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A polycaprolactone nanoparticle formulation of cyclosporin-a improves the prediction of area under the curve using a limited sampling strategy

Jesus Molpeceres *, Mercedes Chacón, Manuel Guzmán, Laura Berges, María del Rosario Aberturas

Department of Pharmacy and Pharmaceutical Technology, University of Alcalá, Alcalá de Henares, Madrid, Spain

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Abstract

Therapeutic monitoring of Cyclosporine (CyA) by using area under the curve (AUC) from abbreviated kinetic profiles is of recent trend in clinical practice due to the potential improvement in transplant and clinical outcome with costs reduction in mind. Several papers describe successful use of the limited sampling strategy to predict AUCs in different transplant populations when treated with Sandimmun or Sandimmun Neoral. However, the same predictive potential is achieved for the latter formulation with lesser effort. The present paper describes the application of the limited sampling strategies to demonstrate the advantages of using CyA incorporated in polymeric nanoparticles (CyA-NP) as compared to two reference Sandimmun formulations which consisted of an emulsion of the oily solution in milk (SIM-EM) and a microemulsion (SIM-Neoral) formerly tried on rats. Two independent data batches were used: group 1 which included 36, 31 and 10 animals receiving SIM-EM, CyA-NP and SIM-Neoral, respectively, and group 2 made of nine and eight rats treated with SIM-EM and CyA-NP. Several limited sampling equations were derived for each formulation from group 1 by stepwise multiple linear regression. Statistical analysis disclosed that CyA concentrations 8 and 32 h after dose administration vouched for 88 and 69% variability in AUC (0–48 h) for CyA-NP and SIM-EM, respectively. When summed up, these two concentrations revealed nearly 97% of AUC (0–48 h) variability. CyA concentrations 8 h post-treatment with SIM-Neoral explained 89% variability in AUC (0–48 h). This value raised to 98% when a second CyA concentration (24 h) was introduced. The equations derived from group 1 were then employed to predict AUCs in group 2. CyA blood levels at 8 h post-treatment confirmed AUC for CyA-NP ($r^2 = 0.98$) to be very precise and unbiased (error = 1.46%, interval -16.2 to 21.33%), while the results for SIM-EM obtained with the CyA concentration at 32 h were $r^2 = 0.93$ plus error = 5.71%, interval -44.33 to 105.94%. Similar results were obtained when the study period was reduced to 24 h. The use of these limited sampling models manifested the coincidence between CyA-NP and SIM-Neoral as well as the advantages of both formulations over SIM-EM when it comes to CyA monitoring.

* Corresponding author. Tel.: +91-8854780; fax: +91-8854658.

E-mail address: mrosario.aberturas@u.alcala.es (M. del Rosario Aberturas)

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1. Introduction

The therapeutic efficacy of cyclosporine (CyA) in preventing graft rejection has contributed immensely to the important development of organ transplantation during the last few years. However, despite its immunosuppressive potential, hepato- and nephrotoxicity have been seen as the major side effects. In addition, a high pharmacokinetic variability makes it necessary for the therapeutic drug monitoring which usually implies blood sampling. Recently, a new microemulsion-based CyA formulation (Sandimmun Neoral) was introduced showing larger area under the curve (AUCs) and higher peak concentrations than those achieved with the oily solution. Nonetheless, no significant differences were found when comparing the trough concentrations (Lindholm, 1995) indicating that similar CyA trough levels in blood may reflect very different AUC values. This fact is consistent with the lack of total correlation between drug levels and rejection episodes or toxicity (Sabbatiello et al., 1996; Rocha et al., 1997; Cantarovich et al., 1997). Thus, the measurement of CyA's AUC has been suggested as a more effective way for estimation of total body drug exposure (Kahan and Grevel, 1988; Awni et al., 1990).

AUC values within a dose administration interval are usually determined from a concentration–time curve including several blood sample concentration data using the trapezoidal rule. Unfortunately, obtaining complete pharmacokinetic profiles from transplant patients is not always an easy task due to ethical, practical and economical reasons. Therefore, reducing the number of blood samples necessary for AUC determinations would be very practical for patient management and would help stave off costs in cyclosporine monitoring. Different authors have already addressed this problem concerning CyA. Thus, Johnston et al. (1990) suggested that the CyA concentrations reached 5 h post-administration accounted for 89% of AUC variability; Cantarovich et al. (1988, 1997) and Rocha et al. (1997) also reported that a single time point sampling at 6 h predicted clinical outcome better than trough concentrations. A completely opposite view expressed Grevel and Kahan (1991), Meyer et al. (1993) and Serino et al. (1994) who reported that AUC and average CyA concentrations at steady state (C_{ssav}) should be derived from abbreviated kinetic profiles including at least three time points in order to secure a more reliable estimate. When the same strategy of abbreviated AUC-based CyA monitoring was applied to Sandimmun Neoral treatment, it was shown that two time points (2 and 6 h post treatment) were sufficient to justify 93% or more variability found in AUCs estimated from the full profile (Amante and Kahan, 1996a,b). Sabbatiello et al. (1996) found out that CyA concentration 9 h after Neoral treatment was the best estimator of AUC ($r^2 = 0.91$). Due to more reproducible CyA absorption kinetics with the new formulation, AUCs obtained from abbreviated profiles often include early time points (Serafinowicz et al., 1996; Rial et al., 1997; Serafinowicz et al., 1997) with subsequent reduction of limited sampling strategy period.

Biodegradable polymeric nanoparticles have proven their ability to increase the oral bioavailability of CyA in rats. Indeed, the average AUC values obtained after CyA dosage (10 mg/kg) as polycaprolactone NP were fourfold and twofold higher than the corresponding AUCs calculated after SIM-EM and SIM-Neoral treatment, respectively (Molpeceres et al., 1998a,b). Therefore, it would be of great interest to know whether CyA administration in the form of polymeric nanoparticles improves the prediction of total body drug exposure from abbreviated AUC profiles, hence, in theory (and in practice) could be potentially useful in reducing the costs of drug monitoring. In view of the above, the objective of the present study consisted on establishing whether polycaprolactone nanoparticles indeed, serve for the reduction of time points needed for AUC estimation under a limited sampling strategy as compared to two reference formulations of Sandimmun.

2. Materials and methods

2.1. Preparation and characterization of CyA dosage forms

The reference formulations were the following: SIM-EM consisted of an emulsion containing 300 μl of the commercial oily solution (100 mg/ml) in 10 ml of whole milk. The emulsion was prepared by using a Polytron homogenizer (Kinematica, Switzerland) during 10 min at 12 500 rpm and room temperature. SIM-Neoral was obtained upon dilution of the microemulsion preconcentrate contained in soft gelatin capsules (100 mg CyA) with phosphate buffer (pH 6.8). CyA loaded nanoparticles (CyA-NP) were produced by the solvent displacement method with slight modifications (Molpeceres et al., 1996). CyA content in each formulation was determined by HPLC (Guzmán et al., 1993) and the amount of drug encapsulated by the nanoparticles was calculated as the difference between the total content in the colloidal suspension and the drug remaining in the supernatant after centrifugation at $35\,000 \times g$ for 60 min at room temperature. The particle size distributions of the colloidal suspension, the microemulsion and the emulsion were measured by frequency spectrum analysis in a UPA/SRA particle size analyzer (Leeds and Northrup, Ireland).

2.2. Study design

Thirty-six (18 males and 18 females), 31 (17 males and 14 females) and 10 (5 males and 5 females) Wistar rats (weight 250–300 g) comprising group 1a, 1b and 1c were obtained from the Central Stabulary of the University (Homologation No E.C. 28005-22A). The rats were housed in metabolical cages and fasted 12 h prior to dosing. CyA was administered by gavage as a single oral dose of SIM-EM, CyA-NP or SIM-Neoral to groups 1a, 1b and 1c, respectively. Four different doses (5, 7.5, 10 and 15 mg/kg) representing a clinically relevant dose range were selected except for group 1c that only received 10 mg/kg. The pharmacokinetic profiles were based on whole blood samples extracted from jugular vein under light isoflurane (Abbot Laboratories) anesthesia at 0 (pretreatment), 0.5, 1, 2, 4, 6, 8, 24, 32 and 48 h after treatment. These profiles were tested for correlation between AUC and the drug concentrations obtained at each time point.

A second part of the study was dedicated to the evaluation of AUC accuracy estimation in two independent groups (groups 2a and 2b) containing 9 (6 males and 3 females) and 8 rats (3 males and 5 females), who were given CyA orally at the same doses as mentioned above (SIM-EM or CyA-NP), respectively. None of these rats were included in the first part of the study.

2.3. CyA assay

Blood samples (150 μl) were collected in 20 μl aqueous solution of EDTA (22 mg/ml), disodium salt, thoroughly mixed and 100 μl of this solution were immediately separated for drug analysis. The samples were stored at -20°C until fluorescence polarization immunoassay (FPIA) analysis with monoclonal antibodies (TDx, Abbot Laboratories).

2.4. Statistical analysis

The AUC for each animal was calculated by the linear trapezoidal rule from 0 time to the last experimentally measured concentration above the limit of detection (25 ng/ml).

The correlations between drug concentrations at each time point and the AUC were assessed by linear regression analysis and the resulted regression significance was estimated by ANOVA.

Stepwise multiple linear regression using blood concentrations grouped by time as the independent variables and AUC as the dependent variable revealed which time points were more adequate to predict AUC with an acceptable safety margin.

The equations derived from the multiple regression analysis in group 1 were then applied to predict AUC in the second group of rats. The agreement between the predicted and experimentally determined AUC was examined using regression analysis and the prediction error (P.E.) calculated from:

$$\text{P.E.} = \frac{\text{Predicted AUC-Measured AUC}}{\text{Measured AUC}} \times 100$$

In addition, since the longest CyA treatment interval in clinical practice is 24 h, the linear and multiple regression analysis were also performed in group 1, but considering the pharmacokinetic data from 0 to 24 h. Prediction errors were also calculated for group 2.

Finally, to verify whether the mathematical models corresponding to each formulation were dependent on the amount of data, 8 males and 8 females from group 1a and the same number of animals from group 1b receiving SIM-EM and CyA-NP, respectively-though at different dose levels, were randomly selected (2 animals per group, gender and dose level). The above mentioned analyses were repeated on this subset of 16 pharmacokinetic profiles per formulation.

3. Results

3.1. Preparation and characterization

Polycaprolactone nanoparticles had mean particle size of 105 ± 44 nm and a maximum encapsulation efficiency of $95.6 \pm 0.8\%$ for a total drug content in the formulation adjusted to 2.5 mg/ml. The diluted emulsion contained 3 mg/ml CyA and the size of the internal phase globules ranged between 4 and 100 μm (mean particle size = 4.33 μm). The microemulsion formulation had the same CyA concentration but the average particle size was in the nanometer range (mean particle size = 27 nm).

3.2. Correlation between CyA concentrations and AUC

The correlation between AUC (0–48 h) and single individual concentrations at different sampling times for all formulations are shown in Table 1. When CyA was administered as CyA-NP, determination coefficients shaved that four distinct time points (6, 8, 24 and 32 h after treatment) individually reflected at least 83% of the variations found in AUC (0–48 h) calculated from the nine time-point. Likewise, two time points (6 and 8 h after treatment) showed a strong correlation ($r^2 > 0.85$) with AUC (0–48 h) after SIM-Neoral treatment. On the other hand, the highest correlation between AUC (0–48 h) and CyA blood concentrations was reached at 32 h after treatment with SIM-EM. Although, the maximum r^2 value was quite lower than those achieved with NP or SIM-Neoral (0.69 versus 0.88 or 0.89, respectively).

When the AUC from 0 to 24 h interval was considered, CyA concentrations at 6 and 8 h after CyA-NP and SIM-Neoral treatment, individually accounted for 93 and 94% AUC variability, respectively. The concentration reached after 8 h post-treatment with SIM-EM also proved to be the best indicator of drug exposure. Again, the correlation with AUC was lower ($r^2 = 0.80$) than those obtained with the other two formulations (Table 2).

The stepwise multiple linear regression indicated that a higher percentage of AUC (0–48 h) variability was explained by using CyA concentrations at 8 and 32 h post-treatment with NP or SIM-EM. CyA concentrations at 8 and 24 h were the best to predict AUC (0-48 h) after SIM-Neoral administration. Nevertheless, a slightly higher r^2 was obtained with SIM-Neoral (0.987 versus 0.971 for CyA loaded NP or 0.964 for SIM-EM) (Tables 3 and 4). Where more concentration time points were considered, the results improved but only slightly for SIM-Neoral. The model equations for AUC estimation when CyA was administered as SIM-EM (Table 4) suggested that an additional time point should be included in order to reach the same determination coefficient as obtained with NP or SIM-Neoral (Tables 3 and 4).

The same statistical methods applied to the 24 h profiles evidenced that CyA concentrations at 4 and 8 h post-treatment with CyA-NP and those at 8 and 24 h post-treatment with SIM-EM allowed for a reliable estimation of AUC, since the r^2 values were 0.968 and 0.949, respectively. Upon including a third time point into the model; 24 and 2 h for CyA-NP and SIM-EM, respectively, it increased the correlation to $> 98\%$ in both cases (Tables 5 and 6). Such a high correlation ($r^2 > 0.985$) was obtained with only two time points (6 and 8 h) after SIM-Neoral treatment (Table 6).

Table 1
Correlation of blood CyA concentration at specific time point and the AUC (0–48 h) determined from the full profile

Time point	SIM-EM				SIM-Neoral				CyA-NP			
	Intercept	Slope	r^2	P	Intercept	Slope	r^2	P	Intercept	Slope	r^2	P
0.5	5863.74	23.59	0.38	<0.001	−1252.56	22.82	0.53	0.017	15191.91	12.47	0.10	0.106
1	4930.77	19.55	0.51	<0.001	4302.84	11.85	0.44	0.037	8578.48	13.85	0.27	0.006
2	5436.12	11.28	0.42	<0.001	5407.48	10.69	0.57	0.011	4845.02	12.78	0.40	<0.001
4	5978.54	9.95	0.36	<0.001	402.87	18.76	0.61	0.007	5046.99	11.61	0.61	<0.001
6	5059.04	13.91	0.44	<0.001	1705.04	21.09	0.85	<0.001	3187.81	17.96	0.86	<0.001
8	3461.22	20.03	0.48	<0.001	289.37	26.97	0.89	<0.001	2015.22	23.37	0.88	<0.001
24	5045.78	27.61	0.63	<0.001	4647.42	40.44	0.75	0.001	6940.81	46.66	0.84	<0.001
32	5328.16	37.31	0.69	<0.001	6396.07	61.12	0.74	0.001	9213.96	63.92	0.83	<0.001
48	6573.15	73.75	0.63	<0.001	9937.87	88.12	0.72	0.002	12470.16	114.25	0.69	<0.001

Table 2
Correlation of blood CyA concentrations at specific time points and the AUC (0–24 h) determined from the full profile

Time point	SIM-EM				SIM-Neoral				CyA-NP			
	Intercept	Slope	r^2	P	Intercept	Slope	r^2	P	Intercept	Slope	r^2	P
0.5	4015.12	17.08	0.43	<0.001	−1163.22	18.51	0.65	0.005	10361.37	12.78	0.22	0.01
1	3202.79	14.64	0.62	<0.001	2717.86	10.36	0.63	0.006	6660.69	10.96	0.38	<0.001
2	3045.18	9.33	0.62	<0.001	4013.34	8.94	0.75	0.001	4252.85	9.96	0.53	<0.001
4	3162.22	8.84	0.63	<0.001	72.98	15.36	0.77	0.001	4107.30	9.30	0.76	<0.001
6	2522.83	12.12	0.73	<0.001	1805.18	16.13	0.93	<0.001	2879.9	14.26	0.93	<0.001
8	1363.01	17.19	0.80	<0.001	879.47	20.31	0.95	<0.001	2235.03	18.15	0.94	<0.001
24	4952.39	13.36	0.33	<0.001	5314.84	25.49	0.56	0.013	7393.15	33.45	0.70	<0.001

When CyA blood concentrations from the second group were substituted into the model equations for predicting AUC (0–48 h), which were based on the single individual concentration with the highest r^2 (C8 and C32 for CyA-NP and SIM-EM, respectively), the mean (range) prediction errors were 1.46% (−16.2 to 21.33%) and 5.71% (−44.33 to 105.94%) for NP as well as for the reference formulation, respectively. However, the model equations for NP that included two and three time-points proved that the errors were −0.92% (−12.5 to 12.5%) and −3.42% (−10.76 to 6.30%), respectively, while the corresponding values for SIM-EM indicated more extended ranges (Fig. 1).

Fig. 2 shows the regression of the measured AUC (0–48 h) values versus predicted AUC in the second group when the model equations in-

Table 3
Mathematical model for the prediction of AUC (0–48 h) obtained by stepwise multiple linear regression after CyA was administered as polymeric NP

Number of time-points in equation	Time points after administration (h)	Model equations AUC (ng × h/ml) estimation[a]	r^2
2	8, 32	14.47 × C8 + 32.97 × C32 + 2999.63	0.971
3	4, 8, 32	3.07 × C4 + 10.38 × C8 + 33.76 × C32 + 1818.26	0.990
4	1, 4, 8, 32	2.48 × C1 + 2.16 × C4 + 10.47 × C8 + 33.91 × C32 + 627.60	0.995
5	1, 4, 8, 24, 32	2.22 × C1 + 2.64 × C4 + 9.36 × C8 + 13.43 × C24 + 17.11 × C32 + 138.01	0.997
6	1, 4, 8, 24, 32, 48	1.81 × C1 + 2.91 × C4 + 9.33 × C8 + 14.96 × C24 + 8.14 × C32 + 14.20 × C48 + 285.63	0.999
7	1, 4, 6, 8, 24, 32, 48	1.36 × C1 + 3.76 × C4-4.54 × C6 + 14.26 × C8 + 16.36 × C24 + 4.29 × C32 + 17.78 × C48 + 33.94	0.999
8	0.5, 1, 4, 6, 8, 24, 32, 48	−0.82 × C0.5 + 1.87 × C1 + 3.81 × C4-5.02 × C6 + 14.93 × C8 + 15.81 × C24 + 4.24 × C32 + 18.34 × C48 + 9.69	0.999

[a] C(i) is the individual concentration corresponding to the ith time point.

Table 4
Mathematical model for the prediction of AUC (0–48 h) obtained by stepwise multiple linear regression after CyA was administered as SIM-EM and SIM-Neoral

Formulation	Number of time-points in equation	Time points after administration (h)	Model equations AUC (ng × h/ml) estimation[a]	r^2
SIM-EM	2	8, 32	15.49 × C8 + 31.93 × C32 + 154.91	0.964
	3	2, 8, 32	3.25 × C2 + 11.74 × C8 + 31.26 × C32 + 44.96	0.981
	4	2, 8, 24, 32	3.74 × C2 + 11.34 × C8 + 10.57 × C24 + 18.31 × C32 − 373.62	0.992
	5	2, 8, 24, 32, 48	3.57 × C2 + 11.02 × C8 + 13.53 × C24 + 10.71 × C32 + 10.46 × C48 − 84.25	0.994
	6	2, 4, 8, 24, 32, 48	2.44 × C2 + 1.85 × C4 + 9.67 × C8 + 14.21 × C24 + 9.32 × C32 + 12.78 × C48 − 238.47	0.997
SIM-Neoral	2	8, 24	18.96 × C8 + 19.01 × C24 − 15.74	0.987
	3	6, 8, 24	7.04 × C6 + 11.27 × C8 + 18.27 × C24-71.06	0.998
	4	2, 6, 8, 24	0.96 × C2 + 5.47 × C6 + 10.96 × C8 + 19.48 × C24 − 50.24	0.998
	5	2, 6, 8, 24, 32	1.82 × C2 + 4.01 × C6 + 10.32 × C8 + 15.68 × C24 + 8.67 × C32 + 232.24	0.999
	6	0.5, 2, 6, 8, 24, 32	1.67 × C0.5 + 1.98 × C2 + 3.85 × C6 + 8.51 × C8 + 13.89 × C24 + 13.01 × C32–129.59	0.999

[a] C(i) is the individual concentration corresponding to the ith time point.

Table 5
Mathematical model for the prediction of AUC (0–24 h) obtained by stepwise multiple linear regression after CyA was administered as polymeric NP

Number of time-points in equation	Time points after administration (h)	Model equations AUC (ng × h/ml) estimation[a]	r^2
2	4, 8	$3.02 \times C4 + 13.99 \times C8 + 1270.47$	0.968
3	4, 8, 24	$3.43 \times C4 + 9.77 \times C8 + 9.94 \times C24 + 1180.85$	0.991
4	1, 4, 8, 24	$1.98 \times C1 + 2.74 \times C4 + 9.56 \times C8 + 10.37 \times C24 + 294.43$	0.998
5	1, 4, 6, 8, 24	$1.80 \times C1 + 3.16 \times C4 - 2.56 \times C6 + 12.38 \times C8 + 10.26 \times C24 + 181.59$	0.999
6	0.5, 1, 4, 6, 8, 24	$-0.83 \times C0.5 + 2.28 \times C1 + 3.22 \times C4 - 3.03 \times C6 + 12.97 \times C8 + 9.96 \times C24 + 161.31$	0.999

[a] C(i) is the individual concentration corresponding to the ith time point.

Table 6
Mathematical model for the prediction of AUC (0–24 h) obtained by stepwise multiple linear regression after CyA was administered as SIM-EM and SIM-Neoral

Formulation	No. of time-points in equation	Time points after administration (h)	Model equations AUC (ng × h/ml) estimation[a]	r^2
SIM-EM	2	8, 24	$15.55 \times C8 + 9.30 \times C24 - 6.19$	0.949
	3	2, 8, 24	$3.33 \times C2 + 11.69 \times C8 + 9.19 \times C24 - 233.29$	0.981
	4	2, 4, 8, 24	$2.20 \times C2 + 1.89 \times C4 + 10.41 \times C8 + 9.46 \times C24 - 233.97$	0.994
	5	0.5, 2, 4, 8, 24	$1.22 \times C0.5 + 1.72 \times C2 + 1.93 \times C4 + 10.54 \times C8 + 9.20 \times C24 - 271.22$	0.995
SIM-Neoral	2	6, 8	$7.62 \times C6 + 11.71 \times C8 + 806.85$	0.985
	3	6, 8, 24	$7.33 \times C6 + 10.34 \times C8 + 3.99 \times C24 + 745.45$	0.992
	4	2, 6, 8, 24	$2.31 \times C2 + 3.55 \times C6 + 9.59 \times C8 + 6.92 \times C24 + 795.55$	0.997
	5	0.5, 2, 6, 8, 24	$1.91 \times C0.5 + 1.99 \times C2 + 4.22 \times C6 + 7.88 \times C8 + 7.04 \times C24 + 220.81$	0.995

[a] C(i) is the individual concentration corresponding to the ith time point.

cluded a single individual time-point (C8 and C32 for CyA-NP and SIM-EM, respectively), two (C8 and C32 for both CyA-NP and SIM-EM) or three concentrations (C4, C8 and C32 for CyA-NP or C2, C8 and C32 for SIM-EM) (Tables 1, 3 and 4). In addition to the higher predictive ability reflected by the CyA-NP model (i.e. for single time point models $r^2 = 0.978$ versus $r^2 = 0.929$), the slope of the linear regression approached the line of identity in most cases, except for the single point (C32) where the model tended to predict lower AUC after SIM-EM treatment (Fig. 2d). Fig. 3 represents the linear regression between estimated AUC and measured AUC from 0 to 24 h. The predictive competence of the models ($r^2 = 0.969$ versus $r^2 = 0.917$, for CyA-NP and SIM-EM, respectively) was not significantly altered, even though different time periods were considered (24–48 h) and different concentration-time points were involved. In this case, the model equations with a single individual time-point included C8 for both CyA-NP and SIM-EM as the best sole estimator of AUC (0–24 h). C4 and C24 were successively added to C8 to obtain the two and three points model for CyA-NP, respectively, while C24 and C2 were the concentrations sequentially incorporated to obtain the two points and three points model for SIM-EM (Tables 2, 5 and 6). Concerning the prediction errors for the 24 h model, there was a lesser error and a less biased AUC (0–24 h) estimation, when using CyA-NP compared to SIM-EM. In fact, the mean predic-

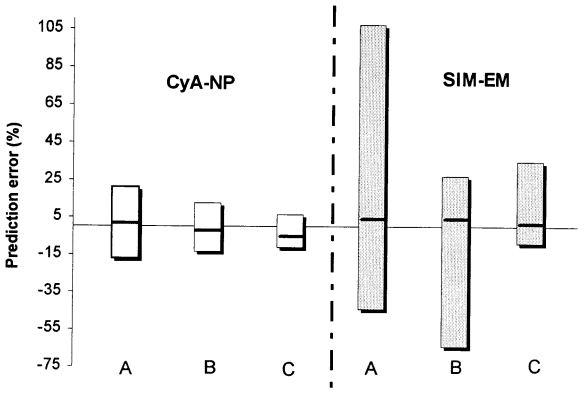

Fig. 1. Percentage mean error (black horizontal line) and range (vertical bars) in AUC prediction from 0 to 48 h using different models. (A) Single time point; (B) two time points; and (C) three time points equations obtained from group 1.

tion errors corresponding to a single, two or three point model were 0.43, −3.44 and −1.81% with ranges −16.19 to 24.32%, −13.07 to 6.73% and −6.53 to 5.86%, respectively. On the contrary, greater error ranges were found in case of SIM-EM treatment, −1.47 to 43.06%, −101.46 to 36.23% and −70.3 to 6.35% for the single, two and three time-points model, respectively. Determination coefficients were always higher for the NP formulation regardless of the time interval of the study.

To validate the mathematical models previously discussed, the study was repeated in a smaller and randomly selected group of rats and the results were (considering the 0–48 h period) compared to those from the whole batch of data (groups 1a and 1b). The statistical analysis revealed CyA concentrations at 4, 6, 8, 24 and 32 h post-treatment with CyA-NP were the best sole estimators of AUC (0–48 h) with r^2 values ranging between 0.80 and 0.93. When more than one time-point was considered the determination coefficient im-

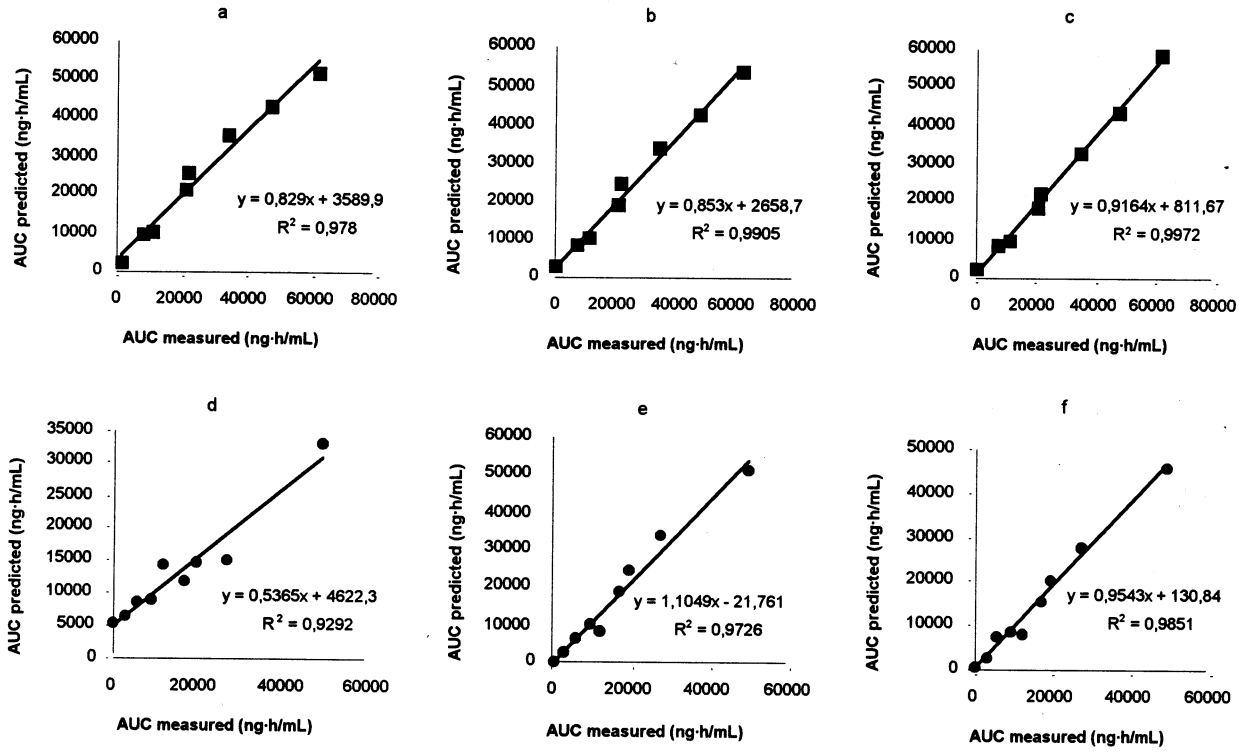

Fig. 2. Correlation between measured and predicted AUC (0–48 h) in group 2. (a) Single time-point (C8), (b) two time-points (C8 and C32), (c) three time-points (C4, C8 and C32) model after CyA-NP dosing, (d) single time-point (C32), (e) two time-points (C8 and C32) and (f) three time-points (C2, C8 and C32) model after treatment with SIM-EM.

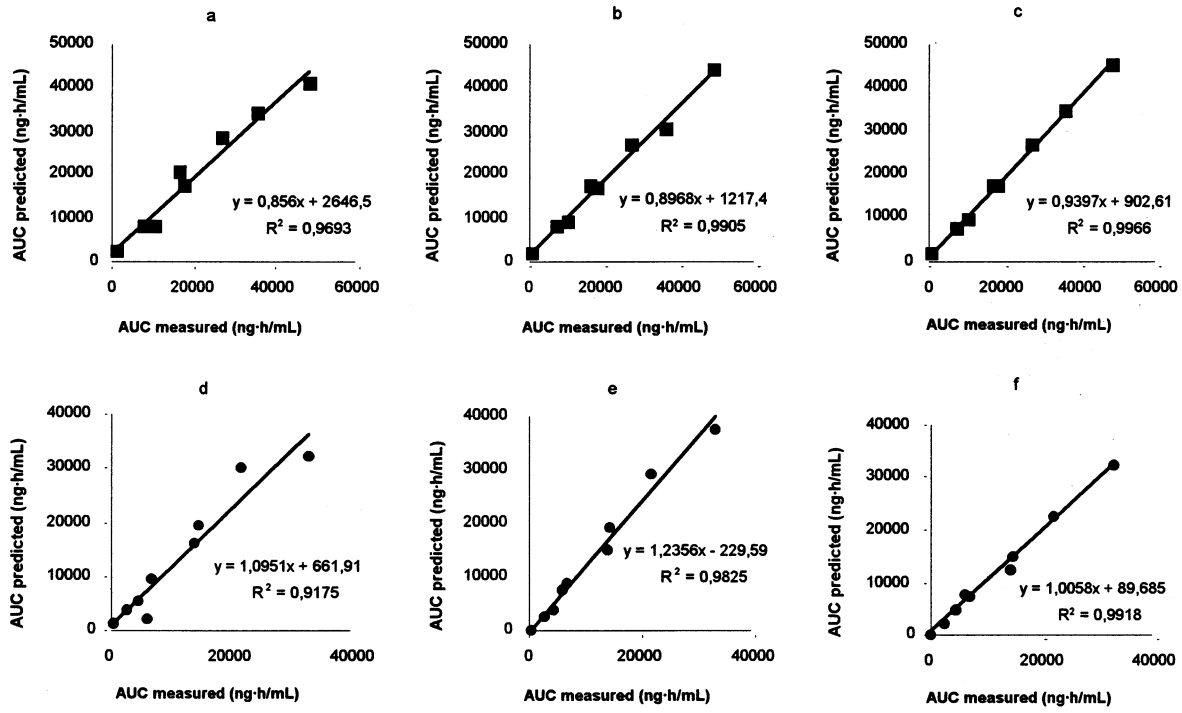

Fig. 3. Correlation between measured and predicted AUC (0–24 h) in group 2. (a) Single time-point (C8), (b) two time-point (C4 and C8), (c) three time-point (C4, C8 and C24) model after CyA-NP treatment, (d) single time-point (C8), (e) two time-point (C8 and C24), and (f) three time-points (C2, C8 and C24) model after treatment with SIM-EM.

proved to 0.99 by using C8 and C24. However, when CyA was administered as SIM-EM, only two individual concentrations (C24 and C32) accounted for 80% or more of AUC (0–48 h) variability with the rest of concentrations showing a very poor correlation. As occurred for CyA-NP, the model containing C8 and C24 taken together proved to be the best approach to explain AUC (0–48 h) variability ($r^2 = 0.98$). Apparently, there is a lack of coincidence between these results and those from the whole batch of data, but C24 and C32 had very similar determination coefficients (Table 1; for the whole batch and undisclosed results for reduced group), consequently, they can be considered interchangeable in this case. In fact, if C24 was substituted by C32 in the model derived from the reduced group, the determination coefficients did not vary significantly (0.993 versus 0.995 and 0.973 versus 0.983 for CyA-NP and SIM-EM, respectively).

4. Discussion

The clinical use of CyA is actually guided by the measurement of blood peak or trough levels and more recently, by the production of pharmacokinetic profiles to obtain AUC or C_{ss} which represent a more useful index of patient exposure to the drug. However, the last approach, although generally accepted as the most appropriate, is not taken by all clinicians. A few years ago, a new microemulsion-based formulation containing CyA was marketed with improved pharmacokinetic behavior but its advantages from a pharmacodynamic point of view remain challenged. In fact, some authors recommend CyA therapy with Neoral only in those patients with erratic and nonstable pharmacokinetics (Bennett et al., 1996; Johnston and Holt, 1996; Pollard, 1996). Furthermore, the higher C_{max} and AUC of the microemulsion formulation may have implications for therapy, as well as for toxic adverse effects

(Friman and Bäckman, 1996). Since, many questions concerning long term effects have not yet been fully answered, we considered the SIM-EM as a valid reference formulation. Very recently, we have demonstrated that polycaprolactone NP increased the AUC after oral treatment of Wistar rats with CyA, even when the microemulsion was taken as the reference formulation (Molpeceres et al., 1998a,b). In this paper, it has been demonstrated that the correlations between AUC and blood concentrations are better for both polycaprolactone NP loaded with CyA and SIM-Neoral than the SIM emulsion, considering the new trends in clinical CyA monitoring. In fact, four single individual concentrations after CyA-NP treatment accounted for 83% or more variability in AUC (0–48 h), while only two (C6 and C8) achieved the same correlation level after SIM-Neoral dosage. Furthermore, C32 (the concentration with the highest correlation) accounted for 69% variability in AUC (0–48 h) after CyA treatment in form of an emulsion. The same results were obtained when the sampling period was reduced to 24 h, the largest sampling time used in clinical practice. C6 and C8 were the most adequate CyA concentrations to explain AUC (0–24 h) variations in more than 93% of the cases. However, single point predictions do not represent a reliable estimate for clinical application.

A model equation based on two concentration time points obtained at 8 and 32 h increased the correlation between CyA concentrations and AUC (0–48 h) to 0.964 and 0.971 after CyA was administered as SIM-EM or CyA-NP, respectively. On the other hand, a two-points model including C8 and C24 represented the best approach to estimate AUC (0–48 h) with a high degree of correlation ($r^2 = 0.987$) after SIM-Neoral treatment. For the 24 h period, the best equations to define AUC from two concentration time points exhibited some modifications. Indeed, C32 was replaced by C24 for SIM-EM model ($r^2 = 0.949$) while, C4 substituted C32 in the CyA-NP model ($r^2 = 0.968$). After SIM-Neoral treatment, C6 and C8 together (the concentrations with the highest individual correlation) defined the best two-points model with no significant alterations of the degree of correlation ($r^2 =$ 0.985). In spite of the fact that determination coefficients corresponding to each formulation were nearly identical, the application of the two-points derived mathematical model to experimental data from an independent population, resulted in a higher predictive capacity for NP as shown by the range of the relative prediction errors, as well as the r^2 values (Fig. 1). The same results were obtained when those individual concentrations showing the highest r^2 values were used to predict the AUC in an independent group of rats; the regression analysis indicated a better predictive capacity for CyA-NP ($r^2 = 0.978$ and 0.969) than SIM-EM ($r^2 = 0.929$ and 0.917), regardless the considered time period (24 or 48 h). Additionally, the slope of the linear regression approached the line of identity in most cases, except for the single point (C32) of the model used to predict AUC after SIM-EM treatment, where the measured AUC (0–48 h) was two-fold higher than the predicted value.

The validity of the models presented here was assessed by repeating the analysis in a reduced group of rats which had been randomly selected from groups 1a and 1b. C8 and C24 were the concentrations for CyA-NP and SIM-EM, respectively, which indicated higher correlation with AUC (0–48 h). When the model was extended to two time-points, both concentrations together represented the AUC variability in more than 98% of the cases. Data from the whole batch indicated that the model containing C8 and C32 represented the best approach to predict AUC (0–48 h) after CyA-NP or SIM-EM treatment. Therefore, there is an apparent lack of coincidence suggesting that the model were not validated because sample size exerts an influence on the blood concentrations that should be considered to predict AUC (0–48 h). However, the similar determination coefficients found for C24 and C32 with AUC (0–48 h) suggest that they could be considered interchangeable. In fact, the application of a two points model derived from the reduced group, where C24 was substituted by C32, did not induce significant changes of the determination coefficients (0.993 versus 0.995 and 0.973 versus 0.983 for CyA-NP and SIM-EM, respectively) suggesting similar predictive ability. Hence, the model can be considered as valid.

In summary, CyA loaded polycaprolactone NP offer a good alternative to other existing CyA formulations as far as drug monitoring is concerned. From a clinical point of view, a single point concentration (8 h post-treatment) is sufficient to predict about 97% variability in AUC after oral administration to rats. However, the impact of different CyA pharmacokinetics that exist between rats and humans on the results presented here should be established. Furthermore, our results constitute a comparison between three different formulations under experimental conditions (single oral dose and sampling time 0–48 h or 0–24 h) which diverges substantially from steady state clinical pharmacokinetics. Consequently, further studies are needed to assess with precision established superiority of CyA-loaded NP under clinical conditions.

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