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Correlation of in vitro dissolution data with in vivo plasma concentrations, for three, orally administered, formulations of sulphamethoxazole-trimethoprim, by statistical moments analysis

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Summary

An in vitro-in vivo correlation was attempted for three orally administered formulations of sulphamethoxazole (SMZ)-trimethoprim (TMP), using first statistical moments for in vitro (mean dissolution time) and in vivo (mean residence time) data. In spite of the fact that both SMZ and TMP possess different absorption, distribution and excretion characteristics, an in vitro-in vivo correlation was achieved between an in vitro and in vivo parameter, since the dissolution rate of the drugs is the rate-limiting factor of the drugs appearance in the blood.

Introduction

Statistical moments represent a group of curve parameters which enable the most general and direct correlation between time functions. A biopharmaceutical time function is a distribution function of the variable 'time', which may be a 'dissolution time', a 'plasma concentration time', or any other kind of 'residence time'.

Statistical moments are characteristic of the shape of the distribution curves, they are only dependent on the observed time course data and are independent of pharmacokinetic compartment models. Zero moment represents the area under the plasma concentration-time curve (AUC). The first moment, which is defined as mean residence time (MRT), is the arithmetic mean which characterizes the overall rate of the process which a drug undergoes from the time of administration to the time of excretion. The second statistical moment, the variance of residence time (VRT), corresponds to the dispersion of the time function.

First and second statistical moments exhibit additive properties, according to which the MRT value (after per os administration) corresponds to (Dengler et al., 1981; Symillides, 1987):

$$MRT_{per\ os} = MDT_{in\ vivo} + MAT + MTT \quad (1)$$

where $MRT_{per\ os}$ is the mean residence time after

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per os administration of a solid formulation (tablets or capsules), $MDT_{in\ vivo}$ denote, the mean dissolution time in vivo, MAT is the mean absorption time and MTT represents the mean transit time.

The additive properties of statistical moments make them useful not only for the evaluation of the rate of bioavailability, but also for in vitro-in vivo correlations.

If it is presumed that:

$$MDT_{in\ vivo} = \beta \cdot MDT_{in\ vitro} \quad (2)$$

and

$$\alpha = MAT + MTT \quad (3)$$

then Eqn 1 becomes:

$$MRT_{per\ os} = \alpha + \beta \cdot MDT_{in\ vitro} \quad (4)$$

According to Eqn 4, the $MRT_{per\ os}$ of a drug in the body correlates linearly with MDT in vitro. If the drug is administered as a solution, then the determination of $MDT_{in\ vivo}$ is possible:

$$MRT_{solution} = MAT + MTT \quad (5)$$

according to Eqns 1 and 5:

$$MDT_{in\ vivo} = MRT_{per\ os} - MRT_{sol} \quad (6)$$

$MDT_{in\ vivo} - MDT_{in\ vitro}$ correlation gives valuable information regarding in vitro simulation of drug dissolution into the GI tract.

In the present investigation, an in vitro-in vivo correlation was attempted for three orally administered formulations of sulphamethoxazole (SMZ)-trimethoprim (TMP), using first statistical moments of in vitro and in vivo data (level B correlation) (Shah, 1990).

Tablets, corresponding to formulation F1, and spheroidal granules encapsulated into hard gelatin capsules, corresponding to formulation F2, were prepared by wet granulation techniques (Athanassiou et al., 1991). The third formulation tested (F3) was a commercially available product (Septrin tablets 400 mg SMZ and 80 mg TMP, Wellcome Hellas, lot no: 890803).

Materials and Methods

Materials

Sulphamethoxazole (Wellcome lot 27755), trimethoprim (Wellcome lot 20273), talc USP NF, sugar USP NF, calcium hydrogen phosphate USP NF, Mg stearate USP NF, polyvinylpyrrolidone (PVP, Gaf), sodium starch glycolate USP NF (Primogel Avebe), Tween 20 (polyoxyethylene 20 sorbitan monolaurate (Atlas), Eudragit 12.5% (Rohm Pharma), acetonitrile, methanol (Prolabo HPLC grade), water (HPLC grade) and hard gelatin capsules (no. 0, Capsugel) were employed. All other reagents were of analytical grade and were used without further purification.

Preparation of tablets

Tablets of formulation F1 were prepared as follows (Athanassiou, 1991): SMZ and TMP powders and 5 g of primogel were thoroughly mixed with a cubic mixer; then the mixture was placed in a Z-blade mixer. PVP and Tween 20 were added as hydroalcoholic solution and after blending, granules were obtained through a sieve granulator. The granules were dried at 30°C for 24 h in a drying oven. The remainder of the primogel and Mg stearate was added and tablets were obtained using a Killian tableting machine.

Preparation of spheroidal granules

Capsules of formulation F2 were prepared according to a previously described method (Athanassiou et al., 1991). SMZ and TMP spheroidal granules were prepared separately and TMP spheres were coated with Eudragit 12.5% solution. The fraction between 600 and 1000 μm was used for filling the capsules. SMZ and TMP spheres were admixed in a 5:1 weight ratio (after drug loading control), and the necessary quantity was encapsulated in hard gelatin capsules (No. 0).

In vitro drug release

Release of SMZ and TMP was determined using a standard USP (Method II) dissolution apparatus (Pharmatest-type PTW/SII, Haiburg, Germany). The dissolution medium was 900 ml of 0.1 N HCl solution. The test conditions were: temperature, 37 ± 0.1°C, basket rotation speed,

75 rpm (according to USP XXI, Sixth Supplement requirements). Aliquots were withdrawn at 5, 10, 15, 20, 30, 40, 50 and 60 min intervals. The samples were assayed using an HPLC method.

In vivo study

This study was performed in accordance with the Declaration of Helsinki. All subjects read the protocol and gave written consent for their participation. Each subject received two tablets or capsules of the tested formulations. Blood samples were collected in heparinized glass tubes, at 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0 and 12.0 h post-dose. The plasma was immediately separated by centrifugation and stored at −20°C until analysis. Plasma concentrations of SMZ and TMP were quantitated simultaneously, by an HPLC method.

HPLC method

The HPLC system used was a Spectra Physics pump model 8800, equipped with a variable-wavelength detector, Spectra 100 (set at λ_{max} = 230 nm) which was connected with a Spectra Physics Chromjet integrator. Aliquots were introduced into a C_{18} column through a Rheodyne 7125 injector with a 50μl loop valve. The mobile phase consisted of acetonitrile: 0.1 M sodium acetate (30 : 70 v/v), adjusted to pH 6.00 by dropwise addition of glacial acetic acid.

For the in vitro study, calibration curves showed good linearity over the concentration range examined (2–24 μg/ml for SMZ and 0.4–4.8 μg/ml for TMP). Regression coefficients were 0.9998 for SMZ and 0.9993 for TMP. For the in vivo study, standard curves were found to remain linear in the range of 8.0–64.0 μg/ml for SMZ and 0.4–2.4 μg/ml for TMP, over the entire period of study. The correlation coefficient ($\pm$SD) of standard curves was 0.998 ($\pm$0.001) and 0.994 ($\pm$0.0012) for SMZ and TMP, respectively.

Statistical moments analysis

First statistical moments for in vitro and in vivo data MDT and MRT, respectively, were calculated algebraically according to Eqn 7, based on the values of the Weibull distribution parameters (Johnson and Leone, 1977; Langebucher and Moller, 1983):

$$\mu_1 = t_0 + T_d \cdot \Gamma(z) \tag{7}$$

and

$$z = 1 + 1/b$$

where t_0 is the lag time, T_d denotes a rate parameter, b is a shape parameter and $\Gamma(z)$ denotes the gamma function. When $b > 1$, then:

$$\Gamma(z) = \Gamma(1 + 1/b) = (1/b)!$$

$$= 1 + C_1(1/b) + C_2(1/b)^2$$

$$+ \ldots + C_8 + \epsilon(1/b)$$

$$0 \leq 1/b \leq 1;\ |\epsilon(1/b)| \leq 3 \times 10^{-7};$$

$C_1 - C_8$, *constants*

When $b < 1$, then:

$$\ln \Gamma(1 + 1/b)$$

$$\approx (z - 0.5)\ln z - z + 0.5 \ln(2\pi)$$

$$+ \frac{1}{12z} - \frac{1}{360z^3} + \frac{1}{1260z^5} \frac{1}{1680z^7}$$

$$+ \ldots z = (1 + 1/b) > 0$$

In vitro-in vivo raw data were turned into cumulative form to obtain t_0, T_d and b and then first statistical moments were calculated with an IBM-PC-based program (Appendix A).

Results and Discussion

$MDT_{in\ vitro}$ and $MRT_{per\ os}$ values for SMZ and TMP of formulations F1, F2 and F3 are shown in Figs. 1 and 2. $MRT_{per\ os}$ values were plotted vs $MDT_{in\ vitro}$ values. The results of regression analysis are shown in Table 1 (Figs 1 and 2). The linearity of the correlation (expressed in terms of

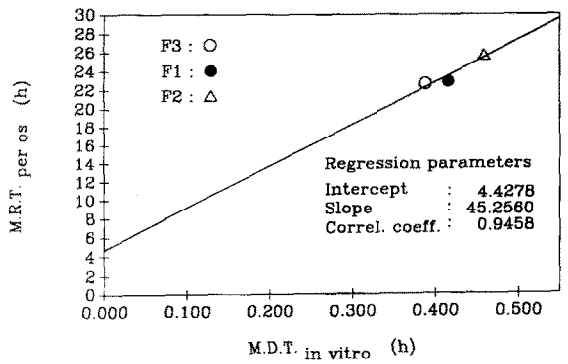

Fig. 1. In vitro-in vivo linear correlation between $MRT_{per\ os}$ and $MDT_{in\ vitro}$ for SMZ of formulations F1, F2 and F3.

correlation coefficient) was better in the case of SMZ than TMP.

For both drugs, due to their low aqueous solubility, the dissolution rate from the formulation is the rate-limiting factor of drug appearance in blood. Trimethoprim, however, due to its greater fat solubility, is capable of crossing biological membranes and penetrating into extravascular and fatty tissues to a greater extent than sulphamethoxazole, reflected in a greater distribution volume (100–120 l for TMP compared to 12–18 l for SMZ) (Patel and Welling, 1980). As a result of the above phenomenon, MRT of TMP was affected to a lower extent by the in vivo dissolution of the drug. Concomitantly, the relatively high value of MDT in vitro of formulation F2 had a negative effect on the linearity of the correlation.

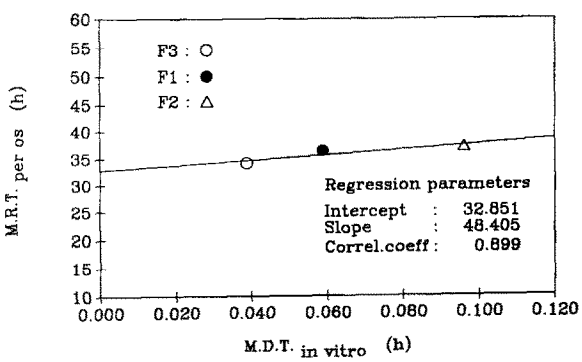

Fig. 2. In vitro-in vivo linear correlation between $MRT_{per\ os}$ and $MDTP_{in\ vitro}$ for TMP of formulations F1, F2 and F3.

TABLE 1

In vitro-in vivo linear correlation results

Regression parameters	SMZ	(±SD)	TMP	(±SD)
Intercept	4.4278	(3.92)	32.851	(3.27)
Slope	45.2560	(6.38)	48.405	(4.31)
Correlation coefficient	0.9458	(0.06)	0.899	(0.01)

Taking into consideration the additive properties of statistical moments, a generalized in vitro-in vivo correlation, between $MDT_{in\ vitro}$ and $MDT_{in\ vivo}$, was attempted for both drugs and all three formulations.

When an in vitro-in vivo correlation is designed for two or more drugs, and several formulations, the in vivo parameter should be independent of the absorption, distribution and excretion characteristics of each drug. $MDT_{in\ vivo}$ is the most suitable parameter in this case. $MDT_{in\ vivo}$ can be determined if the drug is administered as a solution, as previously described. When this is not possible, a 'normalized' expression of the parameter can be used instead.

When a linear correlation was found between $MDT_{in\ vivo}$ and $MDT_{in\ vitro}$ then:

$$MDT_{in\ vivo} = \alpha + \beta \cdot MDT_{in\ vitro} \tag{8}$$

Based on Eqn 8 and on statistical moments; additive properties, it is readily demonstrated that:

$$MRT_{per\ os} - \text{intercept} = MDT_{in\ vivo} - \alpha$$

$$= \beta \cdot MDT_{in\ vitro} \tag{9}$$

where intercept corresponds to the values in Table 1 and α is a constant (Appendix B).

According to Eqn 9, if there exists a linear correlation between $(MDT_{in\ vivo} - \alpha)$ and $MDT_{in\ vitro}$, then the regression line should pass through the origin of the lines.

Taking the above into account $(MDT_{in\ vivo} - \alpha)$ values, corresponding to $(MRT_{in\ vivo}$–intercept) values (for both drugs), were plotted vs $MDT_{in\ vitro}$ values (Fig. 3). The results of the regression anal-

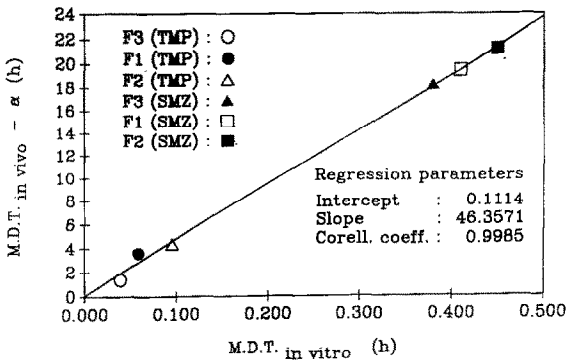

Fig. 3. In vitro-in vivo linear correlation for both SMZ and TMP of formulations F1, F2 and F3.

TABLE 2

In vitro-in vivo correlation results for both SMZ and TMP

Intercept	0.11136 (±0.24)
Slope	46.35712 (±4.82)
Correlation coefficient	0.99885 (±0.001)

ysis of the above correlation are shown in Table 2.

Both SMZ and TMP possess different absorption, distribution and excretion characteristics, as referred to in the literature (Patel and Welling, 1980) and as concluded from the different intercept values in Figs. 1 and 2. In spite of the above fact, an in vitro-in vivo correlation was obtained between a 'normalized' in vivo parameter ($MDT_{in\ vivo} - \alpha$) and MDT_{in} vitro, since the dissolution rate of the drugs, from the formulations is the rate-limiting factor of the drug's appearance in blood.

Appendix A

First statistical moments were calculated with the use of a program which was written in macro-commands of the open spreadsheet package Lotus-123.

The main screen of the program is displayed in the A1 · · H20 range of the spreadsheet. The macro-commands of the program was written in the K1 · · K50 range of the spreadsheet.

Macro-commands located in K1 · · K6 range define the main menu of the program with the six following alternative procedures:

(a) Input	input procedure of new group of data:
(b) File	File retrieve procedure of old group of data and results;
(c) Save	File save procedure of a group of data and results;
(d) Print	Print procedure of a group of data and results (i.e., main screen);
(e) Clear	Clear screen procedure of old group of data and results.
(f) MS-DOS	Return to the operational system (MS-DOS) procedure.

The macro-commands of the data input procedure (K10 · · K14 range) define the titles of the main screen locking the spreadsheet in the C6 cell, they format as hidden the results displayed in the three corresponding named ranges (APOT1-APOT2-APOT3) in order not to appear during the data input and finally the calculated values of the statistical moments formatted appear as fixed numbers of five decimal places. The mathematical functions which calculate the statistical moments are located in the APOT1, APOT2 and APOT3 named ranges. The results of the above calculations are displayed in the main screen after the end of data input (the APOTOL named range, which encloses the above three result ranges, is reset).

The macro-commands of the file retrieve procedure call a file with the old group of data and results, the name of which is given by the user in the 'Name of file to retrieve': prompt. The accepted values of the file's name are defined from the F?? format (?? = 0–99). For any possible error, in the input of the file's name, the corresponding screen (in cell AA1) with the appropriate error message is activated.

The macro-commands of the save file procedure enable the user to save the current set of data and results (i.e., the main screen) to a disk file. The restrictions described in the file retrieve procedure, for the format of the file's name, are still valid.

The macro-commands of the print file procedure enable the user to print the main screen of the program with the current set of data and results. After the end of the printing, the main screen is cleared as happens when the clear screen procedure is activated.

MAIN SCREEN OF THE PROGRAM

```
A1: [W26] /*
                A               B       C       D       E       F       G       H
1   ************************************************************
2     STATISTICAL MOMENTS CALCULATION USING WEIBULL FUNCTION PARAMETERS
3   ============================================================================
4      P A R A M E T E R S     ** 1st GROUP ** 2nd GROUP ** 3rd GROUP   **
5   ============================**===========**===========**=========== **
6         LAG TIME (to)         **           **           **           **
7   ----------------------------**-----------**-----------**----------- **
8      TIME PARAMETER (Td)      **           **           **           **
9   ----------------------------**-----------**-----------**----------- **
10     SHAPE PARAMETER (β)      **           **           **           **
11  ============================================================================
12  ============================================================================
13        R E S U L T S         ** 1st GROUP ** 2nd GROUP ** 3rd GROUP   **
14  ============================**===========**===========**=========== **
15   FIRST STATISTICAL  ; MIN ** **           **           **           **
16         MOMENT       ; HRS ** **           **           **           **
17  ============================================================================
18   THE VALUES OF to AND Td ARE IN MINUTES
19
20 ************************************************************
```

LIST OF THE MACRO-COMMANDS OF THE PROGRAM

```
K8:
        K               L               M               N           O
1   {ONERROR K50}{PANELOFF}/WGZY{PANELON}{INDICATE MENU} {MENUBRANCH MENU}
2   {BRANCH K1}
3
4   INPUT        FILE          SAVE         PRINT         CLEAR
5   Data Input   File Retrieve Save a File Print Screen Clear Screen
6   {BRANCH K10}{BRANCH K17} {BRANCH K32}{BRANCH K42} {BRANCH K43}
7
8
9
10  {INDICATE EDIT}{WINDOWSOFF}{PANELOFF}
11  {GOTO}C6~/WTB/RFHAPOT1~/RFHAPOT2~/RFHAPOT3~
12  {WINDOWSON}{PANELON}/RIDATA~
13  {PANELOFF}/WTC{HOME}{WINDOWSOFF}/RFRAPOTOL~/RFF5~APOT1~/RFF5~APOT2~/RFF5
14  {PANELON}{WINDOWSON}{BRANCH K1}
15
16
17  {INDICATE EDIT}{GETLABEL "NAME OF FILE TO RETRIEVE:",FNAME)~
18  {IF FNAME=""}{BRANCH K1}
19  {IF @LEFT(FNAME,1)<>"F"#OR#@LEFT(FNAME,1)<>"f"}{BRANCH K24}
20  {IF @ISERR(@VALUE(@MID(FNAME,1,30)))=1}{BRANCH K24}
K30
        K               L               M               N           O
21  {ONERROR K25}{WINDOWSOFF}{PANELOFF}/FCCEMRT\ARXEIO\
22  F1
23  ~{WINDOWSON}{PANELON}{BRANCH K1}
24  {BEEP}{BRANCH K17}
25  {BEEP}{GOTO}AA1~
```

```
26  {WINDOWSON}{GET ESCAPE}~
27  {IF ESCAPE="{ESC}"}{BRANCH K29}
28  {BEEP}{BRANCH K26}
29  {HOME}{ONERROR K50}{PANELON}{BRANCH K17}
30
31
32  {INDICATE EDIT}{GETLABEL "NAME OF FILE TO SAVE: ",FNAME}~
33  {IF FNAME=""}{BRANCH K1}
34  {IF @LEFT(FNAME,1)<>"F"#OR#@LEFT(FNAME,1)<>"f"}{BRANCH K40}
35  {IF @ISERR(@VALUE(@MID(FNAME,1,30)))=1}{BRANCH K40}
36  {IF @VALUE(@MID(FNAME,1,3))<1#OR#@VALUE(@MID(FNAME,1,3))>100}{BRANCH K45
37  {INDICATE WAIT}{WINDOWSOFF}{PANELOFF}/FXFMRT\ARXEIO\
38  F1
39  ~WHOLE~R{WINDOWSON}{PANELON}{BRANCH K1}
40  {BEEP}{BRANCH K32}
K41
        K           L           M           N           O
41
42  {INDICATE WAIT}{PANELOFF}/PPAGLLLQ{PANELON}{BRANCH K1}
43  {INDICATE WAIT}{PANELOFF}/FCCEMRT\EMPTY~{PANELON}{BRANCH K1}
44
45  {BEEP}{GOTO}AA1~
46  {WINDOWSON}{GET ESCAPE}~
47  {IF ESCAPE="{ESC}"}{BRANCH K49}
48  {BEEP}{BRANCH K46}
49  {HOME}{BRANCH K32}
50  {BEEP}{BRANCH P6}
51
52
53
54
55
56
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58
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60
```

Appendix B

According to statistical moments' additive properties (Riegelman and Collier, 1980; Tanigawara et al., 1982):

$$MRT_{per\ os} = MRT_{solution} + MDT_{in\ vivo} \qquad (10)$$

from Eqns 8 and 10, the following expression results:

$$MRT_{per\ os} = MRT_{sol} + \alpha + \beta \cdot MDT_{in\ vivo} \qquad (11)$$

According to Eqn 11, $(MRT_{sol} + \alpha)$ corresponds to the intercept of the $MRT_{per\ os}$ and $MDT_{in\ vitro}$ linear correlation (Figs. 1 and 2). Which finally leads to:

$$\begin{aligned} &MRT_{per\ os} - \text{intercept} \\ &= MRT_{per\ os} - (MRT_{sol} + \alpha) \\ &= MRT_{sol} + MDT_{in\ vivo} - MRT_{sol} - \alpha \\ &= MDT_{in\ vivo} - \alpha \end{aligned} \qquad (12)$$

from Eqns 11 and 12, the following is obtained:

$$MDT_{in\ vivo} - \alpha = \beta \cdot MDT_{in\ vitro}$$

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