The Pharmacokinetic-Pharmacodynamic Model of Azithromycin for Lipopolysaccharide-Induced Depressive-Like Behavior in Mice

Kun Hao1, Qu Qi1, Haiping Hao1, Guangji Wang1*, Yuancheng Chen2, Yan Liang1, Lin Xie1

1 State Key Laboratory of Natural Medicines, Key Lab of Drug Metabolism & Pharmacokinetics, China Pharmaceutical University, Nanjing, China, 2 Institute of Antibiotics, Huashan Hospital, Fudan Universitry, Shanghai, China

Abstract

A mechanism-based model was developed to describe the time course of lipopolysaccharide-induced depressive-like behavior and azithromycin pharmacodynamics in mice. The lipopolysaccharide-induced disease progression was monitored by lipopolysaccharide, proinflammatory cytokines, and kynurenine concentration in plasma. The depressive-like behavior was investigated by forced swimming test and tail suspension test. Azithromycin was selected to inhibit the surge of proinflammatory cytokines induced by lipopolysaccharide. Disease progression model and azithromycin pharmacodynamics were constructed from transduction and indirect response models. A delay in the onset of increased proinflammatory cytokines, kynurenine, and behavior test compared to lipopolysaccharide was successfully characterized by series transduction models. The inhibition of azithromycin on proinflammatory cytokines was described by an indirect response model. After lipopolysaccharide challenging, the proinflammatory cytokines, kynurenine and behavior tests would peak approximately at 3, 12, and 24 h respectively, and then the time courses slowly declined toward a baseline state after peak response. During azithromycin administration, the peak levels of proinflammatory cytokines, kynurenine and behavior indexes decreased. Model parameters indicated that azithromycin significantly inhibited the proinflammatory cytokines level in plasma and improved the depressive-like behavior induced by inflammation. The integrated model for disease progression and drug intervention captures turnovers of proinflammatory cytokines, kynurenine and the behavior results in the different time phases and conditions.

Introduction

Increased major depressive-like disorders occur in many diseases (e.g., atherosclerosis, congestive heart failure, rheumatoid arthritis), all of which have a common inflammatory component [1]. For example, depressive-like behaviors were observed in patients undergoing cytokine immunotherapy for the treatment of cancers [2,3]. In these conditions, depressive symptoms were induced by proinflammatory cytokines (PGs), mainly interleukin-1β (IL-1β), interleukin-6 (IL-6), interferon-γ (IFN-γ), and tumor necrosis factor-alpha (TNF-α) [4,5].

Indoleamine 2,3-dioxygenase (IDO) is presented in macrophages and other cells that degrades tryptophan along the kynurenine (KYN) pathway. It was reported thatIDO could be easily activated by PCs [6,7], and its degree of activation (indicated by increased KYN concentration) was correlated to the intensity of depressive symptoms, as observed in cancer patients chronically treated with immunotherapy [7,8]. The increased KYN concentration in circulating induced by PCs was potential to negatively impact serotoninergic neurotransmission in central nervous system (CNS) [6]. Meanwhile, KYN was readily transported across the blood brain barrier into the brain where it could be further metabolized by perivascular macrophages, microglia and astrocytes to generate neuroactive glutamatergic compounds [9,10]. In fact, heightened glutamate activity might play an important role in major depression [11,12]. Several studies evidenced that PCs-IDO -KYN - depressive-like behavior formed a complete relationship of inflammation-induced depressive-like behavior. Many papers reported that acute activation of the peripheral innate immune system in laboratory animals through the administration of the cytokine inducer lipopolysaccharide (LPS) induced depressive-like behavior, which could be attenuated by classical antidepressant administration in some treatments [13,14]. While in order to test whether the activation of PCs by LPS was responsible for development of major depressive disorders, we investigated the depressive-like behavior after the inhibition of PCs in acute immune stimulation. Azithromycin (AZI) is one of the second generation macrolide antibiotics which is prepared semi-synthetically from erythromycin. AZI differs structurally from erythromycin by the presence of methyl-substituted nitrogen at position 9a in the macrolide ring. This modification leads to significant advantages for AZI, such as better stability in acidic pH [15], a longer half-life in serum, better tissue distribution with high peak levels, and a longer mean residence time [16,17]. In the present
study, AZI was chosen to reverse the PCs, because AZI has potent anti-inflammatory effects independent from its microbicidal properties, as it is well known to inhibit macrophage and microglial activation [18,19]. We found that anti-inflammatory effects with AZI approach could abrogate LPS-induced depressive like behavior.

The pharmacokinetic-pharmacodynamic (PK-PD) model is a potential tool for enhancing the efficiency of decision-making in drug development [20]. PK-PD model-based approaches have been rapidly developed because of the insights they provide into how drugs exert their effects, thus improving the knowledge about drug processes and properties for extrapolation and prediction [21]. The PK-PD model has been widely applied in the field of CNS disorders and drugs [22,23]. However, depressive-like behavior induced by PCs is often comorbid complication, which is different with classical depressive-like behavior. The present PK-PD model described the depressive-like behavior induced by PCs and antibiotics intervention, which distinguished from classical antidepressant. The PK-PD model of antibiotics was widely reported to investigate the relationship between the drug concentration and antibacterial effect (minimum inhibitory concentration, etc). However, the PK-PD model of antibiotics on disease progression of depressive-like disorders induced by inflammatory has not been investigated. In the present study, we assessed the potential effect of AZI on the progression of depressive-like behavior in LPS-challenge mice.

Materials and Methods

Animals

All animal care and use were conducted under a license granted by the Jiangsu Science and Technology Office (China) with approval from the Animal Ethics Committee of China Pharmaceutical University. Every effort was made to minimize the stress on the mice. Experiments were performed on 10–14 week-old male CD1 mice obtained from Vital River Laboratories (Beijing, China), whose average body weights were 35–40 g at the beginning of the experiments. Mice were individually housed in standard shoebox cages, with wood shavings litter, in a temperature (23°C) and humidity (45–55%) controlled environment with a 12/12-h modified dark-light cycle (light on 11:00 PM–11:00 AM). Food and water were available ad libitum. Mice were handled individually everyday for 10 days before the experiments.

Chemical

LPS (L-3129, serotype 0127:B8) was purchased from Sigma (St. Louis, MO). AZI was purchased from Dawnrays Pharmaceutical Ltd (Suzhou, China). Other chemicals and solvents were purchased from Nanjing Chemical Reagent Co. Ltd (Nanjing, China).

Study Design

On the day of injection, fresh solutions were prepared by dissolving LPS in sterile endotoxin-free isotonic saline and administered intraperitoneally (i.p.). The dose of LPS (0.8 mg/kg) was selected on the basis of its ability to induce the full spectrum of the acute sickness response and a reliable increase of depressive-like behavior induced by LPS [24]. The TST activity. The TST was conducted as previously described with slight modifications [27]. Briefly, mice were placed individually in a clear cylinder (diameter 10 cm, height 25 cm), containing 15 cm of water at 25±1°C. The water was changed between testing sessions. Mice were forced to swim for 6 min, and the immobility time during the last 5 min was manually measured by a blinded observer. Mice were considered immobile when they ceased struggling, remained floating motionless, and only made those movements necessary to keep their head above the water [28].

TST activity. The TST was conducted as previously described [29]. Briefly, mice were suspended by adhesive tape that was positioned about 2.5 cm from the tail tip with the head 40 cm above the floor. The trial was carried out for 6 min and the duration of immobility was manually recorded by two blinded observers during the final 5 min interval of the test. Mice were considered immobile when they hung passively and motionlessly.
AZI Pharmacokinetics

Fig. 1 showed a general schematic model for the entire PK-PD model. The PK of AZI was described by a one-compartment model with first-order absorption. The drug in the plasma was a driving force for the drug effect. The equations describing the amounts of AZI were as follows:

\[
\frac{dA_a}{dt} = -k_a \cdot A_a; A_a,0 = F_{AZI} \cdot \text{Dose}
\]

\[
\frac{dA_c}{dt} = k_a \cdot A_a - k_e \cdot A_c; A_c,0 = 0
\]

Here, \( A_a \) and \( A_c \) were the amounts of the drug in the central and absorption compartment, respectively. \( A_a,0 \), the initial amount of the drug in absorption compartment; \( A_c,0 \), the initial amount of the drug in central compartment; \( V_{AZI} \) is used to estimate the distribution volume of drug. \( k_a \), the rate constant of the drug from absorption to central compartment; \( k_e \), the rate constant of the drug elimination from central compartment; \( F_{AZI} \), the drug fraction of absorption compartment.

LPS-induced Depressive-like Model and AZI Pharmacodynamics

The animal model with depressive-like behavior induced by LPS in mice is a classical CNS disorder model that is characterized by increased LPS level, inflammation response [30], and KYN concentration in blood. LPS, PCs and KYN levels contributed to the onset of depressive-like behavior in mice. Based on these disease characteristics, LPS, PCs and KYN levels were considered as main disease components in LPS-induced depressive-like disease progression. The dispositions of LPS, PCs and KYN were characterized by transduction models, and the interactions among the three factors were described by indirect response models [31].

Because the LPS were injected i.p., the LPS was assumed to diffuse into circulation by a first-order constant. The disposition of LPS was described by:
The PK-PD Model of Azithromycin

The PK-PD model was constructed in two phases. In the PK phase, non-compartmental analysis by Excel software (Microsoft, Redmond, WA) was first performed for the initial estimated compartmental PK parameters. Fitting of the compartmental PK parameters for AZI was performed using equations 1–2. LPS-induced depressive model was depicted by several transduction compartments of endogenous substances disposition. In the PD model of AZI, the PD was expressed by an indirect response model. The compartmental PD parameters were estimated by the fully integrated model described by equations 5–7. All parameters were estimated by ADAPT II software [34]. The program code of differential functions was showed in appendix (Appendix S1). The naive pool method and bootstrap analysis was used to estimate the model parameters. Individual data were used to estimate the PK-PD parameters by naive pooling approach, which is by maximizing the sum of likelihood (like putting more weight on small values). This method is more advanced in handling large range of values and variability. The goodness-of-fit was assessed by Akaike’s information criterion, examination of residuals, and visual inspection.

Data Analysis

Data were expressed as the mean ± coefficient of variation (CV%). Differences between multiple groups were evaluated by Student’s t-test using SPSS software. Differences were considered statistically significant at P<0.05.

Results

AZI Pharmacokinetics

Table 1 showed the one-compartmental PK parameters of AZI in normal and LPS-challenging mice. The terminal half-lives of AZI in two groups were 17.3 h and 16.5 h respectively. Compared to normal mice, the areas under the concentration-time curves (AUCₜₐₜₜ) increased from 81.7 to 143.6 ug·h/mL, and the peak levels of AZI elevated from 6.1 to 8.9 ug/mL at 2 h. Fig. 2A showed the time-course of AZI levels in plasma after i.g. administration of AZI (100 mg/kg). The predicted plasma concentration curve was well fitted by a one-compartment PK model and estimated parameters.

LPS Pharmacokinetics

To mimic a state that gram-negative bacterial was killed and the LPS was released from the wall of gram-negative bacterial, we injected the LPS to challenge the LPS-PCs-KYN-CNS disorder. No PK difference was observed in normal and AZI-treating mice as shown in table 2. The terminal half-life of LPS in mice were 8.4 and 7.8 h respectively, and the AUCₜₐₜₜ were 2315.2 and 2247.6 Eu·h/mL respectively in normal and LPS-challenge mice. The measured and predicted LPS concentrations in two groups in plasma were shown in Fig. 2B.

LPS Induced-depressive Model

No obvious change of PCs, KYN level and depressive-like behavior was observed in control mice. In the group of AZI only, the time courses of PCs, KYN level and depressive-like behavior were similar to the control mice, so the data were not shown in results. In Fig. 2C, the total PCs concentration was used to model PK-PD parameters. The total peak concentration of PCs at 3 h was 1055 pg/ml after an i.p. LPS injection, [including IL-1β 214 pg/ml, IL-6 287 pg/ml, IFN-γ 176 pg/ml, and TNF-α 376 pg/ml]. After AZI intervention, the increased PCs induced by LPS could be reversed partially. The peak concentration at 3 h decreased to 762 pg/ml, [including IL-1 135 pg/ml, IL-6 202 pg/ml, IFN-γ 126 pg/ml, and TNF-α 299 pg/ml]. As shown in Fig. 2D, the peak time of KYN challenged by LPS appeared at 12 h with an upsurge of 649 ng/mL. In AZI treatment group, the KYN concentration significantly attenuated similar to the total PCs (p<0.05). The depressive-like behavior in CNS including FST and TST were shown in Fig. 3. The peak time of FST and TST were appeared in 24 h, and the depressive-like behavior still had not returned to the baseline until 48 h. The observed profiles of

\[ \frac{dLPS_a}{dt} = -k_{LPS} \cdot LPS_a; \quad LPS_{a,0} = F_{LPS} \cdot Dose' \]  
\[ \frac{dLPS_c}{dt} = k_{LPS} \cdot LPS_a - k_{LPS} \cdot LPS_c; \quad LPS_{c,0} = 0 \]
PCs, KYN level and CNS behavior were reasonably captured by the LPS-induced depressive models and AZI pharmacodynamics.

Final estimated PD parameters and coefficient of variation were summarized in Table 3. We formulated in equation 3–7. PCs₀ and k_{out,PCs} were estimated to 49.8 pg ml⁻¹ and 0.234 h⁻¹; KYN₀ and k_{out,KYN} were 104.3 ng ml⁻¹ and 0.167 h⁻¹. As PD endpoint, the turnover of depressive-like behavior was also be described as a hypothetical input and output rate though the CNS behavior was not a substantial endogenous substance. The FST₀ and k_{out,FST} were 51.3 s and 0.253 h⁻¹, and the TST₀ and k_{out,TST} were 108.2 s and 0.143 h⁻¹. The predictive performance of the model, as shown in Fig. 2 and Fig. 3, was reasonable and adequately reflected the trend and variability of the raw data. The ameliorative effect of AZI on the occurrence of PCs and subsequent results were sufficiently described by the indirect

| Parameter (unit) | Definition | Normal Mice | LPS-Challenging Mice |
|------------------|------------|-------------|----------------------|
| kₐ (h⁻¹) | The absorption rate constant of azithromycin | 0.78 | 0.85 |
| kₑ (h⁻¹) | The elimination rate constant of azithromycin | 0.234 | 0.167 |
| Vᵦₑ/F₁ₐ₀ (L/kg) | Distribution volume of azithromycin | 42.1 | 28.5 |
efficacy of LPS on PCs were also calculated, the estimated SC 50 and Smax were 103.5 Eu/ml and 27.5 for LPS, which showed that the LPS had a high capacity and power to challenge the production of PCs.

Discussion

Depression is a worldwide problem for humans, and commonly selective serotonin reuptake inhibitors and monoamine oxidase inhibitors were used to improve depressive-like behavior [35]. However, their therapeutic effects only manifest in 28%–63% of depressed patients [36]. The classic monoamine hypothesis of depression has been challenged. Accumulating evidences reveals a close linkage between inflammation and depression. Depressive symptoms frequently develop in chronically infected patients accompanied with an increase of PCs [37]. In recent investigation, it has been suggested that inflammation-induced IDO activation resulted in an accumulation of tryptophan metabolite, KYN, ultimately leading to the development of depression [38].

The stimulation of innate immune system and subsequent release of PCs in peripheral by i.p. LPS may also cause neuroinflammation in CNS through both direct (humoral) and indirect (neural) pathway to relay peripheral inflammatory signal to the CNS [39]. A small part of PCs in blood induced by i.p. LPS can gain access through relatively permeable areas of the blood-brain barrier [40]. On the other way, activation of the vagal nerve afferent pathway may also account for CNS inflammation [41]. The neuroinflammation has also activated the IDO in neuronal cells and glial cells and increased brain KYN level. However, the equilibrium percentage of KYN in the brain originated from KYN in the plasma was 78±6% in control mice, while it was 60% in normal rats [42]. The plasma contribution rose to 100±19% with i.p. LPS systemic activation [43]. From these results, the increased KYN level in brain was exclusively derived from the transport of blood KYN level after i.p. LPS, rather than from the synthesis of IDO in brain.

The delay appeared in the time courses among LPS, PCs, KYN, and depressive-like behavior. The different molecular mechanisms contributed to the lag time of multiple PD surrogates and endpoints. It is well known that LPS binds to a LPS binding protein in blood, and the interaction of LPS- LPS binding protein with its receptor triggers the monocyte secretion of several PCs [44]. Some reports have recently shown that the PCs-induced increase of IDO activity was associated with an increased transcription of IDO mRNA. Pharmacological data also showed that the delayed KYN increase induced by synergetic effect of IL-1β, IL-6, IFN-γ and TNF-α were mediated by IDO activation [45]. KYN and KYN metabolites such as quinolinic acid and 3-hydroxykynurenine in brain, which were known to be neurotoxic and thereby might lead to depression-like behaviors through both serotonin and glutamate pathways [46,47].

Macrolide antibiotics are a well established class of antibacterial agents those are active against many gram-positive and gram-negative bacteria. Beyond their antibacterial activity, macrolide antibiotics are reported to exert anti-inflammatory and immunomodulatory activity in vitro and in vivo [48]. It has been reported previously that macrolide antibiotics can affect several steps in the inflammatory process, such as migration of neutrophils, modulation of oxidative burst and production of cytokines [48,49]. AZI is a new broad-spectrum, second-generation macrolide antibiotic for clinical use, which has been widely used to treat a broad array of infectious diseases such as pneumonias, peritonitis and mastitis [50]. AZI reduced the production of PCs, like IL-1β and TNF-α, in response to LPS stimulation, which was responsible for the clinical effectiveness in chronic inflammatory disorders [50,51]. In patients, the LPS was released from the wall of gram-negative bacterial, and occurred in blood when these bacterial were killed by antibacterial agents, so the application of antibacterial agents were double-edged sword to limit the use of antibacterial agents in clinic. In the present study, the administration of LPS mimiced the pathological process of the death of bacteria and the release of LPS from bacteria after AZI administration in systemic bacterial infection. In previous research, minocycline and tetracycline were used to relieve the burst of PCs and improve the depressive-like behavior. Minocycline was regard as an antibiotic with anti-inflammatory action against the PCs after i.p. injection of LPS, which has the great permeability through the blood brain barrier. Consequently, the anti-inflammatory effect of minocycline on CNS and periphery has commonly contributed to the benefit of minocycline on CNS disorder [52]. In the present study, AZI showed a similar effect with minocycline. However, AZI had a weak penetration to distribute from blood to brain, so the benefit effect of AZI on CNS disorder was mainly derived from the effect of AZI on peripheral anti-inflammatory action. Compared to the normal mice, the AZI exposure in LPS loading mice has a significant increase as Fig. 2A. After an i.p. injection of LPS, the inflammation was induced by LPS in peripheral, especially in intestinal tract. The increased inflammatory factor and inflammatory action against the PCs after i.p. injection of LPS, which is responsible for the increased permeability of intestinal tract was benefit for the intestinal microvascular transports of AZI and improved the absorption of AZI after an i.g. administration.

The results in present study could be used to describe the response-concentration-time relationships of AZI by a mathematical model and parameters as opposed to concentration profiles. The estimated PK-PD parameters were based on data from single dose levels of AZI. The large perturbation will make a full change of biological system (mostly nonlinear) and allow a clear characterization of dose-response relationship. Generally, a wide range of dose will benefit model prediction and high dose is essential for inhibitory function. The suppressive fractions of AZI on PCs were 6.5 ug/ml (IC50) and 0.83 (I max). As an inducer, the inuinity and efficacy of LPS on PCs were also calculated, the estimated SC 50 and S max were 103.5 Eu/ml and 27.5 for LPS, which showed that the LPS had a high capacity and power to challenge the production of PCs.

Table 2. The Pharmacokinetic Parameters of LPS in Normal Mice and Azithromycin-Treating Mice.

| Parameter (unit) | Definition | Normal Mice | Azithromycin-Treating Mice |
|------------------|------------|-------------|---------------------------|
|                  |            | Estimated value | CV(%) | Estimated value | CV(%) |
| ke_LPS (h⁻¹)     | The absorption rate constant of LPS | 1.51 | 39 | 1.63 | 37 |
| ke_LPS (h⁻¹)     | The elimination rate constant of LPS | 0.082 | 34 | 0.088 | 33 |
| VLPS/FLPS (L/kg) | Distribution volume of LPS | 16.3 | 32 | 15.5 | 36 |

The PK-PD Model of Azithromycin

DOI:10.1371/journal.pone.0054981.t002
capturing the nonlinear system. Thus, our study designed a very high dose of AZI to support a PKPD characterization. As it is mentioned in eq. 5, the inhibitory function of AZI and the stimulatory function of LPS on the production of PCs were appeared in one equation. Although two functions were included in eq. 5, the only-LPS experimental group (disease control) would allow us to specifically evaluate the effect of LPS on PC production. Sigmoid model would be better than linear model to characterize biological functions, but the final model was chosen based upon several model fitting criteria, such as parameters.

Figure 3. The behavioral profiles of FST (A) and TST (B) after an i.p. LPS (0.8 mg/kg) and AZI treatment (100 mg/kg) in mice. Measured values and model fittings were shown in symbols and lines, respectively. Control mice were shown in closed squares and solid line. The model group was shown in closed circles and dot line. The regulative effect following a single oral dose of AZI was shown in closed triangle with dash line. All observations were reported as Mean ± SD (n = 8).

doi:10.1371/journal.pone.0054981.g003
precision, and visual checking. The linear model could parsimoni-
niously capture the data with reasonable precision of esti-
mates. Additionally, the linear model that used in equation 6 and 7
reduced the estimated parameters and simplified the structure in
the final model, because we mainly focused on the processes of
the stimulation of LPS and the inhibition of AZI in this article. The
Akaike’s information criterion of the final model of LPS and AZI
intervention is 22.31. The residual errors of the AZI, LPS, PCs,
KYN and behavioral test (FST and TST) are all less than 38%.
Initially, various numbers of transit compartments were tested to
describe the production and elimination different physiological
processes.

A number of PK-PD modeling efforts have been reported for
describing the inflammation [53], endogenous amino acid
neurotransmitters [54], and CNS disorder disease progression in
animal and human separately [55]. In our study, the present PK-
PD model was introduced to investigate the interaction of LPS-
PCs-KYN-CNS disorder, as well as incorporating AZI treatment
to evaluate the potential involvement of inflammation on de-
pressive progression.

Supporting Information

Appendix S1 The program code of differential functions
in ADAPT II software.

Acknowledgments

The authors wish to thank Cao YG for help with model construction.

Author Contributions

Conceived and designed the experiments: KH HH GW. Performed
the experiments: KH QQ YL LX. Analyzed the data: KH YC. Contributed
reagents/materials/analysis tools: YL LX. Wrote the paper: KH QQ.

References

1. Pitossi F, del Rey A, Kabiersch A, Besedovsky H (1997) Induction of cytokine
transcripts in the central nervous system and pituitary following peripheral
administration of endotoxin to mice. J Neurosci Res 48: 287–289.
2. Capuron L, Dantzer R (2005) Cytokines and depression: the need for a new
paradigm. Brain Behav Immun (Suppl 1): 119–124.
3. Capuron L, Gunnick JF, Musselman DL, Lawson DH, Reemtsma K, et al. (2002)
Neurobehavioral effects of interferon-alpha in cancer patients: phenomeno-
ology and paroxetine responsiveness of symptom dimensions. Neuropsychophar-
macology 26: 643–652.
4. O’Connor JC, Lawson MA, Andre ´ C, Moreau M, Lestage J, et al. (2009)
Lipopolysaccharide-induced depressive-like behavior is mediated by indole-
amine 2,3-dioxygenase activation in mice. Mol Psychiatry 14: 511–522.
5. Popov A, Abdallah Z, Wickenhauser C, Saric T, Driesen J, et al. (2006)
Indoleamine dioxygenase-expressing dendritic cells form suppurative granulo-
mas following Listeria monocytogenes infection. J Clin Invest 116: 3160–3170.
6. Booj L, Van der Does AJ, Riedel WJ (2003) Monoamine depletion in
psychiatric and healthy populations.review. Mol Psychiatry 8: 951–973.
7. Moreau M, Lestage J, Verrier D, Mormede C, Kelley KW, et al. (2005) Basile
Calmette-Guerin inoculation induces chronic activation of peripheral and brain
indoleamine 2,3-dioxygenase in mice. J Infect Dis 192: 537–544.
8. Fromm F, Moreau M, O’Connor J, Lawson M, Miron C, et al. (2009)
Lipopolysaccharide induces delayed FoxD1/DeltaFosB immunostaining within
the mouse extended amygdala, hippocampus and hypothalamus, that parallel
the expression of depressive-like behavior. Psychoneuroendocrinology 34: 516–531.
9. Guillemin GJ, Smythe G, Takikawa O, Brew BJ (2005) Expression of
indoleamine 2,3-dioxygenase and production of quinolinic acid by human
microglia, astrocytes, and neurons. Glia 49: 15–23.
10. Fukui S, Schwarz R, Rapoport SI, Takada Y, Smith QR (1991) Blood-brain
barrier transport of kynurenines: implications for brain synthesis and
metabolism. J Neurochem 56: 2007–2017.
11. Müller N, Schwarz Mj (2007) The immune-mediated alteration of serotonin and
glutamate: towards an integrated view of depression. Mol Psychiatry 12: 900–
1000.
12. Wichers MC, Koek GH, Rabaey S, Verkerk R, Schaps A, et al. (2005) IDO
and interferon-alpha induced depressive symptoms: a shift in hypothesis from
tryptophan depletion to neurotoxicity. Mol Psychiatry 10: 538–544.
13. Yirmiya R (1996) Endotoxin produces a depressive-like episode in rats. Brain
Res 711: 163–174.
14. Brustolin D, Ribeiro-dos-Santos R, Kast RE, Altschuler EL, Soares MB (2006)
A new chapter opens in anti-inflammatory treatments: the antidepressant
bupropion lowers production of tumor necrosis factor-alpha and interferon-
gamma in mice. Int Immunopharmacol 6: 903–907.
15. Fiese EF, Steffen SH (1990) Comparison of the acid stability of azithromycin and
erythromycin A. J Antimicrob Chemother 25(Suppl A): 39–47.
16. Foulds G, Shepard RM, Johnson RB (1996) The pharmacokinetics of
azithromycin in human serum and tissues. J Antimicrob Chemother 25(Suppl
A): 73–82.
17. Girard AE, Girard AR, English TD, Gooz CR, Cimochowski JA, et al. (1987)
Pharmacokinetics and in vivo studies with azithromycin (CP-62,993), a new
macrolide with an extended half-life and excellent tissue distribution. Antimicrob
Agents Chemother 31: 1948–1954.
18. Scaglione F, Rossoni G (1998) Comparative anti-inflammatory effects of
roxithromycin, azithromycin and clarithromycin. J Antimicrob Chemother 41
Suppl B: 47–50.

Table 3. Parameter Estimates for the LPS-Induced Depressive like Model and Azithromycin Pharmacodynamics.

| Parameter (units) | Definition | Estimated value | CV% |
|------------------|-----------|----------------|-----|
| PC0 (pg ml$^{-1}$) | Initial value of plasma proinflammatory cytokines | 49.8 | 35 |
| kout_PC0 (h$^{-1}$) | Elimination rate constant of plasma proinflammatory cytokines | 0.234 | 38 |
| KYN (ng ml$^{-1}$) | Initial value of plasma kynurenine | 104.3 | 31 |
| kout_KYN (h$^{-1}$) | Elimination rate constant of plasma kynurenine | 0.167 | 35 |
| FST0 (TST0) (s) | Initial value of forced swimming test (tail suspension test) | 51.3 (108.2) | 34 (41) |
| kout_FST(TST) (h$^{-1}$) | Hypothetical elimination rate constant of forced swimming test (tail suspension test) | 0.253(0.143) | 33 (35) |
| Imax | Capacity factor for azithromycin induced inhibition on proinflammatory cytokines | 0.83 | 34 |
| CT (ug/ml) | Concentration of azithromycin producing 50% Imax | 6.3 | 31 |
| Smax | Capacity factor for LPS induced stimulation on proinflammatory cytokines | 27.5 | 42 |
| SC0 (EU/ml) | Concentration of LPS producing 50% Smax | 103.5 | 39 |

doi:10.1371/journal.pone.0054981.t003
19. Ivetic Tkalecic V, Bosnjak B, Hvicbic B, Bosnar M, Marjanovic N, et al. (2006) Anti-inflammatory activity of azithromycin attenuates the effects of lipopolysaccharide administration in mice. Eur J Pharmocol 539: 131–138.

20. Rajman I (2008) PK/PD modelling and simulations: utility in drug development. Drug Discov Today 13: 341–346.

21. Lalonde RL, Kowalski KG, Hutmacher MM, Ewy W, Nichols DJ, et al. (2007) Model-based drug development. Clin Pharmacol Ther 82: 21–32.

22. Ohtani M, Kotaki H, Sawada Y, Iga T (1995) Comparative analysis of bisoprolol- and metoprolol-induced analgesic effects based on pharmacokinetic-pharmacodynamic modeling. J Pharmacol Exp Ther 272: 505–510.

23. Yassen A, Olofsen E, Dahan A, Danhof M (2005) Pharmacokinetic-pharmacodynamic modeling of the antiinflammatory effect of buprenorphine and fentanyl in rats: role of receptor equilibration kinetics. J Pharmacol Exp Ther 313: 1136–1149.

24. Mormede C, Palin K, Kelley KW, Castanon N, Dantzer R (2004) Conditioned taste aversion with lipopolysaccharide and peptidoglycan does not activate cytokine gene expression in the spleen and hypothalamus of mice. Brain Behav Immun 18: 106–200.

25. Lestage J, Verrier D, Palin K, Dantzer R (2002) The enzyme indoleamine 2,3-dioxygenase is induced in the mouse brain in response to peripheral administration of lipopolysaccharide and superantigen. Brain Behav Immun 16: 596–601.

26. Barrett B, Borek-Dobal'sky V, Fjot P, Vainiova-S, Huclová J, et al. (2005) Validated HPLC-MS-MS method for determination of azithromycin in human plasma. Anal Bioanal Chem 383: 213–217.

27. Porsolt RD, Bertin A, Jalfre M (1977) Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 229: 327–336.

28. Pechnick RN, Chesnokova VM, Kariagina A, Price S, Bresee CJ, et al. (2004) Reduced immobility in the forced swim test in mice with a targeted deletion of the leukemia inhibitory factor (Lif) gene. Neuropsychopharmacology 29: 770–776.

29. Svenningsson P, Tzavara ET, Qi H, Carruthers R, Warkin JM, et al. (2007) Biochemical and behavioral evidence for antidepressant like effects of 5-HT6 receptor stimulation. J Neurosci 27: 4201–4209.

30. Watanabe S, Kanada S, Takenaka M, Hamazaki T (2004) Dietary n-3 fatty acids selectively attenuate LPS-induced behavioral depression in mice. Physiol Behav 81: 605–613.

31. Dayneka NL, Garg V, Jusko WJ (1993) Comparison of four basic models of indirect pharmacodynamic responses. J Pharmacokin Biopharm 21: 457–478.

32. Aubert JD, Julius-Euenerz A, Fioroni P, Dayer P, Plan PA, et al. (1998) Function of human alveolar macrophages after a 3-day course of azithromycin in healthy volunteers. Palm Pharmaco Ther 11: 263–269.

33. Steinman L (2008) Nuanced roles of cytokines in three major human brain disorders. J Clin Invest 118: 3557–3563.

34. Homberg JR, Schubert D, Gaspar P (2010) New perspectives on the function to model signal transduction processes in pharmacodynamics. J Pharm 39: 105–121.

35. Kronenberg S, Frisch A, Rotem R, Bertz D, Graup P (2010) New perspectives on the neurodevelopmental effects of SSRIs. Trends Pharmacol Sci 31: 60–65.

36. Muller N, Schwarcz MJ (2007) The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression. Mol Psychiatry 12: 908–1000.

37. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW (2008) From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 9: 46–56.

38. Capuron L, Ravaud A, Neveu PJ, Miller AH, Maes M, et al. (2002) Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. Mol Psychiatry 7: 468–473.

39. Dantzer R, O'Connor JC, Freund GG, Johnson KW, Kelley KW (2008) From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 9: 46–56.

40. Dantzer R, Kelley KW (2007) Twenty years of research on cytokine-induced sickness behavior. Brain Behav Immun 21: 153–160.

41. Steinman L (2008) Nuanced roles of cytokines in three major human brain disorders. J Clin Invest 118: 3557–3563.

42. Gal EM, Seror-Sherman AD (1980) L-Kynurenine: its synthesis and possible regulatory function in brain. Neurochem Res 3: 223–239.

43. Heyes MP, Saito K, Major EO, Milstein S, Markey SP, et al. (1993) A mechanism of quinolinic acid formation by brain in inflammatory neurologic disease: attenuation of synthesis from 1-tryptophan by 6-chloro-tryptophan and 4-chloro-3-hydroxyanthranilic. Brain 116: 1423–1430.

44. Shapiro L, Gelfand JA (1993) Cytokines and sepsis: pathophysiology and therapy. New Horiz 1: 13–22.

45. Fujiwaki H, Saito K, Fujiwaki S, Takamura M, Sudo K, et al. (2006) The signal transducer and activator of transcription I kappaB and interleukin regulatory factor 1 are not essential for the induction of indoleamine 2,3-dioxygenase by lipopolysaccharide: involvement of p38 mitogen-activated protein kinase and nuclear factor-kappaB pathways, and synergistic effect of several proinflammatory cytokines. J Biochem 139: 653–662.

46. Muller N, Schwarcz MJ (2007) The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression. Mol Psychiatry 12: 908–1000.

47. Schwarzw R (2004) The kynurenine pathway of trytophan degradation as a drug target. Curr Opin Pharmacol 4: 12–17.

48. Amosden GW (2005) Anti-inflammatory effects of macrolides—an underappreciated benefit in the treatment of community-acquired respiratory tract infections and chronic inflammatory pulmonary conditions? J Antimicrob Chemother 55: 10–21.

49. Calic O, Ezakovic V, Parham MJ (2001) Anti-inflammatory effects of macrolide antibiotics. Eur J Pharmaco 429: 209–229.

50. Asoyada-Dupuis E, Vallee JP, Bedos M, Muffit J, Picolato JJ (1991) Prophylactic and therapeutic activities of azithromycin in a mouse model of pneumococcal pneumonia. Antimicrob Agents Chemother 35: 1024–1028.

51. Kikuchi T, Hagiwara K, Honda Y, Gomi K, Kobayashi T, et al. (2002) Clarihydrin suppresses lipopolysaccharide-induced interleukin-1 production by human monocytes through AP-1 and NF-kappa B transcription factors. J Antimicrob Chemother 47: 745–755.

52. Henry CJ, Huang Y, Wynne A, Hanke M, Himler J, et al. (2008) Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior, and anhedonia. J Neuroinflammation 13: 5–15.

53. Josa M, Urizar JP, Rapado J, Dios-Vizitica C, Castaieda-Hernandez G, et al. (2001) Pharmacokinetic/pharmacodynamic modeling of antipyretic and anti-inflammatory effects of naproxen in the rat. J Pharmacol Exp Ther 297: 198–205.

54. Chen Y, Gao Y, Zhou N, Liu X (2009) Mechanism based pharmacokinetic pharmacodynamic modeling of bidirectional effect offloxinidemma on plasma homocysteine in rats. Pharm Res 26: 1863–1873.

55. De Lange EC, Ravenstijn PG, Groenendaal D, van Steeg TJ (2005) Toward the prediction of CNS drug-effect profiles in physiological and pathological conditions using microdialysis and mechanism-based pharmacokinetic-pharmacodynamic modeling. AAPS J 7: E532–E543.