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Election of a kinetic model for the study of oral and intravenous administration of busulphan

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ABSTRACT

We have studied the busulphan plasma level curves obtained in six groups of rats when intravenously and orally administered. The most appropriate pharmacokinetic model is the bicompartmental one for both intravenous and oral administration.

KEYWORDS

Pharmacokinetics;
Busulphan;
Oral;
Rats.

INTRODUCTION

This study is part of a research line aimed at predicting human pharmacokinetics from animal data, and focuses on the study of the pharmacokinetics of busulphan in Wistar rats after intravenous and oral administration. For the interpretation of the results from the analysis of the plasma level curves, it is necessary to have an appropriate pharmacokinetic model.

The choice of that model is the aim of this work. The drug used was 1,4-butanediol dimetanosulfonate, marketed as busulphan. It is a synthetic antineoplastic agent belonging to the group of alkylating agents, which has four methylene groups between two methyl-sulfonate groups:

$H_3C\text{-}SO_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}O\text{-}SO_2\text{-}CH_3$

The shortage of available analytical methods for quantification in plasma and other biological fluids has limited the pharmacokinetic studies and has led to the start of the experimental work described in this study. A HPLC analytical method, perfected by our research group, for the quantification of busulphan in plasma samples has been used.

In the case of animals, only some studies on distribution of busulphan to tissues such as brain, liver and lungs using radioactively labelled busulphan have been found. The distribution of busulphan in rat brain has been studied by several authors[1,2], having determined a concentration ratio brain / plasma of 0.74 ± 0.05, and a ratio of values AUC_{brain} / AUC_{plasma} of 0.75.

Similarly, a study in monkeys[1] observed that after intravenous injection of labelled busulphan there was a

TABLE 1 : Characteristics of the different groups of animals used in trials

Trials	Trial groups	Administration of busulphan
Intravenous	1 (age~30-35 days old)n=10	Intravenous (dose = 0.25 mg)
Pharmacokinetic	2 (age~50-55 days old)n=10	Intravenous (dose= 0.5 mg)
Parameters	3 (age~1.5 years old) n=10	Intravenous (dose = 1 mg)
Oral	4 (age ~ 30-35 days old) n=9	Oral (dose = 2.5 mg)
Pharmacokinetic	5 (age~50-55 days old) n=9	Oral (dose = 5 mg)
Parameters	6 (age~1.5 years old) n=9	Oral (dose = 12.5 mg)

Letter "n" refers to the number of animals included in each group

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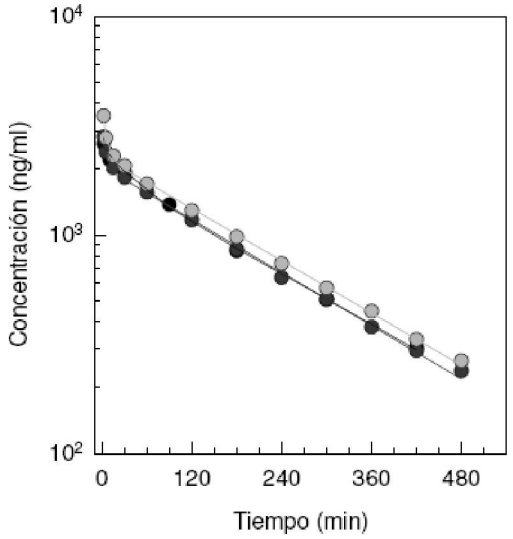

Figure 1 : Plasma level curves obtained after intravenous administration of 0.25 (black circles), 5 (dark grey circles) and 1 (grey circles) mg of busulphan to the animals in groups 1, 2 and 3 respectively. The corresponding theoretical curves obtained after the adjustment of the equation representing a two-compartment disposition model have been represented (Equation 2)

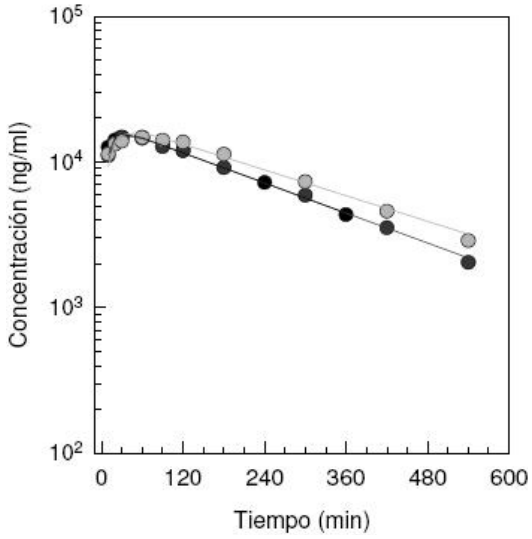

Figure 2 : Plasma level curves obtained after oral administration of 2.5 (black circles), 5 (dark grey circles) and 12.5 (grey circles) mg of busulphan in a solution containing methylcellulose 0.25% to animals of groups 4, 5 and 6, respectively. The curves drawn correspond to the theoretical curves obtained after adjusting a biexponential equation (Equation 4)

TABLE 2 : Results of the adjustment of the equations representing the single compartment, two-compartment and tricompartmental models to the mean plasma levels for the intravenous administration of a dose of 1.6 mg / kg of busulphan to the rats in group 1 (age 30-35 days). The table shows the estimated value of the parameter, its standard deviation (SD), coefficient of variation (CV,%), and dependency between parameters. The units of C_O, A, B and P are ng / ml, those of K_{el}, α, β and π are in min^{-1}

Monocompartmental				
Parameter	**Value**	**S.D.**	**CV (%)**	**Dependency**
C_O	2273	104,0	4,576	0,558
K_{el}	0,00504	0,00024	4,736	0,558
Bicompartmental				
Parameter	**Value**	**S.D.**	**CV (%)**	**Dependency**
A	646,7	173,2	26,78	0,773
α	0,0283	0,0157	55,26	0,819
B	1911,0	172,9	9,05	0,965
β	0,00442	0,00030	6,84	0,930
Tricompartmental				
Parameter	**Value**	**S.D.**	**CV (%)**	**Dependency**
A	10360	$1,53 \cdot 10^9$	$1,47 \cdot 10^7$	1,0000
α	1,799	$7,36 \cdot 10^4$	$4,09 \cdot 10^6$	1,0000
B	829,3	472,5	56,98	0,9957
β	0,0119	0,0063	53,30	0,9910
P	1526	0,0532	34,86	0,9992
π	0,00387	0,00074	19,03	0,9964

TABLE 3 : Results of the adjustment of the equations representing the single compartment, two-compartment and tricompartmental models to the mean plasma levels for the intravenous administration of a dose of 1.6 mg / kg of busulphan to the rats in group 2 (age 50-55 days). The table shows the estimated value of the parameter, its standard deviation (SD), coefficient of variation (CV,%) and dependency between parameters. The units of C_O, A, B and P are ng / ml, those of K_{el}, α, β and are π in min^{-1}

Monocompartmental				
Parameter	**Value**	**S.D.**	**CV (%)**	**Dependency**
C_O	2280	92,42	4,054	0,4412
K_{el}	0,00503	0,00022	4,397	0,4412
Bicompartmental				
Parameter	**Value**	**S.D.**	**CV (%)**	**Dependency**
A	1100	182,9	16,62	0,761
α	0,1950	0,0540	27,72	0,818
B	2057	41,74	2,03	0,750
β	0,00467	0,00009	1,84	0,647
Tricompartmental				
Parameter	**Value**	**S.D.**	**CV (%)**	**Dependency**
A	1161	113,9	9,81	0,8090
α	0,0277	0,0400	14,44	0,8730
B	1554	1110	71,39	0,9999
β	0,00645	0,00235	36,50	0,9994
P	606,7	1131	186,4	0,9999
π	0,00272	0,00206	75,78	0,9997

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TABLE 4 : Results of the adjustment of the equations representing the single compartment, two-compartment and tricompartmental models to mean plasma levels for the intravenous administration of a dose of 1.6 mg / kg of busulphan to the rats in group 3 (age 1.5 years). The table shows the estimated value of the parameter, its standard deviation (SD), coefficient of variation (CV,%) and dependency between parameters. The units of C_O, A, B and P are ng / ml, those of K_{el}, α, β and π are in min^{-1}

Monocompartmental				
Parameter	**Value**	**S.D.**	**CV (%)**	**Dependency**
C_O	2482	107,0	4,313	0,486
K_{el}	0,00485	0,00021	4,223	0,486
Bicompartmental				
Parmeter	**Value**	**S.D.**	**CV (%)**	**Dependency**
A	1698	322,4	18,99	0,773
α	0,0206	0,0519	25,23	0,818
B	2292	43,35	1,89	0,710
β	0,00460	0,00008	1,64	0,632
Tricompartmental				
Parameter	**Value**	**S.D.**	**CV (%)**	**Dependency**
A	2132	293,5	13,77	0,830
α	0,418	0,0705	16,87	0,922
B	472,1	78,49	16,63	0,904
β	0,0339	0,0100	29,59	0,914
P	2153	44,33	2,06	0,955
π	0,00441	0,00006	1,38	0,903

rapid distribution of radioactivity both to the liver and the lungs.

Moreover, a study in rats in our laboratory revealed a wide distribution of antineoplastic to most of the tissues tested (heart, muscle, lung, testis, intestine, stomach, kidney, skin, bone marrow and CSF) and, as a point of interest because there is no comparable studies in humans, concentrations of busulphan in rat milk of the same order as those determined in blood plasma[3] were measured.

In addition to that, recent studies have shown that busulphan is extensively metabolized in the liver of rats, and that the major metabolic pathway is the reaction with glutathione catalyzed by glutathione S-transferase[4,5].

After intravenous administration of 15 mg / kg of [1,4-^{14}C] busulphan to rats, the urinary excretion (unchanged drug and metabolites) globalized a 70% of the total radioactivity after 72 hours. The amount of unchanged busulphan excreted in urine was minimal (6% of the total radioactivity). Three major metabolites were

TABLE 5 : AIC values obtained after the adjustment of the single compartment, two-compartment and tricompartmental models to the average plasma concentration data for the intravenous administration of 1.6 mg / kg of busulphan to groups of animals 1 (30-35 days), 52 (50-55 days) and 63 (1.5 years)

Age	Monocompartmental AIC	Bicompartmental AIC	Tricompartmental AIC
30-35 days	5,612	-3,231	-16,122
50-55 days	31,476	-22,629	-14,271
1.5 years	17,879	-8,445	-28,935

TABLE 6 : Comparison of the results of the test based on the Snedecor's F between one-compartment and two-compartment models for an intravenous administration for each of the three ages tested. If the Fcalculated is lower than the Ftabulated the most simple model is chosen

Monocompartmental - bicompartmental			
Age	**Fcalculated**	**Ftabulated**	**Selected model**
30-35 days	7,90	19,16 [F(0,05, 3, 2)]	Monocompartmental
50-55 days	45,99	19,37 [F(0,05, 8, 2)]	Bicompartmental
1.5 years	46,06	19,37 [F(0,05, 8, 2)]	Bicompartmental

isolated and quantified: 3-hidroxisulfolano (39%), tetrahydrothiophene 1-oxide (20%) and sulfolano (13%). The results of this study show that busulphan is extensively metabolized and that most of the metabolites are eliminated via the kidney[4].

MATERIAL AND METHODS

Trials

The intravenous administrations of busulphan were made from solutions thereof in dimethyl sulfoxide (DMSO) of 5mg/ml.

The oral administrations of busulphan were made from suspensions thereof in 0.25% methylcellulose. The suspensions for each of the doses were prepared as follows:

a. Suspension 1 (2.5 mg / ml): 25 mg of busulphan and 10 ml 0.25% methylcellulose
b. Suspension 2 (0.25 mg / ml): 1 ml of Suspension 1 (2.5 mg / ml) and 9 ml of 0.25% methylcellulose.

TABLE 1 summarizes the characteristics of the different groups of animals used in trials.

The selection of the most appropriate model in each case was made taking into account several criteria. First of all, we evaluated the dependency or redundancy of the constitutive parameters of the model, which was used as a discriminating criterion between models.

TABLE 7 : Comparison of the results of the test based on the Snedecor's F between two-compartment and tricompartmental models for an intravenous administration for each of the three ages tested. If the Fcalculated is lower than the Ftabulated the most simple model is chosen

Bicompartmental - tricompartmental			
Age	**Fcalculated**	**Ftabulated**	**Selected model**
30-35 days	5,08	18,51 [F(0,05, 1, 2)]	Bicompartmental
50-55 days	18,16	19,33 [F(0,05, 6, 2)]	Bicompartmental
1.5 years	20,09	19,33 [F(0,05, 6, 2)]	Tricompartmental

TABLE 8 : Results of the adjustment of the equations representing one-compartment and two-compartment models to the mean plasma levels for the oral administration of a dose of 16.6 mg / kg of busulphan to the rats in group 4 (age 30-35 days) . The table shows the estimated value of the parameter, its standard deviation (SD), coefficient of variation (CV,%) and dependency between parameters. The units of C^O, M and L are ng / ml, those of K_{el}, K_a, α and β are in min^{-1}

Monocompartmental				
Parameter	**Value**	**S.D.**	**CV (%)**	**Dependency**
C^O	18600	661,1	3,554	0,7117
K_{el}	0,00398	0,00021	5,310	0,6257
K_a	0,103	0,015	14,73	0,3884
Bicompartmental				
Parameter	**Value**	**S.D.**	**CV (%)**	**Dependency**
M	-10130	284,0	2,804	0,9348
α	0,0415	0,00190	4,578	0,9712
L	20110	124,1	0,617	0,9704
β	0,00427	0,00002	0,571	0,8933
K_a	0,606	0,661	109,0	0,8128

Secondly we used the AIC criterion; the third and last test was performed based on the Snedecor's F for each of the three age groups tested. In this last test comparisons were made between one-compartment and two-compartment models on the one hand, and between two-compartment models and tricompartmental ones on the other, in the case of intravenous administration. For oral administration comparisons were made between one-compartment and two-compartment models.

Equations

The equations representing the plasma concentrations versus time are:

$$C = C_0 \cdot e^{-Kel \cdot t} \quad (1)$$

$$C = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} \; (\alpha > \beta) \quad (2)$$

$$C = P \cdot e^{-\pi \cdot t} + A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t} \; (\pi > \alpha > \beta) \quad (3)$$

For single compartment models, two-compartment ones and tricompartmental models, respectively.

In the case of oral administration of the antineoplastic, the following expressions of plasma concentration versus time were taken into account:

$$C = C^0 \cdot (e^{-Kel \cdot t} - e^{-Ka \cdot t}) \quad (4)$$

$$C = L \cdot e^{-\beta \cdot t} - (L + M) \cdot e^{-Ka \cdot t} + M \cdot e^{-\alpha \cdot t} \quad (5)$$

Corresponding to one-compartment and two-compartment models, respectively.

RESULTS

Intravenous administration

Plasma levels obtained after intravenous administration of the drug at different times are shown in figure 1.

TABLE 2, 3 and 4 show the results of adjusting the average levels of plasma concentration to the equations for single compartment models, two-compartment ones and tricompartmental models obtained after fast intravenous administration of 1.6 mg / kg of busulphan to rats in groups 41, 52 and 63 respectively. In these tables the estimated values of the parameters are included, as well as the coefficient of variation thereof and the dependency between them.

On the other hand TABLE 5 summarizes the resulting AIC values of the adjustment.

In addition to that, the results of the test based on the Snedecor's F made for each of the three age groups tested are shown in TABLE 6 and 7.

Oral administration

Plasma levels obtained after oral administration of the drug at different times are shown in figure 2.

TABLE 8, 9 and 10 show the results of the adjustment of the average levels to the equations for single compartment and two-compartment models, groups 4, 5 and 6 respectively.

The AIC values resulting from the adjustment of the equations representing the single compartment and two-compartment models to the plasma concentration average values obtained after an oral dose of 16.6 mg / kg of busulphan to three groups of animals with ages of 30-35 days (group 74), 50-55 days (group 15) and 1.5 years (group 86), are shown in TABLE 11.

Moreover, the test based on the Snedecor's F done for each of the three age groups tested are shown in TABLE 12.

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TABLE 9 : Results of the adjustment of the equations representing one-compartment and two-compartment models to the mean plasma levels for the oral administration of a dose of 16.6 mg / kg of busulphan to rats in group 5 (age 50-55 days) The table shows the estimated value of the parameter, its standard deviation (SD), coefficient of variation (CV,%) and dependency between parameters. The units of C^O, M and L are ng / ml, those of K_{el}, K_a, α and β are in min^{-1}

Monocompartmental				
Parameter	**Value**	**S.D.**	**CV (%)**	**Dependency**
C^O	18470	469,4	2,541	0,7580
K_{el}	0,00395	0,00009	2,309	0,6927
K_a	0,0887	0,0076	8,542	0,4306
Bicompartmental				
Parameter	**Value**	**S.D.**	**CV (%)**	**Dependency**
M	-5978	$1,81 \cdot 10^{14}$	$3,03 \cdot 10^{12}$	1,0000
α	0,0890	$1,91 \cdot 10^{5}$	$2,15 \cdot 10^{8}$	1,0000
L	18470	782,6	4,238	0,8782
β	0,00395	0,00013	3,386	0,7998
K_a	0,0890	78750	$8,85 \cdot 10^{7}$	1,0000

TABLE 10 : Results of the adjustment of the equations representing one-compartment and two- compartment models to the mean plasma levels for the oral administration of a dose of 16.6 mg / kg of busulphan to the rats in group 6 (age 1.5 years) The table shows the estimated value of the parameter, its standard deviation (SD), coefficient of variation (CV,%) and dependency between parameters. The units of C^O, M and L are ng / ml and those of K_{el}, K_a, α and β are in min^{-1}

Monocompartmental				
Parameter	**Value**	**S.D.**	**CV (%)**	**Dependency**
C^O	19770	1010	5,11	0,7877
K_{el}	0,00338	0,00023	6,71	0,7373
K_a	0,0699	0,0106	15,17	0,4168
Bicompartmental				
Parameter	**Value**	**S.D.**	**CV (%)**	**Dependency**
M	13470	$1,83 \cdot 10^{11}$	$1,36 \cdot 10^{9}$	1,0000
α	0,0710	2828	$3,98 \cdot 10^{6}$	1,0000
L	19720	1666	8,449	0,8913
β	0,00337	0,00033	9,703	0,8226
K_a	0,0707	1199	$1,70 \cdot 10^{6}$	1,0000

DISCUSSION

Intravenous administration

The graphical representation in semilogarithmic coordinates of the mean plasma levels of busulphan versus time after intravenous administration shows an initial curved layout (Figure 1) for the three age groups tested, which seems to be indicative of a two-compartment disposition model of the antineoplastic. However, three kinetic models were tested: single compartment, two-compartment and tricompartmental models, by adjusting the equations representing these models to the mean plasma concentrations.

The selection of the optimal kinetic model was made by applying the MAICE test to the mean plasma levels obtained for each of the three groups (TABLE 5). Based on AIC values resulting from the adjustment, it was observed that for the age group 50-55 days the MAICE criterion favours the two-compartment model. For the age groups 30-35 days and 1.5 years the two-compartment model was also selected because, for although the tricompartmental model showed a better AIC value, it was discarded because the adjustment showed high coefficients of variation and high values for dependency between parameters.

The test based on the Snedecor's F was also applied for the model selection. For each of the three age groups tested, comparisons between one-compartment models and two-compartment ones on one hand, and between two-compartment models and tricompartmental ones on the other were performed, resulting in most of the cases that the two-compartment model (TABLE 6 and 7) was favoured.

Therefore we can conclude that a two-compartment pharmacokinetic model is suitable for describing the disposition of busulphan in the rat according to the criteria used (MAICE and Snedecor's F), at least at the dose used in this study. We proceeded then to adjust the equation for the two-compartment model to the individual values of the antineoplastic plasma levels. Only one animal of the age group 30-35 days adjusted to the one-compartment model, as it was the only case in which the curve of plasma levels did not show two clear exponentials.

Oral administration

The graphical representation in semilogarithmic coordinates of the mean plasma levels of busulphan versus time after oral administration shows an initial curved layout (Figure 2) for the two age groups tested, which seems to be indicative of a two-compartment disposition model of the antineoplastic. However, two kinetic models were tested: one-compartment and two-compartment models by adjusting the equations representing these models to the mean plasma concent-

TABLE 11 : AIC values obtained after the adjustment of single compartment and two-compartment models to the mean plasma concentration data for the oral administration of 16.6 mg / kg of busulphan to animals in group 4 (30-35 days), 15 (50-55 days) and 86 (1.5 years)

Age	Monocompartmental AIC	Bicompartmental AIC
30-35 days	-1,249	-41,095
50-55 days	-10,503	-6,503
1.5 years	5,455	9,455

TABLE 12 : Comparison of the results of the test based on the Snedecor's F between one-compartment and two-compartment models for an oral administration for each of the three age groups tested. If Fcalculated is lower than the Ftabulated the most simple model is chosen

Monocompartmental- bicompartmental			
Age	Fcalculated	Ftabulated	Selected model
30-35 days	524,15	19,00 [F(0,05, 2, 2)]	Bicompartmental
50-55 days	0	19,30 [F(0,05, 5, 2)]	Monocompartmental
1.5 years	0	19,30 [F(0,05, 5, 2)]	Monocompartmental

rations.

It should be noted that in the case of the extravasal administration of a drug, one can only speak of apparent models, because the disposition may be masked by the absorption process, by contrast with to what happens with the intravenous administration.

The selection of the optimal apparent kinetic model was made in the same way as in the case of intravenous administration. Based on AIC values resulting from the adjustment (TABLE 11) it was observed that for age groups 50-55 days and 1.5 years, the MAICE criterion favours a biexponential equation (monocompartmental apparent model). For the age group 30-35 days a biexponential equation was also selected (apparent monocompartmental model), for although the two-compartment model showed the best AIC value, it was discarded because the adjustment showed high coefficients of variation and high values for dependency between parameters. The test based on the Snedecor's F was also applied for the apparent model selection. For each of the three age groups tested, comparisons between biexponential and triexponencial equations were performed, resulting that the biexponential equation was favoured for the age groups 50-55 days and 1.5 years, whereas the triexponencial equation was favoured for the age group 30-35 days. However, the biexponential equation was selected for this last test group for the same reason as when the MAICE test was applied (apparent monocompartmental model) (TABLE 12).

Thus we can conclude that the profile of plasma levels of busulphan in the rat after oral administration is biexponential (Figure 2) according to the criteria used (MAICE and Snedecor's F), at least at the dose used in this study. We proceeded then to adjust the biexponential equation (apparent monocompartmental model) to the individual values of plasma levels of the antineoplastic.

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