D/F ($L \cdot h^{-1} \cdot kg^{-1}$)	Distribution clearance	8.363 (37.1)	1.489 (26.9)	-
$t_{0.5}$ (min)	Terminal half-life	20	12	47

^afixed based on the results of preliminary model fitting

Table 2.

Pharmacodynamic parameter estimates for effects of BRL-50481, rolipram, and cilostazol in male BALB/c mice challenged IV with ConA.

Parameter (Units)	Definition	Final Estimate (CV% or Reference)
P_{i0}	Initial value of TNF α production variable	475.5 (38.6)
k_{deg} (h $^{-1}$)	Decay rate constant of TNF α production variable	3.56 (37.1)
$k_{out_TNF\alpha}$ (h $^{-1}$)	TNF α elimination rate constant	0.179 (6.4)
P'_{i0}	Initial value of IL-17 precursor production variable	251.4 (45.9)
k'_{deg} (h $^{-1}$)	Decay rate constant of IL-17 precursor production variable	1.393 (43.9)
k_{out_IL17} (h $^{-1}$)	IL-17 precursor elimination rate constant	0.2653 (9.3)
$IC_{50_TNF\alpha}$ (μ M)	Increase in concentration of cAMP above baseline at 50% of I_{max_TNF}	2.81 (43.7)
IC_{50_IL17} (μ M)	Increase in concentration of cAMP above baseline at 50% of I_{max_IL17}	1.89 (35.9)
$I_{max_TNF\alpha}$	Maximum inhibition of TNF α production by cAMP	1 (fix a)
I_{max_IL17}	Maximum inhibition of IL-17 precursor production by cAMP	1 (fix a)
K_{i_BRL} (μ M)	Inhibition constants of compounds against corresponding PDE	0.185 (fix a) (Smith et al., 2004)
K_{i_ROL} (μ M)		0.121 (fix a) (Ohsawa et al., 1998)
K_{i_CIL} (μ M)		0.57 (fix a) (Zhang et al., 2002)
V_{max_PDE7} (μ M $\cdot$ h $^{-1}$)	Maximum activities of PDE type against cAMP in a mouse T-cell	28.6 (SP b)
V_{max_PDE4} (μ M $\cdot$ h $^{-1}$)		84.4 (9.8)
V_{max_PDE3} (μ M $\cdot$ h $^{-1}$)		30.0 (15.2)
V_{max} (μ M $\cdot$ h $^{-1}$)	Maximum activity of PDE against cAMP in a mouse T-cell	143 (fix a) (Yang et al., 2003)
K_{m_PDE7} (μ M)	Michaelis constants of cAMP for each PDE type	0.1 (fix a) (Han et al., 1997)
K_{m_PDE4} (μ M)		1.5 (fix a) (Huston et al., 1997)
K_{m_PDE3} (μ M)		0.18 (fix a) (Bender and Beavo, 2006)
$\tau_{TNF\alpha}$ (h)	Mean transit time of TNF α	1.474 (12.7)
τ_{IL17} (h)	Mean transit time of IL-17 precursor	0.344 (15.1)
S_{ALT} (L 2 $\cdot$ ng $^{-2}$)	Stimulation coefficient of ALT production by TNF α and IL-17	0.0092 (16.9)

$S_{AST} (\text{L}^2 \cdot \text{ng}^{-2})$	Stimulation coefficient of AST production by $\text{TNF}\alpha$ and IL-17	0.0105 (17.8)
$k_{out_ALT} (\text{h}^{-1})$	ALT elimination rate constant	0.0555 (24.4)
$k_{out_AST} (\text{h}^{-1})$	AST elimination rate constant	0.1080 (16.8)
^a fixed based on the assumptions or literature data, ^b secondary parameter		

Table 3.

Pharmacokinetic parameters and K_i values of GRMS-55 used in the simulation.

Parameter (Units)	Definition	Final Estimate (CV%)
Vd/F ($L \cdot kg^{-1}$)	Apparent volume of distribution	1.81 (fix ^a)
k_a (h^{-1})	Absorption rate constant	1.49 (fix ^a)
CL_T/F ($L \cdot h^{-1} \cdot kg^{-1}$)	Apparent total clearance	8.31 (fix ^a)
K_{i_PDE3} (μM)	Inhibition constants of GRMS-55 against corresponding types of PDE	10.8 (fix ^a)
K_{i_PDE4} (μM)		26.0 (fix ^a)
K_{i_PDE7} (μM)		4.9 (fix ^a)

^afixed based on literature data

Figure 1

Figure 2

Figure 3

Figure 4

Intracellular cAMP concentration (uM)

Figure 5

Figure 6

Figure 7