Review in Pharmacokinetic Models on Corticosteroids

Nabil Semmar* and Nicolas Simon

Laboratory of Medical Pharmacology, Medical School of Marseilles, UPRES EA 3784, 27 bd Jean Moulin, 13385 Marseilles Cedex 5, France

Abstract: The pharmacokinetics of corticosteroids provides a large set of mathematical models which led to analyse many kinetic profiles corresponding to many clinical and/or physiological situations. In this paper, we present a review on the usefulness, advantages and limits of such models which could find a large application in medicinal chemistry.

Key Words: Corticosteroids, Pharmacokinetic models, Release, Absorption, Distribution, Elimination processes.

INTRODUCTION

Pharmacokinetics describes the relationship between the dose and the unbound drug concentration at the site of action (a drug receptor), and the time course of drug in the body [1]. Drug disposition is a broad term that covers all the processes by which the body handles foreign chemicals including drugs. These are absorption into systemic circulation, distribution and metabolism in the body, and elimination from the body _ often abbreviated as ADME. For a given drug, the ADME system can be characterized by a pharmacokinetic (PK) model, which is a hypothetical structure using mathematical terms to concisely describe quantitative relationships, namely to parameterize the essential factors governing the kinetic process. Simplifying assumptions are made to describe a complex biological system concerning the movement of drugs. The system is entirely defined by the determination of the PK parameters of drug, leading to describe how the drug is handled by the body.

The PK models of corticosteroids are various, and led to fit many kinetic profiles resulting from different clinical situations and/or physiological states. These models were applied with the aim of:

- describing the basic kinetics of an endogenous corticosteroid,
- quantifying its disturbance after administration of exogenous drugs,
- modeling the time-concentration profiles of exogenous corticosteroids.

The main of the present paper is to present a review on the diversity of the PK models of corticosteroids, which could find large applications in medicinal chemistry. The choice of corticosteroids was motivated on the hand by the fact that these compounds offer a large variety of PK models, and on the other hand by the fact that they are very similar to natural compounds (i.e. steroids, steroid alkaloids, triterpenoids). Moreover, the various PK models of corticosteroids showed important inter-individual variabilities of the PK parameters [2]; this highlights large handling ranges of these compounds by the human body.

Technically, the corticosteroids' term refers to both glucocorticoids and mineralocorticoids, but it is often used as a synonym for glucocorticoids which will be especially presented in this paper. Glucocorticoids (GC) play a number of important physiological roles. In corticotherapy, they are administrated by different ways (parenteral, oral, cutaneous, inhalation, etc.).

SYNTHESIS, STRUCTURES, ACTIVITIES OF GLUCOCORTICOIDS

Glucocorticoids could be separated into endogenous (naturally produced by the body) and exogenous (synthetic) compounds. Their basic chemical structure consists of 21 carbon atoms with 4 rings: three 6-carbon rings and a five-carbon ring (Fig. **1**).

They are lipophilic low-molecular weight compounds derived from cholesterol. Endogenous GC are synthesized mainly by endocrine glands such as the gonads (testis and ovary), the adrenals and (during gestation) by the fetoplacental unit. They are steroid hormones which are released into the blood circulation to (1) regulate whole body homeostasis, and (2) to maintain balance in the body's host-defense system to protect against over-reaction to environmental change in the invasion of foreign substances. This control includes profound effects on metabolism of carbohydrates, proteins, and lipids through gene regulation and post-transcriptional effects, trafficking and functions of lymphoid cells, and effects on inflammatory molecules [3-5]. They are also involved in maintenance of the integrity of the cardiovascular system, central nervous system, and skeletal muscle function. Exogenous GC are administrated as drugs to decrease or to prevent tissue responses to inflammatory processes, thereby reducing development of symptoms of inflammation without affecting the underlying cause. Corticosteroids inhibit accumulation of inflammatory cells including macrophages, monocytes, endothelial cells, fibroblasts, and lymphocytes at sites of inflammation, in part by induction of lipocortin, a protein that inhibits phospholipase A2 [6]. As a result, there is a decrease in the production and release of cytokines, an inhibition of the synthesis of arachidonic acid–derived mediators of

*Address correspondence to this author at the Laboratory of Medical Pharmacology, Medical School of Marseilles, UPRES EA 3784, 27 bd Jean Moulin, 13385 Marseilles Cedex 5, France;
E-mail: nabilsemmar@yahoo.fr

1389-5575/06 $50.00+.00

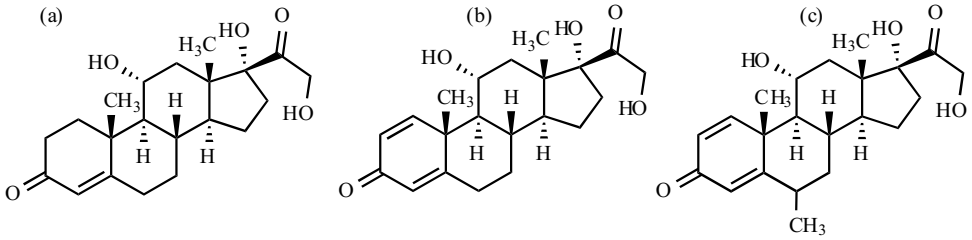

Fig. (1). Structures of hydrocortisone (a) (natural corticosteroid), prednisolone (b) and methylprednisolone (c) (synthetic corticosteroids).

inflammation (leukotrienes and prostaglandins), and a decrease in the extravasation of leukocytes to areas of injury [6-9]. An immunosuppressant effect of corticosteroids also may contribute to the anti-inflammatory effect, possibly because they involve inhibition of specific functions of leukocytes [6].

Concerning the anti-inflammatory activity of steroids, the essential features consist of: 1) a 2-carbon chain at C-17; 2) methyl groups at C-18 and C-19; 3) ketone oxygen at C-3; 4) an unsaturated bond between C-4 and C-5; 5) a hydroxyl group at C-11; 6) a ketone oxygen at C-20. Changes in these positions lead to a loss of biological activity. Substitutions in other sites may modify the biological activity, imparting greater anti-inflammatory activity [5, 6; 10-13]:

Structural chemical properties	Biological activity variations
Double bond 1-2 (prednisone, prednisolone)	increases the anti-inflammatory activity
Methylation at C6 (eg. methyprednisolone)	increases anti-inflammatory activity and improves pulmonary penetration.
Fluorination at C9 (triamcinolone, dexamethasone)	improves anti-inflammatory activity
Hydroxylation or methylation at C16	greatly reduces anti-inflammatory activity
Hydroxylation at C17	very important for anti-inflammatory activity
Hydroxylation at C21	very important for anti-inflammatory activity

These are all important considerations in the formulation and design of synthetic steroids. A variety of synthetic glucocorticoids have been created for therapeutic use. Cortisol or hydrocortisone (HCn), a natural GC, is the standard of comparison for GC potency.

PHARMACOKINETICS of GLUCOCORTICOIDS

(A) Absorption

Absorption is a PK process which characterizes a *per os* administration way of drug. In a PK model, it is quantified by the absorption coefficient which gives the average speed of drug transport from intestine to systemic circulation. The drug fraction (in %) which transits the gastro-intestinal system to reach the systemic circulation is called bioavailability (F). It is calculated from the ratio of area under concentration vs. time curve (AUC) of the drug after *per os* administration on corresponding AUC obtained after an intravenous (IV) bolus administration:

$$F = \frac{AUC\ \ of\ \ per\ \ os\ \ curve}{AUC\ \ of\ \ bolus\ \ curve} \times 100 \quad (1)$$

All corticosteroids are absorbed readily from the gastrointestinal tract. In consequence, their absorption after oral administration is rapid and their bioavailability is high (78% for prednisone, 98% for prednisolone), but with a wide range among normal subjects, reflecting an important inter-subject variability [14, 32]. Water-soluble esters are given by an IV way to achieve absorption rapidly, while intramuscular injection provides more prolonged effects [15]. Hemi-synthesis derivatives are used after small modification (e.g. substitution in 17α position) of the initial structure in order to improve the bioavailability after a *per os* administration. Corticosteroids are also well absorbed from several sites of topical application, and large doses can lead to systemic absorption.

(B) Distribution

In blood, corticosteroids are mostly bound to plasma proteins, and only unbound drug has access to the tissues to be biologically active. The concentration of the unbound drug is:

$$Cu = fu * C \quad (2)$$

where Cu is the unbound drug concentration, fu is the unbound fraction to plasma proteins, and C is the total concentration in the blood. The affinity of drug for the tissues could be quantified by its distribution volume (V). The volume V depends on the free fraction of drug in plasma (fu), the free fraction in tissue (fu_t), the volume of tissue (V_T) and the volume of plasma (V_P) [16, 17]:

$$V = V_p + \left(\frac{fu}{fu_t} \right) \cdot V_T \quad (3)$$

Because V_p and V_T are physiologic constants, V will depend primarily on the ratio fu/fu_t. Subsequently, alterations in fu (e.g. by displacement) can have a profound impact on drug pharmacokinetics [17].

A high V means a high affinity of drug for tissue which could lead to a longer pharmacological action duration. Inversely, a low V means a high affinity of drug for plasma, which supports fast drug circulation and elimination. Because corticosteroids are lipophilic, they diffuse easily

Table 1. Classic Pharmacokinetic Parameters of Different Corticosteroids Estimated from Different Models or Cited by Different; References k_e: Elimination Constant Rate; CL: Clearance; V: Vollume of Distribution: $T_{1/2}$: Plasmatic Half-life; fu: unbound fraction

Corticosteroid	(PK Model) [reference]	k_e (h^{-1})	CL (L/h)	V (L)	$T_{1/2}$ (h)	fu (%)
Hydrocortisone	[12]		18	34		20
	[15]					30
	[18]	0.64±0.42				
	(5) [48]	0.18±0.03				
	(9a, 9b) [48]	0.445				
	(16) [48]	[0.64-0.69]				
	(23, 24, 25) [39]				1.32±0.28	
	(26) [45]	[0.24-0.65]				
	(32) [50]				1.15±0.33	
	[49]		18	39.9	[1.7-1.8]	
	(28, 29) [50]			13.7±2.5	1.1±0.3	
	[55]			33.7		
Prednisolone	[12]		6	93		25
	(31) [41]	0.30±0.04				
	(30) [37]			49.5±7.5	3.52±0.84	
Methyl-prednisolone	[19]		25.2±4.8	80.96±10.40	2.28±0.37	
	[12]		21	80		23
	(31) [41]	0.30±0.04	22.7±2.9	77.1±10.8		
Methyl-prednisolone succinate	(31) [44]		18.8±0.7	77.2±3.5	3.0±0.2	
Dexamethasone	(32, 33) [41]	0.57±0.26	18.8±4.9	37.6±16.5		
	[12]		17	57		32
	(32, 33) [37]			212.0±61.9	4.06±1.66	
Betamethasone	[12]		9	98		36
Flunisolide	[20]		58	96	1.6	20
Triamcinolone acetonide	[20]		37	103	2.0	29
Triamcinolone	[12]		29	119		60
Budesonide	[20]		84	183	2.8	12
Beclomethasone dipropionate	[20]		230		0.1-0.2	13
Fluticasone propionate	[20]		69	318	7.8	10
	[18]				[7.7-8.3]	
Fluocortolone	[12]		32	61		13

through the cell membranes, and therefore have high volumes of distribution. Ranges of V are summarized in (Table **1**): 10-40 L for hydrocortisone, 42-93 L for prednisolone, 65-92 L for methyl-prednisolone, and 21-274 L for dexamethasone [12, 15, 18-20, 37].

In their target tissues, corticosteroids are concentrated by an uptake mechanism which relies on their binding to intracellular proteins (or " receptors "). They have also a high binding to plasmatic proteins, albumin and cortisol-binding globulin (also termed transcortin) [21-27]. Only 10 to 20% represents the free or unbound fraction (fu) which is biologically active, i.e. able to reach the cellular receptor [20, 21, 28].

(C) Metabolism and Elimination

Small changes in steroid structure result in large differences in biological activity [25, 29-31]. Most of the peripheral conversion or metabolism of corticosteroids occurs in the liver, and to some extent in the kidneys, which

are the major sites of hormone inactivation and elimination, or catabolism [15, 32, 33].

In PK models, elimination can be characterized by two parameters: the half-life ($T_{1/2}$) and the clearance (CL). The half-life (min or h) gives the time necessary to eliminate the half of the administrated drug amount. Although it quantifies how rapidly the plasma concentration changes, it does not indicate the magnitude of this concentration. A long $T_{1/2}$ means a long residence time for the drug in the body leading to a long action duration. The clearance ($mL.min^{-1}$ or $L.h^{-1}$) is defined as the volume of plasma that is totally cleared of drug in time unit; it is calculated from a ratio between distribution volume V (mL or L) and half-life $T_{1/2}$ (min or h):

$$CL = V * \frac{\ln(2)}{T_{1/2}} = V * \frac{0.69}{T_{1/2}} = V * k \quad (4)$$

where k is the elimination rate constant ($time^{-1}$). In theory, the faster the systemic clearance, the greater margin of safety in the use of a drug (higher therapeutic index).

The relationships between corticosteroid plasma concentrations (bound and free) and clearance are complex and not fully determined [22]. The plasma half-lives of corticosteroids have been assessed under different circumstances, including intravenous and oral routes, low and high doses [14, 34]. Approximate plasmatic half-lives values are 0.5 hours for cortisone, 1-2 hours for hydrocortisone, 1.0-3.5 hours for prednisone, 2.5-4.4 hours for prednisolone, 1.9-2.7 hours for methyl-prednisolone, and 2.4-5.7 hours for dexamethasone [14, 15, 19, 29, 34-39].

Prednisone, prednisolone (active metabolite of prednisone) and Me-Prednisolone are the most used drugs in corticotherapy because their short $T_{1/2}$ made they are easy to manipulate [25, 40].

A summary of the values of classic PK parameters given by different PK models is presented in the (Table **1**). The presented parameter values concern healthy voluntaries except methyl prednisolone succinate [44] (asthmatic patients) and they could be significantly different in other clinic and/or physiologic situations. Tacking into account the parameter values for one GC such as HCn, (Table **1**) shows an important inter-individual variability for the PK parameters: (i) the elimination rate constant k_e for HCn varies between 0.24 and 0.65 h^{-1} [45], and in other works the average k_e-value was 0.18 h^{-1} [48]; (ii) the volume of distribution of dexamethasone has very different values according to cases, 37.6 L [41] and 212 L [37], highlighting an important variability between patient groups. GC differing by small variations in their chemical structures, showed high differences between their PK parameter values. For example, the volume of distribution varies around 30L for HCn, 50L for prednisolone, whereas that of Me-Prednisolone, which has both a supplementary double bond and methyl, varies around 80L. Moreover, the plasma half-life varies over a substantial range as well, from 0.1 hours for Beclomethasone dipropionate to over 7.5 hours for fluticasone. Note that the PK parameter values could be affected by many factors such as age, gender, race, clinical situation, physiological state, etc. [53, 78-81].

PHARMACOKINETIC PROFILES

The PK profiles vary with the drug and with the administration way. In the case of oral administration, the absorption phase is represented, in time-concentration curve, by an increasing phase until a peak (Fig. **2b**). The corresponding kinetic profile is called first-order absorption profile. The absorption phase is obviously absent in the case of intravenous bolus (Fig. **2a**) or infusion (Fig. **2c**), because the drug is entirely and directly administrated in the blood; one talks about zero-order absorption.

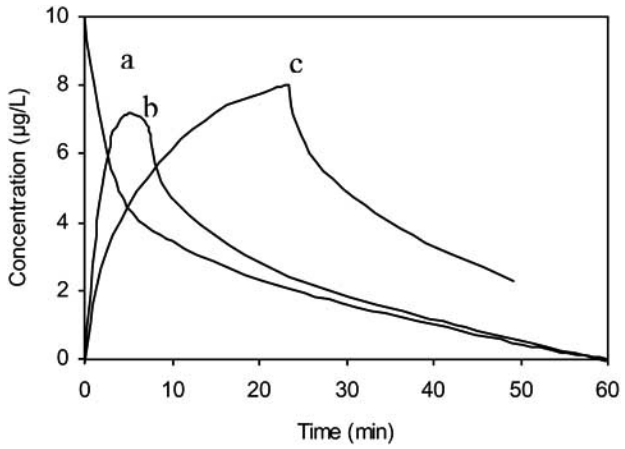

Fig. (2). Three plasma concentration-time profiles representing an intravenous bolus (a), per os (b) and intravenous infusion (c) administration of drug; the peak is "flattened" in the case of oral administration, and pointed in infusion.

Distribution, metabolism and elimination are processes responsible for decreasing of the plasmatic drug concentration. In the case where a drug is quickly distributed in tissue, its plasmatic concentrations decrease according to two kinetic steps which correspond to distribution and elimination phase respectively. The PK profile (log(concentration) vs time) of such a drug will show two linear decreasing phases α and β which correspond to two homogeneous compartments (Fig. **3**).

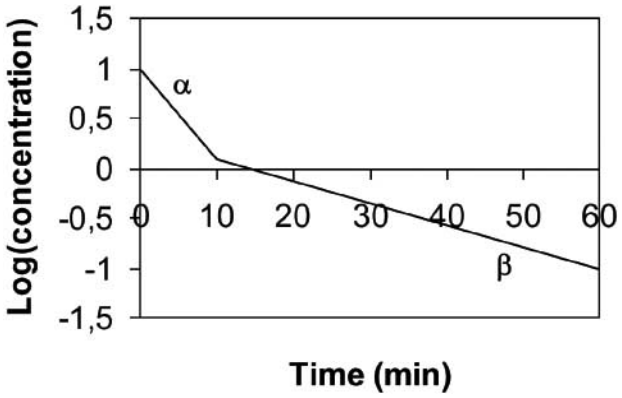

Fig. (3). Semi-logarithmic concentration-time profile showing two linear decreasing phases corresponding to distribution and elimination compartments in the body, respectively.

However, if the drug has a slow distribution in tissue, its hepatic and/or renal elimination will be the major processes of the plasmatic concentrations decreasing. In that case, the semi-logarithmic profile of the drug will show only one decreasing phase (β).

PHARMACOKINETIC MODELS

Many PK models were applied in order to achieve the best fitting of kinetic data, and to quantify the inter-individual variability. The pharmacokinetics of GC is known by an important inter-individual variability [2]. The choice of a PK model depends on many factors: administration way of drug (intravenous, oral, inhalation, cutaneous), general biological processes (considered as mechanistic trends; e.g. passive diffusion, hepatic metabolism, etc.), molecular phenomena (considered as random real events; e.g. pulsatile hormonal secretion), time range of data observation, etc. .

The numerous PK models of GC concerned three application cases: first, modeling or simulation of the kinetics of endogenous corticosteroids (e.g. hydrocortisone) tacking into account molecular, physiological and/or biorhythmic concepts [18, 20, 48, 55-56, 60, 70]; second, modeling of the kinetics of endogenous corticosteroids mediated by exogenous corticosteroids [39, 45, 74]; third, modeling of the kinetics of exogenous corticosteroids [19, 37, 41-43, 44, 46-47, 49-55, 75].

Concerning the PK models, there are many mathematical structures: compartment, polynomial, trigonometric, linear release, chaotic and stochastic models**.** Compartment models are the most popular in pharmacokinetics; they are determinist models (by opposition to probabilist or stochastic), based on the mass conservation concept. They consist in a sum of exponential terms which fits the concentration-time curve. Trigonometric and harmonic models were especially applied to fit biorhythmic process such as circadian variations of endogenous GC.

PK MODELS FOR BASELINE ENDOGENOUS HYDROCORTISONE

(A) One-Compartment with Zero-Order Absorption Model

This is the simplest kinetic model where the whole body is considered as a single compartment in which the GC distributes rapidly, so that one observes only a single elimination phase (one compartment).

A monoexponential self-suppression model was applied to fit the negative feedback mechanism modulating the hydrocortisone (HCn) release [48]. Hence, the "inhibitory" HCn concentration was used to describe the change of HCn release. The disposition of this "inhibitory" cortisol (C_{inh}) that is present in blood at the time of the acrophase was described by a monoexponential equation:

$$C(t) = C_z e^{-k_e t} \tag{5}$$

where t is the time after the last acrophase, C_Z is the concentration at the acrophase, and k_e is the elimination rate constant of HCn.

This very simplistic model fitted less well the elimination of HCn with time, compared to more sophisticated models [48], described below.

(B) One-Compartment with First-Order Absorption Model

An empirical approach to describe baseline cortisol levels is to use a sum of a positive and a negative exponential which describe the input and the output phase respectively [48]:

$$C(t) = be^{\beta t} + ae^{-\alpha t} \tag{6}$$

where a, b, α and β are positive constants, and the sum (a + b) is equal to the cortisol concentration in the acrophase (from which the time *t* is accounted in this model). At t=24h, C(t) will be back to the initial concentration. Since the α-term will approach 0 at t=24h (Fig. **4**), it follows that $\beta = \frac{\ln\left(\frac{a+b}{b}\right)}{24}$. Therefore, only three parameters (α, a, b) are necessary to describe the curve.

This model allows reasonable description of the cortisol baseline data [48]. Although plasma HCn disappears biexponentially, monoexponential model is often applied because the rapid phase is unnoticed if HCn is measured even at a 10 min interval, a normal procedure in the studies of daily production of plasma HCn.

POLYNOMIAL MODELS

The mean baseline of circadian HCn concentrations was simply simulated by a polynomial function of fifth order [56]. This model is simple but difficult to interpret:

$$C(t) = a_0 + a_1 t + a_2 t^2 + a_3 t^3 + a_4 t^4 + a_5 t^5 \tag{7a}$$

where $a_0 \ldots a_5$ are the polynome's coefficients. By combining this model with that giving the variation of concentration C(t) by time unit:

$$dC/dt = R(t) - k*C(t) \tag{7b}$$

a polynomial function describing the HCn secretion rate R(t) (concentration/time) under baseline conditions was derived:

$$R(t) = a_1 + k*a_0 + (2a_2 + k*a_1)t + (3a_3 + k*a_2)t^2 + (4a_4 + k*a_3)t^3 + (5a_5 + k*a_4)t^4 + k*a_5*t^5 \tag{8}$$

where k is the first-order elimination rate of HCn ($time^{-1}$). The simulated values of R(t) were then noised and fitted by other PK models in order to examine and compare their accuracy.

TRIGONOMETRIC MODELS

Tacking into account the circadian cycle of HCn, the secretion rate R (concentration/time) of HCn under baseline conditions was described by a single cosine function [48, 56, 58]:

$$R(t) = R_{av} + R_{amp} \cos\left[\frac{2\pi}{24}(t - t_z)\right] \tag{9a}$$

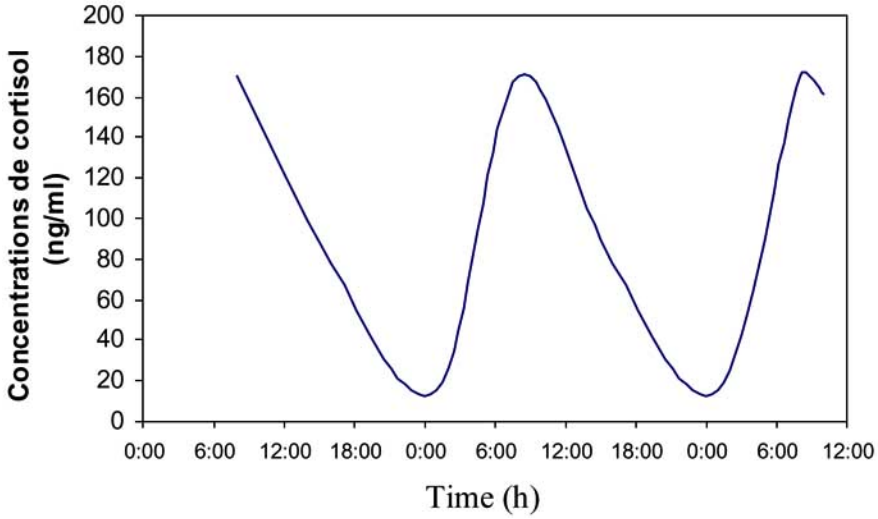

Fig. (4). Circadian variations of plasma hydrocortisone concentrations showing an asymetric profile [20, 55, 77].

where R_{av} is the average secretion rate of HCn, R_{amp} is the amplitude of the secretion rate, and t_z is the time of acrophase. If $t=t_z$, then cos(0)=1 and $R=R_{av}+R_{amp}$. If $t=t_z\pm12$, then cos(π)=-1 and $R=R_{av}-R_{amp}$. The resulting change in cortisol concentration C under baseline conditions is given by [48]:

$$\frac{dC}{dt} = R(t) - k_e C \tag{9b}$$

where k_e is the elimination rate constant.

The cosine model predicts symmetrical behavior giving correct results on a major part of the circadian cycle; however, it fails to predict the short increasing phase due to the asymetric profile of HCn [56], (Fig. **4**).

Single cosine function is a particular case of a more general model: the harmonic model, in which the circadian cycle of HCn baseline C(t) is represented by Fourier series [46, 56, 57]:

$$C(t) = a_0 + \sum_{n=1}^{\infty}\left[a_n \cos\left(\frac{2\pi nt}{24}\right) + b_n \sin\left(\frac{2\pi nt}{24}\right)\right] \tag{10}$$

where a_0, a_n and b_n are Fourier coefficients which can be obtained by fitting the above equation to baseline or placebo data. The value of n represents the frequency of the harmonic function. For example, when n=0, the harmonic function describes a steady state baseline value of a_0, when n=1, the harmonic function has a period of 24h; when n=2, the period is 12h; and so on.

A two harmonic model (n=2) worked very well for fitting of most HCn data except for the three first 3 hr [56]. Booth the curvature and asymmetry were well fitted. The second harmonic captures a small peak near 12 hr. The yielded coefficients were: a_1 (55.3) and b_1 (88.8) for the 24-hr period and a_2 (87.5) and b_2 (124) for the 12-hr period as well as a_0 (33.14) [56].

In order to improve the modeling of the asymmetric profile of endogenous HCn, its circadian variation was described by 3 cosine functions [56, 59]. The first equation described the increase of the secretion rate R from t_{min} to t_{max}:

$$R(t) = R_{av} + R_{amp}\cos\left[\frac{2\pi(t - 2t_{\min} + t_{\max})}{2(t_{\max} - t_{\min})}\right] \tag{11}$$

The second described the decrease between t_{max} and 24h (end of last cycle):

$$R(t) = R_{av} + R_{amp}\cos\left[\frac{2\pi(t - t_{\max})}{2(t_{\min} - t_{\max} + 24)}\right] \tag{12}$$

The third equation described the decrease of R from 0h (beginning of next cycle) to t_{min}:

$$R(t) = R_{av} + R_{amp}\cos\left[\frac{2\pi(t - t_{\max} + 24)}{2(t_{\min} - t_{\max} + 24)}\right] \tag{13}$$

The equations 11-13 produced a t_{min} of 16.7 hr., t_{max} of 20.4 hr., R_{av} = 55.7, and R_{amp} = 53.5 ng/(mL/hr). This model provided a better fitting of the early data and readily accommodates the asymmetry [56].

LINEAR RELEASE RATE MODEL

Various mathematical functions were used to characterize the normal physiologic secretion rate of HCn, which follows a 24-h circadian pattern. Whereas most functions require the simultaneous fitting of placebo and treatment data, the linear release model can sufficiently describe the baseline HCn concentrations in the absence of treatment data. Briefly, the model suggests that the HCn release rate follows a linear decrease from the acrophase time (t_{max}) to a value of zero at some time t_{min}, and a linear increase from t_{min} to t_{max} [12, 18, 20, 39, 48, 49, 60, 76], (Fig. **5**).

The release rate R (concentration/time) from time t_{max} to t_{min} is given by the following equation [48]:

$$R(t) = \frac{Q_{\max}}{V^*(t_{\max} - t_{\min} - 24)}(t - t_{\min}) = R_{\max}\frac{(t - t_{\min})}{t_{\max} - t_{\min} - 24} \tag{14}$$

where Q_{max} is the maximum release rate (amount/time) of HCn, and V is the distribution volume of HCn.

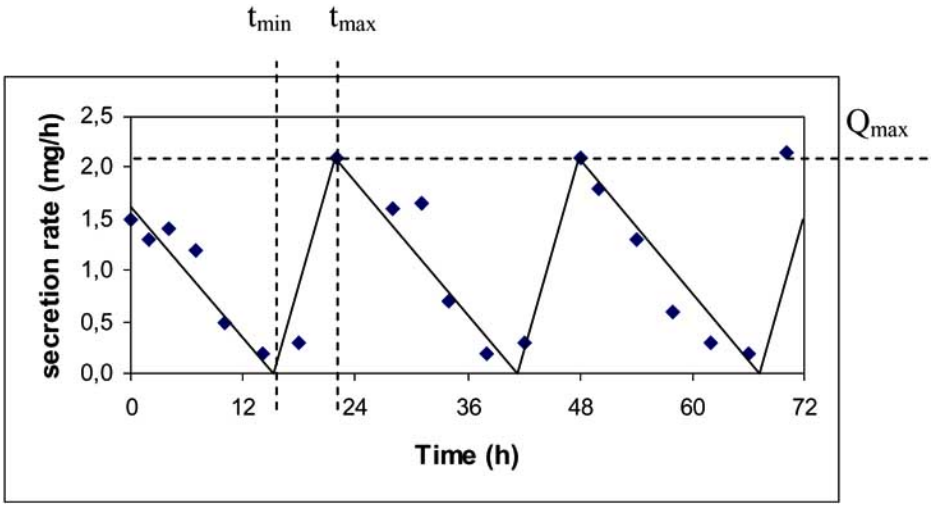

Fig. (5). Linear variation of hydrocortisone release rate; t_{min} and t_{max}: times of the minimum and maximum (Q_{max}) values of the secretion rate [48].

From t_{min} to t_{max}, the secretion rate increases linearly :

$$R(t) = \frac{Q_{max}}{V * (t_{max} - t_{min})} * (t - t_{min}) = R_{max} \frac{(t - t_{min})}{t_{max} - t_{min}} \quad (15)$$

The endogenous release rate R(t) can be converted into concentration C(t):

$$C(t) = \frac{R(t)}{k_e} \quad (16)$$

where k_e is the elimination rate.

The equations 14-16 produced a t_{min} of 16.1-16.9 hr., t_{max} of 20-21 hr., Q_{max} = 2808-3180µg/h, and k_e = 0.64-0.69 h^{-1}. Compared with the previous models, exponential (Eq. 5-6) and single cosine (Eq. 9a), this model provided a better data fit for HCn baseline, and readily accommodated the asymmetry [48, 56]. However, it fitted HCn baseline data slightly less well than the three cosines approach (Eq. 11-13) [56].

CHAOTIC MODELS

The hypothalamic-pituitary-adrenal axis (HPA) is one of the most studied hormonal systems. Most attempts to model blood hydrocortisone concentrations in pharmacokinetics/pharmacodynamics studies are purely phenomenological and focus on describing the circadian rhythm using periodic mathematical functions. This ignores the pulsatility of HCn secretion [61], thus producing smooth periodic curves [48, 56, 62]. However, the available experimental data are not at all smooth and there is strong evidence that plasma HCn secretion is characterized by pulsatility and irregularity apart from diurnal variation [61, 63-67].

Some authors gave a particular attention to the non-smooth and irregular variation of HCn highlighted by experimental data [63, 77]. Such kinetic variations reflect a chaotic nature of the pulsatile secretion of HCn [68-69]. Dokoumetzidis *et al*., [70] developed a PK model for HCn taking into account its non-linear dynamics due to negative feedback. Hydrocortisone concentration was described by a nonlinear time-delay differential equation [71] with two terms, namely, a secretion rate term which adheres to the negative feedback mechanism [72] and drives the pulsatile secretion, and a first-order output term:

$$\frac{dC}{dt} = k_1 \frac{a^n C_{lag}}{a^n + C_{lag}^{\ n}} - k_2 C \quad (17)$$

where C is the concentration of HCn, C_{lag} is the value of C at time (t-T), T is the time range between two pulsatile secretions, n is an exponent, k_1 and k_2 are the input and

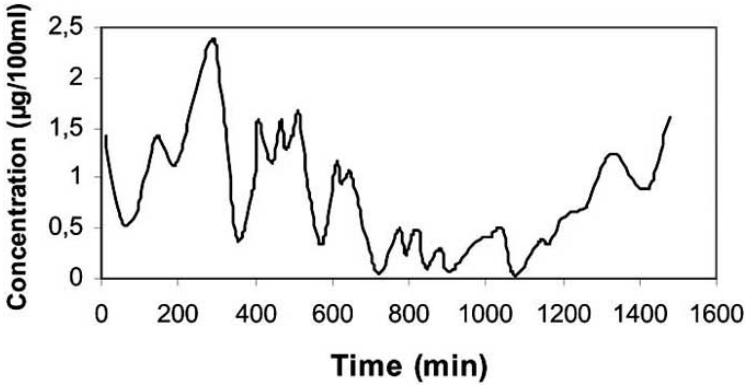

Fig. (6). Chaotic circadian profile of hydrocortisone simulated by equations (17) and (18), and highlighting a periodic profile characterized by a chaotic variation due to a pulsatile secretion process.

output rate constants, respectively. The circadian rhythm of HCn secretion is implemented phenomenologically by considering the parameter *a* in the equation (17) as a simple cosine function of the 24 h period:

$$a = A\cos\left[(t - f)\frac{2\pi}{1440}\right] + B \quad (18)$$

where A and B are constants with concentration units, f is a constant with time units and t is time in min.

The stimulated profile generated from equations (17) and (18) makes to be able to describe both the circadian rhythm of HCn and its pulsatile secretion nature, (Fig. **6**). Model parameters take the values k_1=0.0666 min^{-1}, k_2=0.0333 min^{-1}, n=10, f=250 min and T=70 min.

The physical meaning of the time delay in equation 17 is that the HCn concentration, C, affects other physiological parameters of HPA system, which in turn affect, *via* the feedback mechanism, HCn concentration and, thus, HCn controls its own secretion [56].

This model offered an opportunity to refer to some implications of the presence of nonlinear dynamics. Apart from the jagged HCn concentration profile, elements such as the sensitive dependence from the initial conditions, as well as the parameters of the system, played an important role and may explain the inter- and intra-subject variability observed in the secretion of HCn. Thus, a change in the initial conditions or the parameter values of equations 17 and 18 may be depicted in a relatively large change of the final profile.

STOCHASTIC MODELS

By opposition to all previous models (deterministic) which derive from mass conservation equations, the stochastic models are based on probability density functions (pdf) which are attributed to some events playing a key role in the concentration-time variation. Such key roles are formalized by the following relationship:

$$C(t) = AUC * pdf \quad (19)$$

The pdf were used to describe many random events: time and/or number of random recirculation of drug particles before elimination, number of secretory events occurring in an interval of time, waiting times between secretory events, amplitude or total amount of hormone contained in the secretion event initiated at time t, etc. [73]. Among the numerous pdf, Gamma and Weibull functions were used to model the endogenous HCn and exogenous GC respectively [74, 75].

GAMMA MODEL

Engel *et al.* [74] carried out a population analysis on stimulated HCn by an intravenous administration of ACTH in veal. Interest was focused on variation in HCn profiles both within and between animals. Potential effects of age, of animal diet and housing system on the profiles are addressed as well. The HCn concentration-time curves showed a peak followed by a decreasing phase, and they were modeled by a gamma function:

$$C(t) = at^d e^{-kt} + C_b \quad (20)$$

where t is time, a is a statistical parameter inherent to the gamma function, d describes the initial increase and k describes the final decrease back to the base level C_b.

A quadratic function of the equation 20 gave a better fitting of these data [74]:

$$C(t) = at^d e^{-kt^2} + C_b \quad (21)$$

PK MODELS FOR ENDOGENOUS HYDROCORTISONE DISTURBED BY EXOGENOUS CORTICOSTEROIDS

During a corticotherapy, the endogenous cortisol levels are considered as a suitable marker to quantify the degree of systemic activity. In other words, the alterations in cortisol plasma levels as the consequence of exogenous drug administration was used as a surrogate marker to quantify overall systematic corticosteroid activity. This concern led to develop PK models to quantify the disturbance of the endogenous HCn.

(A) One-Compartment with Zero-Order Absorption Model

The influence of the time of dexamethasone (DEX) administration on the suppression of plasma HCn was investigated [39]. After a single IV administration of DEX, plasma HCn concentrations versus time were fitted using a zero-order absorption model, an infusion model:

$$C(t) = a + b + c \quad (22)$$

with

$$a = \frac{Inf_1}{V \cdot k} e^{-k \cdot t} \quad (23)$$

$$b = \frac{Inf_2}{V \cdot k} \quad (24)$$

$$c = \frac{Inf_3}{V \cdot k}\left(1 - e^{-k \cdot (t - t_{on})}\right) \quad (25)$$

where V and k are the distribution volume and the elimination rate constant of HCn respectively, Inf_1 is the hypothetical infusion rate of HCn before DEX administration (amount.$time^{-1}$), Inf_2 is the non-suppressible HCn baseline infusion rate that remains after DEX administration (amount.$time^{-1}$), and Inf_3 is the HCn infusion rate after restart of HCn production (amount.$time^{-1}$) at time t_{on} (hours after DEX administration).

The parameter values of the HCn production rates estimated with this model (Eq. 23-25) were Inf_1 (74.7 ± 22.3 µg/h), Inf_2 (3.3 ± 0.5 µg/h), Inf_3 (47.4 ± 11.5 µg/h), t_{on} (04h 06min ± 00h 42 min), $T_{1/2}$ (1.32 ± 0.28 h) [39]. This model showed that suppression of HCn production by DEX was instantaneous. Using a comparable composite model, Dubois *et al*. [37] found the elimination rate constant of cortisol after DEX to be apparently higher than after Pnl. Also, this model highlighted that a monoexpo-

nential curve is not efficient enough to fit well this rapid decline of HCn plasma concentrations [39].

A one-compartment infusion model was also performed by **Milad and Jusko** [45] tacking into account the releasing rate R of endogenous HCn stimulated by an adrenocorticotropin infusion:

$$C(t) = \frac{R}{k}\left(1 - e^{-kt}\right) + C_0 e^{-kt} = C_{ss}\left(1 - e^{-kt}\right) + C_0 e^{-kt} \qquad (26)$$

where C_0 is the concentration at initial time t=0, k the elimination rate. The second term ($C_0 e^{-kt}$) describes the elimination process through time from pre-existing concentration C_0 at t=0. The first term describes a more complex process combining both stimulation and elimination, governed by releasing rate (R) and elimination rate (k) respectively. At t=0, this term is null, and thus only pre-existing concentration C_0 is eliminated; at unlimited time (t=∞), the second term become null and the first term is equal to the steady state concentration C_{ss}=R/k.

The model assumes that cortisol formation and secretion from the adrenal gland occurs at a constant rate (R) when maximally stimulated by ACTH. The estimated parameter values were k (0.245-0.65 h^{-1}) and R (7.97-14.62 nmol/min). The authors recommended the use of this model to assess to the extent of adrenal suppression after long-term corticosteroid therapy or in the presence of disease states [45]. They highlighted that with increasing duration of corticosteroid therapy, the R decreased (<36 mo, R=4.69 nmol/min; >36 mo, R = 3.03 nmol/min).

(B) One-Compartment with First-Order Absorption Model

After intravenous administration of triamcinolone acetonide (TCA), the suppression of endogenous HCn was described by an empirical model [48]:

$$C(t) = be^{\beta t} + ae^{-\alpha t} - Z_{TCA}C_{TCA} \qquad (27)$$

where a, b, α, β and Z_{TCA} are positive constants, and C_{TCA} the concentration of exogenous corticosteroid (TCA). This model gave good cortisol data fits for only the situation when the exogenous steroid is administrated in the acrophase.

(C) Two-Compartment with Zero-Order Absorption Model

The endogenous cortisol suppression by DEX was studied using a two-compartment open model. Considering, the body as the sum of two compartments, concentration-time profiles were fitted by a sum of two negative exponential terms [50]:

$$C(t) = C_a e^{-\alpha t} + C_b e^{-\beta t} \qquad (28)$$

where C_a and C_b are intercept constants representing the concentrations at the initial time in distribution compartment (tissue, organ) and in elimination (blood) compartment respectively , and α and β are two hybrid rate constants (or macro-constants) characterizing the distribution and the elimination phases respectively. The detailed analytical equation of this model is given by [50]:

$$C(t) = \frac{D}{V}\left[\left(\frac{k_{21} - \beta}{\alpha - \beta}\right)e^{-\beta t} + \left(\frac{k_{21} - \alpha}{\beta - \alpha}\right)e^{-\alpha t}\right] \qquad (29)$$

where C(t) and V are the concentration and the distribution volume of the glucocorticoid (HCn) in the central compartment (1), k_{21} is the apparent transfer rate constant of HCn from the peripheral compartment to the central compartment.

PK MODELS FOR EXOGENOUS CORTICOSTEROIDS

(A) One-Compartment with Zero-Order Absorption Model

After intravenous (IV) administration of methylprednisolone (MPnl), prednisolone (Pnl) and triamcinolone acetonide (TCA), plasmatic concentrations decreased according to a simple mono-exponential function [12, 37, 41-43]. Therefore, the fitting of concentration-time curves *C*(*t*) was carried out using a one-compartment model with a zero order absorption:

$$C(t) = C_0 e^{-kt} = \frac{A}{V}e^{-kt} \qquad (30)$$

where A is the total amount received in the blood by bolus at time t=0, C_0 is the blood concentration at initial time t=0, V is the distribution volume of the exogenous GC, and k is its elimination rate from the blood.

(B) One-Compartment with First-Order Absorption Model

After oral administration, plasmatic concentrations of Pnl and MPnl showed a peak followed by only one elimination phase [41, 44, 53]. Therefore, a one-compartment PK model with first order absorption was applied to fit the kinetics of Pnl.

$$C(t) = \frac{D * F}{V}\left(\frac{k_a}{k_a - k}\right)\left(e^{-kt} - e^{-k_a t}\right) = C_b\left(\frac{k_a}{k_a - k}\right)\left(e^{-kt} - e^{-k_a t}\right) \qquad (31)$$

where D is the total dose given per os, V is the distribution volume of drug, F is bioavailability which means the fraction of the administrated dose which transits the gastro-intestinal system to reach the systemic circulation, k_a is the absorption rate from intestine to blood, k is the elimination rate, and C_b is the bioavailable concentration.

This model has two exponential terms, increasing ($-e^{-k_a t}$) and decreasing (e^{-kt}), representing the absorption and the elimination phases respectively.

This model was also used to fit plasma concentration-time of MPnl administrated in the form of prodrug, methylprednisolone sodium succinate (MPnls), as a rapid intravenous injection [19, 44, 46]. The MPnls was rapidly metabolized with a first-order rate (k_f) to its active form MPnl. In the model equation, the first-order absorption coefficient k_a was replaced by the first order rate constant k_f for the formation of MPnl from the MPnls. The model

assumes 100% conversion of MPnls to MPnl and negligible inter-conversion between MPnl and its metabolite, methylprednisone.

An analogous model was used to simulate the penetration of dermatologic corticosteroids through the skin [47]. By assuming a drug availability F=100%, and using one-compartment with first order absorption, the concentration-time equation is written as:

$$C(t) = \frac{D}{V}\frac{k_p}{k_p - k_e}\left(e^{-k_e t} - e^{-k_p t}\right) \quad (32)$$

where C(t) is the drug concentration at the receptor site, D the applied dose of drug, V the distribution volume at the receptor site, k_p the penetration rate constant, and k_e the elimination rate constant. The penetration rate constant k_p which derives from Fick's first law of diffusion is defined as follow:

$$k_p = D_B \cdot A \cdot \frac{PC_{B/V}}{e_B \cdot V_V} \quad (33)$$

where D_B is the diffusion coefficient of the drug in the barrier startum corneum, A the application area, $PC_{B/V}$ the (stratum corneum/vehicle) partition coefficient of the drug, e_B the thickness of the stratum corneum, and V_V the volume of the applied preparation. The ratio V_V/A represents the thickness of the ointment layer. The PK parameters used for the simulations according to Eq. (32) were: D (10mg), V (10ml), k_e (0.2 h^{-1}), k_p (0.05 and 0.5 h^{-1}) [47].

According to Eq. (33) the formulation volume is part of the penetration rate constant and can therefore affect the kinetics of drug penetration. A high formulation volume leads to infinite dose conditions and thus zero order kinetics. With regard to penetration kinetics it is essential to guarantee zero order kinetics during the exposure time periods in order to prevent drug depletion. Moreover, in contrast to suspension-type penetrations, solution-type formulations have to be applied in an excess amount to guarantee infinite dose conditions and thus zero order kinetics [47].

(C) Two-Compartment with Zero-Order Absorption Model

This model is considered to be more appropriate (realistic) than the one-compartment model, though the kinetics of several GC xenobiotics were adequately described by the one-compartment model.

After IV administration of HCn [49-51], Pnl [52] and DEX [37, 41], in healthy patients, the plasmatic concentrations of these GC decreased bi-exponentially. The disappearance of these GC in the systemic circulation was then studied using the equations (28-29).

(D) Two-Compartment with First-Order Absorption Model

After *per os* administration, three separate phases are observed for a xenobiotic that distributes in the body according to a two-compartment model. The additional phase to the distribution and elimination phases is the absorption phase, leading to a supplementary parameter, absorption rate constant k_a .

A two-compartment model was applied after a single oral administration of prednisolone [53, 54], and after inhaled flunisolide [55], which showed a biexponential decreasing phase after a peak. Flunisolide is a synthetic corticosteroid which is inhaled in the treatment of asthma. The variation in plasma concentration with time is complex, but can be expressed by the equation:

$$C(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} - C \cdot e^{-k_a t} \quad (34)$$

with:

$$A = \left(\frac{k_a * D * F}{V}\right)\left(\frac{k_{21} - \alpha}{(k_a - \alpha)(\beta - \alpha)}\right)$$

$$B = \left(\frac{k_a * D * F}{V}\right)\left(\frac{k_{21} - \beta}{(k_a - \beta)(\alpha - \beta)}\right) \quad (35, 36, 37)$$

$$C = \left(\frac{k_a * D * F}{V}\right)\left(\frac{k_a - k_{21}}{(\alpha - k_a)(\beta - k_a)}\right)$$

where D is the dose administrated *per os*, F is the bioavailability, V is the distribution volume of the central compartment, k_a is the absorption rate constant, k_{21} is the transfer rate constant from the compartment 2 (peripheral) toward the compartment 1 (central) and α and β are hybrid rate constants (or macro-constants) which can be expressed according to the micro-constants k, k_{12} and k_{21}.

(E) Stochastic Models

Weibull Model

Heikkilä [75] suggested analytical equation based on a generalized Weibull pdf. The model was applied on Pnl data in 12 healthy patients recorded for 24h after *per os* administration. The equation has 4 parameters α, β, κ, γ which govern the variation of concentration-time profile:

In Eq. 38, $\alpha > 0$ and $\beta > 0$ correspond to the peak concentration C_{max} and peak time T_{max} respectively, $\gamma > 1$ is a parameter which governs the kurtosis of the curve, and $\kappa > \max(2, \gamma)$. The variation of one parameter under fixation of the three others leads to the following profiles:

$$C(t) = \alpha\left(\frac{t}{\beta}\right)^{\gamma-1}\left[\frac{\left(\frac{t}{\beta}\right)^{\gamma}}{2} + \frac{1}{2}\right]^{\left(\frac{\kappa}{\gamma}\right)-2} * \exp\left\{-\frac{\kappa-2}{\kappa-\gamma}\left\{\left[\frac{\left(\frac{t}{\beta}\right)^{\gamma}}{2} + \frac{1}{2}\right]^{\left(\frac{\kappa}{\gamma}\right)-1} - 1\right\}\right\} \quad (38)$$

Variation of parameter	Resulting profile C(t)
α	Variation of C_{max}
β	Variation of T_{max}
κ	Variation of the slope of the 1st decreasing phase
γ	Flattening of the peak C_{max}

DISCUSSION AND CONCLUSION

The pharmacokinetic of glucocorticoids offer a large diversity of mathematical models which could be widely applied in medicinal chemistry. These models complement each others to allow analyses on various time ranges:

On a relatively long time duration, e.g. 24h or days, harmonic or/and linear release rate models are well adapted to fit periodic profiles due to circadian variations. Polynomial models offer a more simplistic way to fit different curvature profiles by selecting different polynomial degrees. On middle time duration, e.g. several minutes or some hours, compartment models are adequate to fit ADME systems. This approach is more practical in clinical situations. However, on relatively short time duration, rapid biological processes (e.g. pulsatile secretions) can be taken into account by chaotic models. Alternatively to the previous deterministic models, the stochastic models reason on the number of particles rather than amount. They ignore the mass transfer processes and specify a probability density function of residence time of the drug molecules in the body. Although they are non heuristic, they have the advantage to be flexible by the variation of few parameters, leading them to be adapted to many clinical or physiological situations.

The PK models of GC highlighted a high variability of numerical results. This illustrates their usefulness to highlight a physiological diversity between subjects (patients), which help to use better the drug and to adapt well its posology during a therapy. These mathematical tools could be applied in medicinal chemistry in order to help to understand the behavior and the "fate" of natural products in biological systems.

ABBREVIATIONS

GC = Glucocorticoids

HCn = Hydrocortisone

Pnl = Prednisolone

DEX = Dexamethasone

MPnl = Methylprednisolone

IV = Intravenous

PK = Pharmacokinetic

ADME = Absorption-Distribution-Metabolism-Elimination

pdf = Probability density function

AUC = Area under concentration-time curve

REFERENCES

[1] Shargel, L.; Yu Rescigno, A.B.C. *Applied Biopharmaceutics and Pharmacokinetics*. McGraw Hill: New York, **1999**; p. 36.

[2] Abel, S. M.; Maggs, J. L.; Back, D. J.; Park B. K. *J. Steroid Biochem. Mol. Biol.*, **1992**, *43*, 713.

[3] Boumpas, D. *Ann. Intern. Med.*, **1993**, *119*, 1198.

[4] Fauci, A.; Dale, D.; Balow, J. *Ann. Intern. Med.*, **1976**, *84*, 304.

[5] Hollenberg, S.M.; Weinberger, C.; Ong, E.S.; Cerelli, G.; Oro, A.; Lebo R., Thompson E.B., Rosenfeld M.G., Evans R.M. *Nature*, **1985**, *318*: 635.

[6] Schimmer, B.P.; Parker, K.L. In *Goodman and Gilman's the pharmacological basis of therapeutics*, Hardman, J.G.; Limbird, L.E.; Goodman, A.G. Eds; McGraw Hill: New York, **2001**; pp. 1459-85.

[7] Reynolds, P.D.; Hunter, J.O. *Dig. Dis.* **1993**; *11*, 334.

[8] Hanauer, S.B. *Lancet*, **1993**, *342*, 412.

[9] Hanauer, S.B. *N. Engl. J. Med.*, **1996**, *334*, 841.

[10] Baxter, J.D.; Rousseau, G.G. In *Glucocorticoid hormone action*, Baxter J.D., Rousseau G.G. Eds; Springer-Verlag: New York, **1979**; p1.

[11] Liddle, G.W. *J. Clin. Endocrinol. Metab.*, **1960**, *20*, 1539.

[12] Mager, D.E.; Jusko, W.J. *J. Pharm. Sci.*, **2002**, *91*, 2441.

[13] McLean, H.M.; Khalil, M.A.; Heilan, A.S.; Lee, H.J. *J. Pharm. Sci.*, **1994**, *83*, 476.

[14] Pickup, M. *Clin. Pharmacokinet*, **1979**, *4*, 111.

[15] McKay, L.; Cidlowski, J.A. In *Cancer Medicine*, Bast, R.C.; Kufe, D.W.; Pollock, R.E.; Weichselbaum, R.R.; Holland, J.F.; Frei, E. Eds.; Baltimore, MD: Williams and Wilkins, **2000**, 730-742.

[16] Wilkinson, G.R.; Shand, D.G. *Clin. Pharmacol. Ther.*, **1975**, *18*: 377.

[17] Gibaldi, M.; Mc Namara, P.J. *Eur. J. Clin. Pharmacol.*, **1978**, *13*, 373.

[18] Rohatagi, S.; Bye, A.; Falcoz, C.; Mackie, A.E.; Meibohm, B.; Mollmann, H.; Derendorf, H. *J. Clin. Pharmacol.*, **1996a**, *36*, 938.

[19] Booker, B.M.; Magee, M.H.; Blum, R.A.; Lates, C.D.; Jusko, W.J. *Clin. Pharmacol. Ther.*, **2002**, *72*, 370.

[20] Derendorf, H.; Hochhaus, G.; Meibohm, B.; Möllmann, H.; Barth, J. *J. Allergy Clin. Immunol.*, **1998**, *101*, S440.

[21] Peets, E.A.; Stam, M.; Symchowicz, C. *Biochem. Pharmacol.*, **1969**, *18*, 1655.

[22] Begg, E.; Atkinson, H.; Gianarakis, N. *Med. J. Austral*., **1987**, *146*, 37.

[23] Frey, J. *Endocrine Rev.*, **1987**, *8*, 453.

[24] Rocci, M.L.Jr.; D'Ambrosio, R.; Johnson, N.F.; Jusko, W.J. *Biochem. Pharmacol.*, **1982**, *31*, 289.

[25] Frey, B.M.; Frey, F.J. *Clin. Pharmacokinet.*, **1990**, *19*, 126.

[26] Rohatagi S.; Barth, J.; Mollmann, H.; Hauchhaus, G.; Soldner, A.; Mollmann, C.; Derendorf, H. *J. clin. Pharmacol.*, **1997**, *37*, 916.

[27] Boudinot, F.D.; Jusko, W.J. *J Steroid Biochem.* **1984**, *21*, 337.

[28] Al Katheeri, N. A.; Wasfi, I. A.; Lambert, M.; Saeed, A. *Vet. Res. Commun.*, **2004,** *28*, 525.

[29] Axelrod, L. *Medicine*, **1976**, *55*, 39.

[30] Polley, H.; Mason, H. *JAMA*, **1950**, *143*, 1474.

[31] Tsang, S.; Garovoy, M.; Benet, L. *Int. J. Immunopharmacol*, **1985**, *7*, 731.

[32] Bromberg, M.B.; Carter, O. *Muscle Nerve*, **2004**, *30*, 20.

[33] Jenkins, J.; Sampson, P. *BMJ*, **1967**, *2*, 205.

[34] Gambertoglio, J.; Amend, W.; Benet, L. *J. Pharmacokinet. Biop.*, **1980**, *8*, 1.

[35] Weitzman, E.D.; Fukushima, D.; Nogeire, C.; Roffwarg, H.; Gallagher, T.F.; Hellman, L. *J. Clin. Endocrinol. Metab.*, **1971**, *33*, 14.

[36] Walsh, J.P.; Dayan, C.M. *Ann. Clin. Biochem.*, **2000**, *37*, 279.

[37] Dubois, E.F.L.; Derks, M.G.M.; Schweitzer, D.H; Zwinderman, A.H.; Dekhuijzen, P.N.R.; Boxtel, C.J.V. *Eur. J. Clin. Pharmacol.*, **2004**, *60*, 315.

[38] Jusko, W. J.; Ferron, G. M.; Mis, S. M.; Kahan, B. D.; Zimmerman, J.J. *J. Clin. Pharmacol.*, **1996,** *36***,** 1100.

[39] Koopmans, R.P.; Braat, M.C..P.; Oosterhuis, B.; van Boxtel, C.J. *J. Pharmacol. Exp. Ther.*, **1992**, *262*, 503.

[40] Szefler, S.J.; Ebling, W.F.; Georgitis, J.W.; Jusco, W.J. *Eur. J. Clin. Pharmacol.*, **1986**, *30*, 323.

[41] Mager, D.; Lin, S.X.; Blum, A.; Lates, C.D.; Jusko, W.J. *J. Clin. Pharmacol.*, **2003**, *43*, 1216.

[42] Tornatore, K.M.; Gililand-Johnson, K.K.; Farooqui, M. *J. Clin. Pharmacol.*, **2004**, *44*, 1003.

[43] Yates, Ch.R.; Vysokanov, A.; Mukherjee, A.; Ludden, T.M., Tolley, E.; Meduri, U.; Dalton, J.T. *J. Clin. Pharmacol.*, **2001**, *41*, 415.
[44] Parker, J.; P.T. Daley-Yates; Wood, S.A. *Br. J. Clin. Pharmacol.*, **1997**, 43, 589.
[45] Milad, M.A.; Jusko, W.J. *Ann. Pharmacother.*, **1993**, *27***,** 1195.
[46] Chow, F.S.; Sharma, A.; Jusko, W.J. *J. Pharmacokinet. Biop.*, **1999**, *27*, 559.
[47] Leopold, C.S. *Eur. J. Pharm. Biopharm.*, **2003**, *56*, 53.
[48] Rohatagi, S.; Bye, A.; Mackie, A.E.; Derendorf, H. *Eur. J. Pharm. Sci.*, **1996b**, *4*, 341.
[49] Derendorf, H.; Möllmann, H.; Barth, J.; Möllmann, C.; Tunn, S.; Krieg, M. *J. Clin. Pharmacol.*, **1991**, *31*, 473.
[50] Kraan, G.P.B.; Dullaart, R.P.F.; Pratt, J.J.; Wolthers, B.G.; De Bruin, R. *J. Steroid Biochem. Molec. Biol.*, **1997**, *63*, 139.
[51] Barton, R.N.; Horan, M.A.; Clague, J.E.; Rose; J.G. *Arch Gerontol Geriat*, **1999**, *29*, 95.
[52] Krzyzanski, W.; Jusko, W.J. *J. Pharmacokinet. Pharmacodyn.*, **2001**, *28*, 57.
[53] Magee, M.H.; Blum, R.A.; Lates, C.D.; Jusko, W.J. *J. Clin. Pharmacol.*, **2001**, *41*, 1180.
[54] Petersen, K. B.; Jusko, W. J. *Cancer Chemother. Pharmacol.*, **2003**, *51*, 465.
[55] Meibohm B.; Hochhaus, G.; Möllmann, H.; Barth, J.; Wagner, M.; Krieg, M.; Stöckmann, R.; Derendorf, H. *J. Pharmacokinet. Biop.*, **1999**, *27*, 127.
[56] Chakraborty, A.; Krzyzanski, W.; Jusko, W.J. *J. Pharmacokinet. Biop.*, **1999a**, *27*, 23.
[57] Krzyzanski, W.; Chakraborty, A.; Jusko, W.J. *Chronobiol. Int.*, **2000**, *17*, 77.
[58] Milad, M.A.; Ludwig, E.A.; Lew, K.H.; Kohli, R.K.; Jusko, W.J. *Am. J. Ther.*, **1994**, *20*, 567.
[59] Chakraborty, A.; Blum, R.A.; Culter, D.L.; Mis, S.; Jusko, W.J. *J. Clin. Pharmacol.*, **1999b**, *39*, 624.
[60] Meibohm, B.; Hochhaus, G.; Möllmann, H.; Barth, J.; Wagner, M.; Krieg, M.; Stöckmann, R.; Derendorf, H. *J. Clin. Pharmacol.*, **1997**, *37*, 704.
[61] Kraan, G.P.B.; Dullaart, R.P.F.; Pratt, J.J.; Wolthers, B.G.; Drayer, N.M.; De Bruin, R. *J. Clin. Endocrinol. Metab.*, **1998**, *83*, 1247.
[62] Krishnaswami, S.; Hochhaus, G.; Derendorf, H. *AAPS Pharmsci*, **2000**, 2, Article 22 (http://www.pharmsci.org).
[63] Hellman, L.; Nakada, F.; Curti, J.; Weitzman, E.D.; Kream, J.; Roffwarg, H.; Ellman, S.; Fukushima, D.K.; Gallagher, T.F. *J. Clin. Endocrinol. Metab.*, **1970**, *30***,** 411.
[64] Linder, B.L.; Esteban, N.V.; Yergey, A.L.; Winterer, J.C.; Loriaux, D.L.; Cassorla, F. *J. Pediatr*. **1990**, *117*, 892.
[65] Chrousos, G.P.; Torpy, D.J., Gold, P.W. *Ann. Intern. Med.*, **1998**, *129*, 229.
[66] Vgontzas, A.N.; Mastorakos, G.; Bixler, E.O.; Kales, A; Gold, P.W.; Chrousos, G.P. *Clin. Endocrinol.*, **1999**, *51*, 205.
[67] Ilias, I.; Vgontzas, A.N.; Provata, A.; Mastorakos, G. *Endocrine Regulations*, **2002**, *36*, 63.
[68] Veldhuis, J.D.; Pincus, S.M. *Eur. J. Endocrinol.*, **1998**, *138*, 358.
[69] Lenbury, Y.; Pacheenburawana, P. *Biosystems,***1991**, *26*, 117.
[70] Dokoumetzidis, A.; Iliadis, A.; Macheras, P. *Br. J. Clin. Pharmacol.*, **2002**, *54*, 21.
[71] Mackey, M.C.; Glass, L. *Science*, **1977**, *197*, 287.
[72] Goodwin, B.C. *Adv. Enzyme Regul.*, **1965**, *3*, 425.
[73] Brown, E.N.; Meehan, P.M.; Dempster, A.P. *Am. J. Physiol. Endocrinol. Metab.*, **2001**, *280*, E450.
[74] Engel, B.; Reenen, K.V.; Buist, W. *Biometrical J.*, **2003**, *45*, 866.
[75] Heikkilä, H.J. *J. Biopharm. Stat.*, **1999**, *9*, 89.
[76] Rohatagi, S. ; Zech, K.; Nave, R.; Hochhaus, G.; Jensen, K.; Barrett, J.S. *J. Clin. Pharmacol.*, **2003**, 43, 365.
[77] Veldhuis, J.D.; Iranmanesh, A.; Lizarralde, G.; Johnson, M.L. *Am. J. Physiol.*, **1989**, *257*: E6-14.
[78] Tornatore, K.M.; Reed, K.A.; Venuto, R.C. *Pharmacotherapy*, **1993**, *13*, 481.
[79] Inagaki, K.; Inagaki, M.; Kataoka, T.; Sekido, I.; Gill, M.A.; Nishida, M. *Ther. Drug Monit.* **2002**, *24*, 722.
[80] Hill, M.R.; Szefler, S.J.; Ball, B.D.; Bartoszek, M.; Brenner, M. *Clin. Pharmacol. Ther.*, **1990**, *48*, 390.
[81] Charmandari, E.; Hindmarsh, P.C.; Johnston, A.; Brook, C.G.D. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 2701.

Received: November 17, 2004 **Revised: July 05, 2005** **Accepted: July 12, 2005**