Detectable prednisolone is delayed in pericardial fluid, compared with plasma of patients with tuberculous pericarditis: A pilot study

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ABSTRACT

Background: In patients with tuberculous pericarditis [TBP] adjunctive prednisolone reduces the incidence of constrictive pericarditis. It is unknown whether prednisolone permeates adequately into pericardial fluid. Drug measurements in pericardial fluid require invasive procedures, and thus less invasive methods are needed to perform full pharmacokinetic characterization of prednisolone in large numbers of patients. We sought to evaluate the relationship between prednisolone concentrations in pericardial fluid, plasma, and saliva.
Methods: Plasma, pericardial fluid, and saliva samples were collected at 7 time points from TBP patients randomized to 120 mg prednisolone or placebo. Compartmental pharmacokinetic parameters, peak concentration [C_{max}], and 0–24 h area under the concentration-time curve [AUC_{0-24}] were identified in plasma, saliva and pericardial fluid.
Results: There were five patients each in the prednisolone and placebo groups. Prednisolone concentrations were best described using a one compartment model. The absorption half-life into plasma was 1 h, while that into pericardial fluid was 9.4 h, which led to a median time-to-maximum concentration in plasma of 2.0 h versus 5.0 h in pericardial fluid [$p = 0.048$]. The concentration-time profiles in pericardial fluid versus plasma exhibited system hysteresis. The pericardial fluid-to-plasma C_{max} peak concentration ratio was 0.28 ($p = 0.032$), while the AUC_{0-24} ratio was 0.793. The concentration-time profiles in saliva had a similar shape to those in plasma, but the saliva-to-plasma C_{max} was 0.59 [$p = 0.032$].
Conclusion: The prednisolone AUC_{0-24} achieved in pericardial fluid approximates that in plasma, but the C_{max} is low due to delayed absorption. Saliva can be used as surrogate sampling site for pericardial fluid prednisolone.

1. Introduction

Tuberculous pericarditis (TBP) results from infection of the pericardium by *Mycobacterium tuberculosis* (Mtb) [1]. TBP accounts for about 1% of all tuberculosis (TB) cases, but 26% of patients die within six months of diagnosis, rising to approximately 40% in those with human immunodeficiency virus (HIV) co-infection and signs of advanced immunosuppression [2]. About 60% of TBP patients have a pericardial effusion requiring aspiration [3]. Pericardial aspiration may be indicated in patients with large pericardial effusions, especially if cardiac tamponade is present [4]. The mainstay of the management of TBP is anti-TB chemotherapy, which may be augmented by adjunctive corticosteroids in those without advanced HIV related immunosuppression [3,4]. Adjunctive corticosteroids have been shown to reduce the incidence of constrictive pericarditis, hasten resolution of symptoms, and reduce hospitalization [3]. Prednisolone is the preferred glucocorticoid in this setting [5].

Mtb infection leads to pericardial inflammation, which is thought to be responsible for many of the debilitating symptoms patients experience in TBP [1]. Adjunctive prednisolone attenuates this inflammatory response. However, there is a lack of consensus on the optimal dose for adjunctive corticosteroid therapy. Part of the problem is that the degree prednisolone penetration into the pericardium is unknown. Drug pharmacokinetics and penetration concentration gradients into TB lesions by antibiotics are major determinants of microbial and clinical outcomes [6–10].

Pericardiocentesis represents a rare opportunity to explore the relationships between the prednisolone concentrations in the pericardial compartment and plasma, which has not previously been determined. However, we were also interested in finding less invasive sampling procedures to use as future surrogates of serum and pericardial fluid prednisolone concentrations. The pharmacokinetics of prednisolone have been characterized in plasma and saliva [5]. Prednisolone administered orally is rapidly absorbed, with a time to maximum concentration [T_{max}] of 1–3 h [11], and 80% protein-bound, predominantly by globulin and albumin [12,13]. Unbound prednisolone diffuses passively across cell membranes to the nucleus, where it binds to glucocorticoid receptor type II, forming a complex, which subsequently adheres to the glucocorticoid response

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element, which down-regulates transcription of pro-inflammatory cytokines [14,15]. This down-regulation of pro-inflammatory pathways is concentration-dependent. The aim of the study was to identify the prednisolone penetration into pericardial fluid, and saliva.

2. Patients and methods

Our study was a sub-study of the now published Investigation of the Management of tuberculous Pericarditis (IMPI) trial, which sought to evaluate the effect of adjunctive prednisolone on the six months incidence of the combined outcome of mortality tamponade constrictive pericarditis of patients with TBP [3]. All participants were required to provide written informed consent. The study was approved by the University of Cape Town Research Ethics Committee and was registered with clinicaltrials.gov (NCT00810849).

All patients with TBP over the age of 18 years, presenting to a tertiary hospital in Cape Town as part of IMPI study were invited to participate in the sub-study. Patients who had received corticosteroids within the previous month and those who had been on TB treatment for more than a week were excluded. The sub-study was restricted to patients who required pericardiocentesis as part of their standard of care to relieve hemodynamic compromise caused by the pericardial effusion [3]. Participants were categorized as definitive TBP or probable TBP on the basis of pre-specified published criteria by Pandie et al. [16] A definite diagnosis required microbiological evidence of *Mtb* infection in the pericardium, whereas a probable diagnosis was based on the presence of a lymphocyte predominant exudate with raised adenosine deaminase (ADA) or a Tygerberg diagnostic index score of six or greater [16].

2.1. Intensive PK sampling procedure

All participants had a clinical history and physical examination performed and base-line investigations which included a full blood count, serum urea, and electrolytes. An echocardiogram confirmed the presence of a pericardial effusion and determined whether the pericardial effusion exceeded a cross-sectional diameter of 10 mm, hence amenable to aspiration. Participants underwent fluoroscopy and electrocardiogram (ECG) guided pericardial aspiration, using local anesthesia and the Seldinger technique, during which a pig-tail catheter was left in the pericardial space for 24 h to permit ongoing drainage of residual pericardial fluid.

The 120 mg dose of prednisolone was administered orally under supervision after pericardiocentesis [10]. Patients who were randomized to the control arm, received anti-TB therapy without prednisolone. All patients continued their usual concomitant medication, for example

their antiretroviral treatment. Following pericardial aspiration and within 5 min after the administration of either prednisolone or placebo, a baseline plasma, pericardial fluid and saliva sample was collected and subsequently at the following time points; baseline, 0.5-, 1-, 2-, 3-, 5-, 8- and 24 h for a total of 8 time-points in each patient for each matrix. Plasma, pericardial and saliva samples were stored at −80 °C.

2.2. Drug concentration assay

Prednisolone concentrations were determined with a liquid chromatography tandem mass spectrometry assay developed in the Division of Clinical Pharmacology, University of Cape Town. The assay was validated in plasma, and cross-validated in blank pericardial fluid and artificial saliva. Samples were processed with a liquid-liquid extraction method using ethyl acetate, followed by high performance liquid chromatography with MS/MS detection using an AB SCIEX API 4000 instrument. An Agilent Zorbax-SB Phenyl Rapid Resolution HT 1.8 μm, 2.1 × 100 mm analytical column was used. Prednisolone and prednisolone-d8 (internal standard), were monitored at mass transitions of the protonated precursor ions m/z 361.2 and m/z 369.3 to the product ions m/z 147.2 and m/z 150.2, respectively. The calibration curves fitted quadratic (weighted by 1/concentration^2) regressions over the ranges 1.95 ηg/ml to 1000 ηg/ml. A 5-fold dilution was validated for samples above the upper limit of quantification.

2.3. Compartmental pharmacokinetic analysis

Prednisolone concentrations were modeled using ADAPT 5 (Biomedical Simulations Resource, California, USA) software of D'Argenio et al. [17] We used the maximum likelihood expectation maximization algorithm. We modeled the concentrations using a one-compartment and a two-compartment model with first-order input and elimination, as described in our prior publications [18–21]. Akaike information criteria (AIC), Bayesian information criteria (BIC) and parsimony were then used to choose the best number of pharmacokinetic compartments. The model-derived concentration-time profiles were then used to identify the 0–24 h area under the concentration-time curves [$AUC_{0–24}$].

2.4. Statistical analysis

The statistical analysis was done using GraphPad Prism version 7 (Graphpad, California, USA). The non-parametric Mann-Whitney and Fisher's exact test were used to compare baseline characteristics between prednisolone and placebo arms and to compare the pericardial

Table 1
Baseline characteristics of participants who underwent intensive pharmacokinetic sampling.

Parameter	Median of all participants (range) or proportion (%)	Median or proportion in Placebo arm, N = 5	Median or proportion in Treatment arm, N = 5	p value
Demographic and clinical parameters				
Gender: Male	4(40%)	2(40%)	2(40%)	1.0
Age in years	30(24–59)	29 (24.1–56.3)	31 (24.0–58.8)	0.999
Weight in kg	60(40–82)	53 (40–82)	66 (52–72)	0.278
Prednisolone dose mg/kg	N/A	0	1.81 (1.67–2.31)	N/A
Positive AFB	7(70%)	4(80%)	3(60%)	0.431
HIV	7(70%)	4(80%)	3(60%)	0.431
Proportion on HAART	1(14%)	1(20%)	0(0%)	N/A
Plasma				
CD4 count in cell/m^3	149(42–874)	159 (50–485)	139 (42–874)	0.999
Creatinine in μmol/L	78(20–257)	80 (20–257)	65 (43–97)	0.547
Globulin in g/L	51(30–56)	46 (30–57)	55 (36–56)	0.999
Pericardial fluid				
Total protein in g/L	62(50–70)	58 (54–67)	66 (55–70)	0.175
Adenosine deaminase in U/L	57(26–133)	87 (52.5–133)	51 (25.9–119.4)	0.190

Abbreviations: AFB, acid fast bacilli; HIV, human immunodeficiency virus; HAART, highly active anti-retroviral therapy; U/L, units per liter.

and plasma prednisolone C_{max} and T_{max}. The median and proportions were used as the measures of central tendency.

3. Results

A total of 37 potential participants were screened for the study. Ten participants met all inclusion criteria and were enrolled, while 27 participants were excluded; 14 did not require pericardiocentesis, 11 did not provide consent for sub-study and 2 participants withdrew consent due to discomfort of pigtail catheter. The patients' demographic and clinical characteristics are shown in Table 1. Seven patients had microbiological confirmation of TBP and 3 had probable TBP. Seven participants had HIV co-infection and were referred for initiation of anti-viral treatment following enrolment. There were no differences between baseline characteristics in respect of selective clinical and biochemical factors for the treatment and placebo groups.

3.1. Comparison of prednisolone concentrations based on naïve pooling

All five participants randomized to prednisolone had prednisolone concentrations above limits of detection in saliva, plasma and pericardial fluid, while the prednisolone concentrations were below limits of detection in all three matrices in participants who were assigned to placebo. The prednisolone concentrations versus time plots are shown in five patients (Fig. 1A). Prednisolone was rapidly absorbed into plasma, and was detectable within 30 min of administration. The shape of the pericardial fluid concentration-time profile versus plasma was consistent with system hysteresis as evidenced by the relationship between the C_{max} and T_{max} among the three matrices shown in Fig. 1B and C. The ratio of the pericardial to plasma and salivary to plasma C_{max} (pericardial/plasma or saliva/plasma) were median 0.28 with range 0.12 to 0.82 and median 0.59 with a range of 0.20 to 0.99 respectively. Fig. 1 shows a rapid rise in prednisolone concentration plasma and saliva, with a higher permeation in plasma compared to saliva while the rise
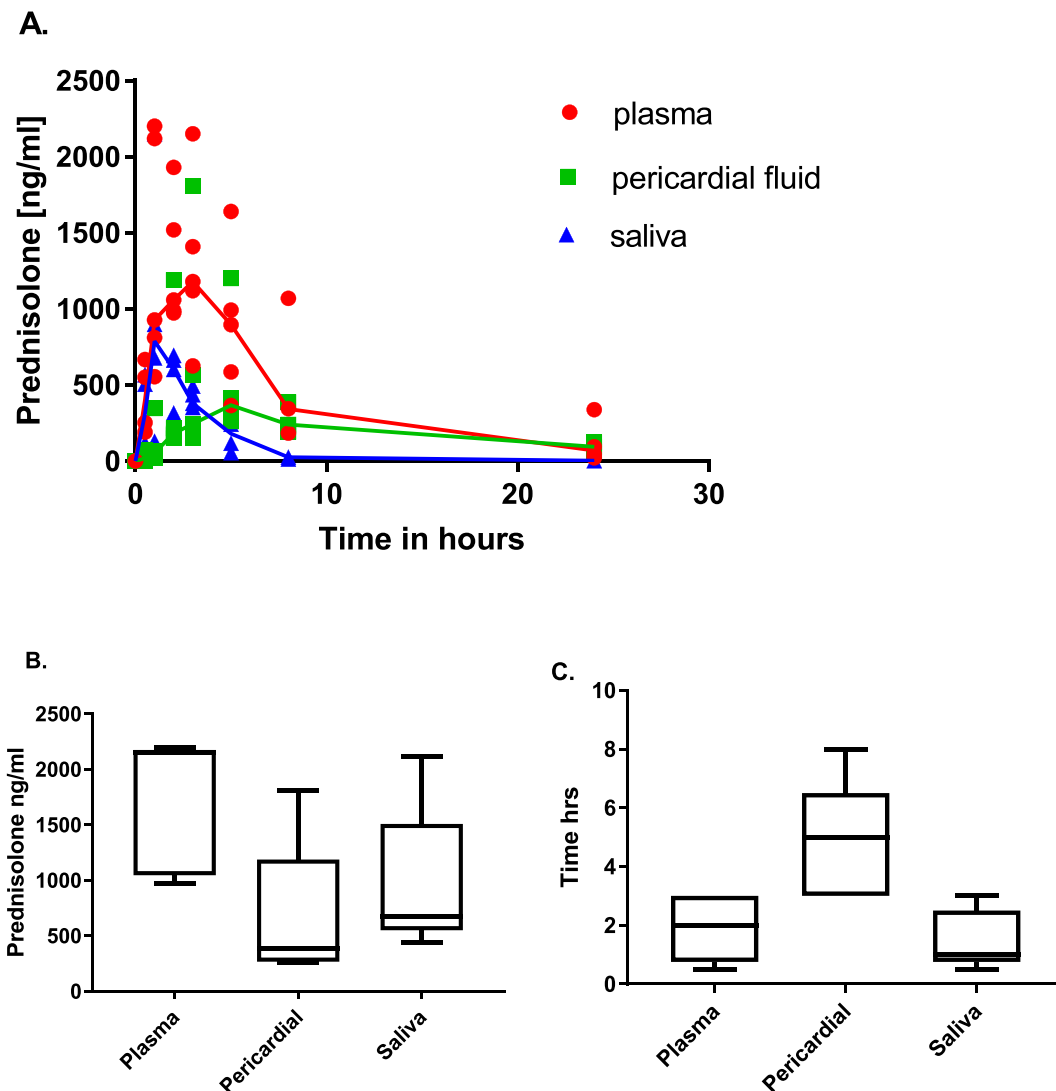

Fig. 1. Naïve pooled prednisolone concentrations over a 24 h period. A. Prednisolone concentration time graphs over a 24 h period for each of the five participants that were assigned to the treatment arm. The plasma, pericardial fluid and saliva matrices were represented by red, green and blue respectively. Each matrix was represented by a line of best fit and the data points for each of the 5 participants. B. The box and whisker plot comparing the prednisolone C_{max} of the three compartments; plasma, pericardial fluid and saliva. The median plasma, pericardial and saliva median C_{max} was 2150.0 μg/ml, 389.0 μg/ml and 679.0 μg/ml respectively. Plasma C_{max} was higher than pericardial C_{max} ($p = 0.032$). Plasma C_{max} was higher than saliva C_{max} ($p = 0.032$). There was no difference between pericardial and saliva prednisolone C_{max} ($p = 0.151$). C. The box and whisker plot comparing the prednisolone T_{max} of the three compartments; plasma, pericardial fluid and saliva. The median plasma, pericardial and saliva median T_{max} was 2.0 h, 5.0 h and 1.0 h respectively. Pericardial T_{max} was higher than plasma T_{max} [$p = 0.048$]. Pericardial T_{max} was higher than saliva T_{max} ($p = 0.024$). There was no difference between plasma and saliva prednisolone T_{max} ($p = 0.810$).

Table 2
Compartmental model choices using information criteria.

Matrix	Number of compartments in model	Akaike information criteria	Bayesian information criteria
Plasma	One	80.011	92.453
	Two	87.866	106.530
Pericardial fluid	One	−35.243	−22.800
	Two	−27.200	−8.536

in pericardial concentration was more circumspect but more sustained. The relative peaks were highest in plasma, followed by saliva and lastly pericardial fluid.

3.2. Compartmental pharmacokinetic modeling based comparisons

Given the system hysteresis for the plasma concentrations and pericardial concentrations, we modeled each compartment separately from each other. Table 2 shows that based on Akaike Information Criteria and Bayesian information Criteria, a one compartment model best explained plasma concentrations, consistent with what has been reported in the literature in larger studies of prednisolone plasma pharmacokinetics [14,22]. Prednisolone pericardial concentrations were also best explained by a one-compartment model. The prednisolone plasma and pericardial pharmacokinetic parameter estimates for the one compartment model are shown in Table 3. The table shows that the clearance and apparent volume were similar in pericardial fluid and plasma, and by parsimony are considered as a single pharmacokinetic compartment. The major differences were in the absorption rate constant (K_a), which is the rate of prednisolone absorption from the gastrointestinal tract, the site of administration. The plasma K_a of 0.693 h^{-1} in Table 3 translates to an absorption half-life of exactly 1.0 h into plasma, while the pericardial K_a of 0.0735 h^{-1} translates to an absorption half-life of 9.431 h into pericardial fluid. Thus, the rate of absorption into pericardial fluid differed between plasma and pericardial fluid. In addition, Table 3 means that the largest between-patient variability among all pharmacokinetic parameters, measured as % coefficient of variation (CV), was the 99.59% that was encountered in pericardial fluid K_a. The physiological implication of the K_a is that it determines the T_{max} which together with the volume distribution are determinants of the C_{max} [23]. Thus, the physiological basis for the prednisolone system hysteresis curves, was a slow rate of absorption into pericardial fluid versus a fast rate into plasma [24]. The pharmacokinetic-model derived T_{max} is shown in Fig. 2C, which shows that in all cases the paired values for each patient demonstrated greater T_{max} in pericardial fluid compared to plasma, with medians of 2.49 h versus 4.09 h [$p = 0.032$].

The pharmacokinetic model-derived prednisolone C_{max} and AUC_{0-24} in plasma and the pericardial fluid were used to derive the drug penetration ratios, with results shown in Fig. 2D. The median pericardial fluid-to-plasma C_{max} ratio was 0.218 with between-patient % CV of 96.93%. The median pericardial fluid-to-plasma AUC_{0-24} ratio was 0.793 [95% confidence interval: 0.419 to 1.269] with between-patient % CV of 40.55%. Thus, while the peak concentration in pericardial fluid was lower than in plasma, the total daily drug concentration (AUC_{0-24}) was comparable

Table 3
Estimation of plasma and pericardial fluid prednisolone one compartment model parameters.

Parameter	Plasma		Pericardial fluid	
	Mean	Standard deviation	Mean	Standard deviation
Clearance (L/h)	17.2	7.86	13.7	0.483
Volume of distribution (L)	37.6	1.54	25.7	1.58
Absorption constant (h^{-1})	0.693	0.039	0.0735	0.0732

between the two matrices, which means although delayed, most prednisolone eventually reaches pericardial fluid.

4. Discussion

Our first major finding was quantification of prednisolone concentrations that are achieved in pericardial fluid in patients with TBP. We found that the compartmental pharmacokinetic parameters for a one compartment model were similar between pericardial fluid and plasma, so that these two matrices were likely one pharmacokinetic compartment [25]. Prednisolone metabolism and clearance is by 11-β-hydroxysteroid dehydrogenase (11βHSD), in the liver and kidneys, which could explain the similar clearances between blood and pericardial fluid [26,27]. Similarly, the volumes demonstrate that the plasma and pericardial fluid in reality form a single "bucket". We found that there was a delayed entry into the pericardial fluid, resulting in a shape best described by system hysteresis. System hysteresis occurs when the output (pericardial fluid concentrations) lags behind the input (in this case drug dose and plasma concentrations), a concept first described in electromagnetism [24]. This resulted in pericardial fluid prednisolone peak concentrations that were only 21% those in plasma. However, AUC_{0-24} ratios had a 95% confidence interval that crossed 1, another factor consistent with system hysteresis: dependence of the system on history [history being the input and plasma AUCs that were achieved earlier]. The therapeutic implications are as yet unclear, and will depend on the PK/PD driver for prednisolone anti-inflammatory effect. If efficacy is peak concentration-driven then efficacy will be compromised, however if it is AUC or time above threshold driven then efficacy will not be compromised. In the latter case, it would not be necessary to sample pericardial fluid concentrations as instead plasma AUC_{0-24} based calculations would be good surrogates for those in pericardial fluid.

Second, we also found that the saliva prednisolone concentration-time profile was more closely aligned to that of plasma than that of the pericardial matrix which means that there was little delay to peak concentration between the plasma and saliva. This less invasive method could help with more convenient way for pharmacokinetic sampling [28]. However, there was reduced prednisolone penetration into saliva for both C_{max} and AUC_{0-24}. Thus, if saliva is to be used as a surrogate for plasma, a correction factor would need to be applied for both concentrations; if it to be used as a surrogate for pericardial fluid concentration then a correction factor will need to be calculated for AUC_{0-24}. A possible drawback of using saliva as a surrogate for pericardial concentrations of prednisolone is the potential for contamination. There is also concern that saliva prednisolone may contain residual prednisolone left over from ingestion of prednisolone and may result in over-estimation of saliva pharmacokinetic profiles [28,29].

5. Limitations

Our study has several limitations, including a small sample size limiting the power of the study. Second, the study participants received a very high dose of prednisolone 120 mg, previous TBP studies used lower doses of prednisolone [13,14]. Therefore, the findings of this study may not be generalizable to other conditions requiring prednisolone therapy. Third, it is also not understood what effect if any the inflamed pericardium has on pericardial concentrations of prednisolone. Other serosa have been shown to be more permeable to prednisolone when inflamed, as for example the meninges during meningitis [30,31]. Third, it remains to be determined in clinical studies what the concentration thresholds in plasma and pericardium are that reduce inflammation and are associated with prednisolone efficacy for factors such as prevention of constrictive pericarditis [9,32,33]. If these were known, then the optimal dose could be determined based on the penetration ratios that we identified here.
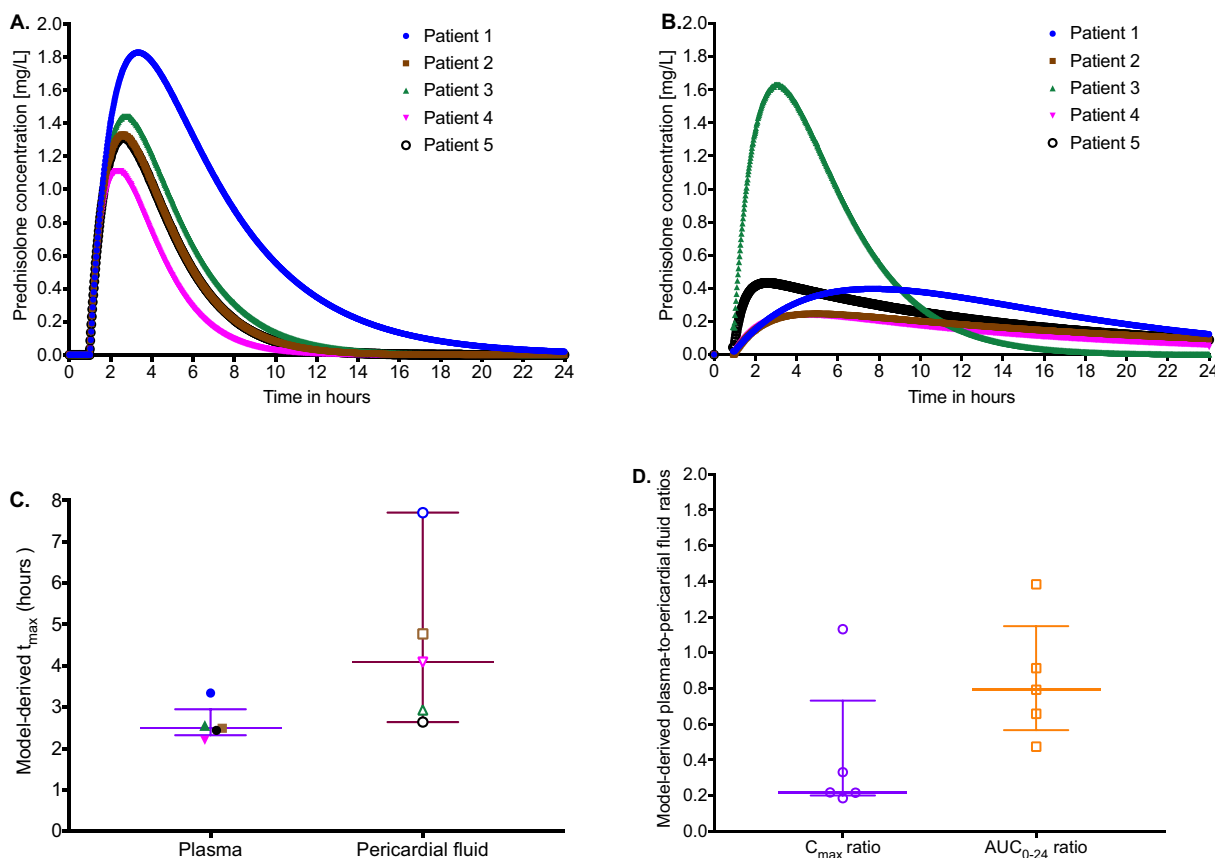
Fig. 2. The prednisolone pharmacokinetic model for plasma and pericardial fluid. A. Pharmacokinetic model-predicted concentration-time curves for plasma. There was rapid absorption of prednisolone into the plasma compartment followed by a rapid elimination. B. Pharmacokinetic model-predicted concentration-time curves for pericardial fluid, shows delayed time to peak concentration due to reduced absorption into pericardial fluid compartment. C. Pharmacokinetic model-derived T_{max} are shown paired for each patient [color coded], with closed symbol in plasma and open symbol in pericardial fluid. D. Pharmacokinetic model-derived pericardial fluid-to-plasma concentrations in each patient, demonstrate a low peak concentration penetration ratio but an AUC_{0-24} with confidence intervals crossing 1.

6. Conclusion

In conclusion, we have demonstrated 1] that prednisolone penetrates into pericardium in a delayed fashion, 2] that the AUC_{0-24} in pericardial fluid approximates that in plasma, and 3] that saliva can be a surrogate matrix for prednisolone pharmacokinetic sampling, but AUC_{0-24} measures would need a correction factor to equal those in pericardial fluid.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcha.2018.12.008.

References

[1] B.M. Mayosi, L.J. Burgess, A.F. Doubell, Tuberculous pericarditis, Circulation 112 (23) (2005) 3608–3616.

[2] B.M. Mayosi, C.S. Wiysonge, M. Ntsekhe, et al., Mortality in patients treated for tuberculous pericarditis in sub-Saharan Africa, S. Afr. Med. J. 98 (1) (2008) 36–40.

[3] B.M. Mayosi, M. Ntsekhe, J. Bosch, et al., Prednisolone and Mycobacterium indicus pranii in tuberculous pericarditis, N. Engl. J. Med. 371 (12) (2014) 1121–1130.

[4] B.M. Mayosi, Interventions for treating tuberculous pericarditis, Cochrane Libr (2002) https://doi.org/10.1002/14651858.CD000526.

[5] N. Teeninga, Z. Guan, J. Freijer, et al., Monitoring prednisolone and prednisone in saliva: a population pharmacokinetic approach in healthy volunteers, Ther. Drug Monit. 35 (4) (2013) 485–492.

[6] K. Dheda, L. Lenders, G. Magombedze, et al., Drug penetration gradients associated with acquired drug resistance in tuberculosis patients, Am. J. Respir. Crit. Care Med. 198 (9) (2018) (ja).

[7] K. Dheda, T. Gumbo, G. Maartens, et al., The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis, Lancet Respir. Med. 5 (4) (2017) 291–360.

[8] J.G. Pasipanodya, H. McIlleron, A. Burger, P.A. Wash, P. Smith, T. Gumbo, Serum drug concentrations predictive of pulmonary tuberculosis outcomes, J. Infect. Dis. 208 (9) (2013) 1464–1473.

[9] S. Swaminathan, J.G. Pasipanodya, G. Ramachandran, et al., Drug concentration thresholds predictive of therapy failure and death in children with tuberculosis: bread crumb trails in random forests, Clin. Infect. Dis. 63 (suppl_3) (2016) S63–S74.

[10] J. Shenje, F.I. Adimora-Nweke, I.L. Ross, et al., Poor penetration of antibiotics into pericardium in pericardial tuberculosis, EBioMedicine 2 (11) (2015) 1640–1649.

[11] M. Pickup, Clinical pharmacokinetics of prednisone and prednisolone, Clin. Pharmacokinet. 4 (2) (1979) 111–128.

[12] F. Frey, Kinetics and dynamics of prednisolone, Endocr. Rev. 8 (4) (1987) 453–473.

[13] J.Q. Rose, A.M. Yurchak, W.J. Jusko, Dose dependent pharmacokinetics of prednisone and prednisolone in man, J. Pharmacokinet. Biopharm. 9 (4) (1981) 389–417.

[14] B.M. Frey, F.J. Frey, Clinical pharmacokinetics of prednisone and prednisolone, Clin. Pharmacokinet. 19 (2) (1990) 126–146.

[15] T. Wilckens, Glucocorticoids and immune function: physiological relevance and pathogenic potential of hormonal dysfunction, Trends Pharmacol. Sci. 16 (6) (1995) 193–197.

[16] S. Pandie, J.G. Peter, Z.S. Kerbelker, et al., Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous pericarditis compared to adenosine deaminase and unstimulated interferon-γ in a high burden setting: a prospective study, BMC Med. 12 (1) (2014) 101.

[17] D.Z. D'argenio, A. Schumitzky, A program package for simulation and parameter estimation in pharmacokinetic systems, Comput. Programs Biomed. 9 (2) (1979) 115–134.

[18] R.G. Hall, M.A. Swancutt, C. Meek, R. Leff, T. Gumbo, Weight drives caspofungin pharmacokinetic variability in overweight and obese people: fractal power signatures beyond 2/3 or 3/4, Antimicrob. Agents Chemother. (2013) https://doi.org/10.1128/AAC.01490-12 (AAC. 01490-12).

[19] R.G. Hall, M.A. Swancutt, T. Gumbo, Fractal geometry and the pharmacometrics of micafungin in overweight, obese, and extremely obese people, Antimicrob. Agents Chemother. 55 (11) (2011) 5107–5112.

[20] R. Hall, J. Pasipanodya, M. Swancutt, C. Meek, R. Leff, T. Gumbo, Supervised machine-learning reveals that old and obese people achieve low dapsone concentrations, CPT: Pharmacometrics & Systems Pharmacology 6 (8) (2017) 552–559.

[21] R. Hall, J. Pasipanodya, C. Meek, R. Leff, M. Swancutt, T. Gumbo, Fractal geometry-based decrease in trimethoprim-sulfamethoxazole concentrations in overweight and obese people, CPT: Pharmacometrics & Systems Pharmacology 5 (12) (2016) 674–681.

[22] S. Al-Habet, H. Rogers, Pharmacokinetics of intravenous and oral prednisolone, Br. J. Clin. Pharmacol. 10 (5) (1980) 503–508.

[23] R.N. Upton, L.E. Mather, W.B. Runciman, C. Nancarrow, R.J. Carapetis, The use of mass balance principles to describe regional drug distribution and elimination, J. Pharmacokinet. Biopharm. 16 (1) (1988) 13–29.

[24] J.A. Ewing, I. Experimental researches in magnetism, Proc. R. Soc. Lond. 38 (235–238) (1885) 58–62.

[25] M.E. Burton, Applied Pharmacokinetics & Pharmacodynamics: Principles of Therapeutic Drug Monitoring, Lippincott Williams & Wilkins, 2006.

[26] A. Vermeulen, The metabolism of 4–14C prednisolone, J. Endocrinol. 18 (3) (1959) 278–291.

[27] E. Rusvai, A. Naray-Fejes-Toth, A new isoform of 11 beta-hydroxysteroid dehydrogenase in aldosterone target cells, J. Biol. Chem. 268 (15) (1993) 10717–10720.

[28] I. Ross, N. Levitt, J.-S. Van der Walt, et al., Salivary cortisol day curves in Addison's disease in patients on hydrocortisone replacement, Horm. Metab. Res. 45 (01) (2013) 62–68.

[29] I. Ross, M. Lacerda, T. Pillay, et al., Salivary cortisol and cortisone do not appear to be useful biomarkers for monitoring hydrocortisone replacement in Addison's disease, Horm. Metab. Res. 48 (12) (2016) 814–821.

[30] R. Duma, A. Berry, S. Smith, J. Baggett, E. Swabb, T. Platt, Penetration of aztreonam into cerebrospinal fluid of patients with and without inflamed meninges, Antimicrob. Agents Chemother. 26 (5) (1984) 730–733.

[31] M.R. Holdiness, Cerebrospinal fluid pharmacokinetics of the antituberculosis drugs, Clin. Pharmacokinet. 10 (6) (1985) 532–534.

[32] C. Modongo, J.G. Pasipanodya, B.T. Magazi, et al., Artificial intelligence and amikacin exposures predictive of outcome in multidrug-resistant tuberculosis patients, Antimicrob. Agents Chemother. (2016) https://doi.org/10.1128/AAC.00962-16 (AAC. 00962-16).

[33] S. Srivastava, J.G. Pasipanodya, G. Ramachandran, et al., A long-term co-perfused disseminated tuberculosis-3D liver hollow fiber model for both drug efficacy and hepatotoxicity in babies, EBioMedicine 6 (2016) 126–138.