A COMPARATIVE ANALYSIS OF TILMICOSIN PRODUCTION VARIETIES APPLIED TO PIGS

Kaloyan Yankov, Miglena Kazakova

Faculty of Technics and Technologies, Trakia University, Yambol,

38 Graf Ignatiev str., 8602, Yambol, Bulgaria

e-mail: kaloyan.yankov@trakia-uni.bg

Abstract: *In the present work, a comparative analysis of two production forms of the antibiotic tilmicosin was performed after intravenous administration. The experiment was conducted on two groups of pigs, each of 10 animals, and the pharmacokinetic curves were received. A mathematical model of the curves is identified and on its basis, the dynamic parameters of the processes are calculated. A statistically significant difference (p -level <0.05) was found in only two parameters - initial value concentration and time-correction parameter. A statistically significant difference only of one dynamic parameter is found - the power of the pharmacokinetic curve. The final conclusion is that the two forms of tilmicosin have the same effect on the experimental animals.*

Keywords: *pharmacokinetics, tilmicosin, identification, mathematical model, dynamic parameters.*

1. INTRODUCTION

Tilmicosin is an antibiotic for use in veterinary medicine. In pigs, it is used for the treatment and prevention of bacterial respiratory diseases associated with *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Ornithobacterium rhinotracheale*, *Pasteurella multocida* and others. drug-sensitive micro-organisms.

Tilmicosin is available from pharmaceutical manufacturers with different brand names. The identification of differences in the action of drugs containing the same active ingredient is a laborious and complex task. The statistical comparison of the pharmacokinetic curves is not always appropriate due to the small number of parameters that can be compared.

When the pharmacokinetic curve is presented with an algebraic equation, then the functional analysis can be used to compare and evaluate the action of the drug. Differences can thus be found or, on the contrary, the identity and indistinguishability of drugs are proven.

The purpose of this work is to determine whether there is a difference in the action of two tilmicosin forms. By introducing a mathematical model of the pharmacokinetic curves of the two forms and calculating additional parameters for their evaluation, a statistically significant difference will be sought for them.

2. MATERIAL AND METHODS

2.1. Experimental animals

In order to preserve the anonymity of the manufacturer and of the trade name of the product, each drug will bear the name of the group of animals to which "Drug1 (D1)" and "Drug2 (D2)" have been applied. The data for drugs D1 and D2 administered to 10 male and female healthy pigs, 9-10 weeks of age with a body weight of 9-14.5 kg.

2.2. Order of the experiment

Blood samples were investigated after intravenous (iv) injection of tilmicosin at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72 hours according to the procedure presented in [1,2]. Concentration was determined by HPLC with fluorescence detection. The experimental protocol of the study was approved by the Institutional Animal Care and in accordance with the national regulations, and European Directive of 22.09.2010 (210/63/EU) concerning the protection of animals used for scientific and experimental purposes.

2.3. Data processing

Statistical processing was performed with STATISTICA 13, Statsoft Inc. A p-level < 0.05 was accepted as statistically significant. The Shapiro-Wilk test was used to verify normal distribution of data. For each data set, an arithmetic mean value, standard deviation SD, skewness and kurtosis are calculated. A statistically significant difference between the parameters of both drugs was sought with the Student-Fisher t-test. The identification of pharmacokinetic curves and the obtaining of the values of the dynamic processes is done with a program Korelia-Ident.

2.4. Identification of pharmacokinetic curves

The change in drug concentration in iv administration is characterized by a steep growth front and a decreasing downward branch. The increase in blood concentration may be presented as Dirac function, therefore it cannot be a characteristic of the action of the preparation or of the body's response. For that reason, the object of study will be the downstream branch of the process presenting the elimination phase. The concentration change curve is represented in Figure 1 and can be described by the exponential equation [6]:

$$C(t) = (C_0 - C_\infty) \cdot e^{-k \cdot t} + C_\infty \quad (1)$$

where: k is the rate of absorption of the drug;
 $C_0 = C(0)$ – initial concentration;
 $C_\infty = C(t \rightarrow \infty)$ – infinite asymptote.

Figure 1. Pharmacokinetic curve of a drug and parameters of the process

2.5. Dynamic parameters for estimation of kinetic curves

The graph of a process allows direct measuring a number of parameters. However, the presence of a mathematical model makes it possible to calculate features inaccessible from the graphic. Using a mathematical model, it is possible to apply control theory to analyze, evaluate, and compare processes [4,5].

Inverse function. The pharmacokinetic curve is a monotone, continuous function that ensures the existence of an inverse function. It gives the time to reach the desired concentration C_L :

$$t(C_L) = \frac{1}{k} \cdot \ln \left(\frac{C_0 - C_\infty}{C_L - C_\infty} \right) \quad (2)$$

Steady-state level of concentration (C_s). This is the residual concentration of the drug. The value is chosen by assuming a neighborhood of convergence δ relative to the initial concentration:

$$C_s(\delta) = C_\infty + \delta \cdot |C_0 - C_\infty| \quad (3)$$

Settling time (t_s). Time to reach the steady-state level of concentration C_s . It is obtained from formula (2), taking into account that $C_L = C_s$:

$$t_s(C_s) = \frac{1}{k} \cdot \ln \left(\frac{1}{\delta} \right) = t_s(\delta) \quad (4)$$

Half-time for elimination $t_{1/2}$. The time required to eliminate one-half of the quantity of drug:

$$t_{1/2}(\delta) = \frac{1}{k} \cdot \ln \left(\frac{2}{1 + \delta} \right) \quad (5)$$

Table 1 lists practical values for calculating the settling time t_s and half-time $t_{1/2}$ as a function of the absorption constant k for several commonly used values of δ .

Table 1. Dependence between t_s , $t_{1/2}$ and the rate constant k

δ	0,1	0,05	0,02	0,01
t_s	2,3/k	3/k	3,9/k	4,6/k
$t_{1/2}$	0,598/k	0,644/k	0,673/k	0,683/k

Area under curve – AUC for the interval $[0, t_s]$ is criteria for bioavailability:

$$AUC = \int_0^{t_s} ([C(t) - C_s(\delta)]) dt = \int_0^{t_s} \{C(t) - [C_\infty + \delta(C_0 - C_\infty)]\} dt \quad (6)$$

Mean Residence Time (MRT). Used to determine the mean residence time of the drug in the animal's body:

$$MRT = \frac{\int_0^{t_s} t \cdot C(t) dt}{\int_0^{t_s} C(t) dt} \quad (7)$$

Process Energy E. The defined process energy $y(t)$ is:

$$E = \int_0^{t_s} |y(t)|^2 dt \quad (8)$$

Process power P. This indicator averages energy **E** over a certain period:

$$P(\delta) = \frac{E}{t_s} = \frac{k \cdot E}{\ln(1/\delta)} \quad (9)$$

The listed parameters will be used to evaluate and compare the pharmacokinetic curves of the drugs. A statistically significant difference between the medications of the two manufacturers will be searched [3].

3. RESULTS

3.1. Pharmacokinetic parameters

The pharmacokinetic curves of the two forms of tilmicodin are presented in Figure 2. Statistical data for drug D1 are in Table 2 and for drug D2 - in Table 3. Visually, the differences in kinetic curves of the two drugs are negligible. This determines the need to introduce process identification and compare their dynamic parameters to look for statistically significant differences.

Figure 2. Kinetics of mean tilmicodin concentrations

Table 2. Statistical parameters for drug D1

Time, h	2	3	4	6	8	12	24	48	72
Average concentration C $\mu\text{g/ml}$	1,202	0,897	0,674	0,523	0,335	0,290	0,123	0,065	0,050
Standard deviation SD	0,157	0,142	0,130	0,122	0,091	0,075	0,036	0,015	0,000
Skewness	-0,515	0,316	-0,224	-0,208	-0,042	-0,062	0,238	0,969	-
Kurtosis	-0,579	-1,124	-1,028	-1,002	-1,379	-1,176	-0,795	0,027	-

Table 3. Statistical parameters for drug D2

Time, h	2	3	4	6	8	12	24	48	72
Average concentration C $\mu\text{g/ml}$	1,245	0,955	0,682	0,507	0,340	0,266	0,125	0,065	0,050
Standard deviation SD	0,182	0,175	0,125	0,122	0,087	0,082	0,039	0,016	0,000
Skewness	0,040	0,115	-0,438	-0,366	-0,201	-0,184	0,136	0,572	-
Kurtosis	-1,537	-1,413	-0,666	-1,353	-1,316	-1,613	-1,107	-1,287	-

3.2. Identification of a mathematical model

To identify the model, a neighborhood $\delta=0.05$ is chosen. In order to increase the reliability of the model, corrective parameter β is introduced into the exponential equation (1):

$$y(t) = (C_0 - C_\infty)e^{-k \cdot (t+\beta)} + C_\infty \quad (10)$$

Identification for each animal was performed with the Korelia-Ident program [7,8]. The data are in Table 4. The identification vector has been calculated, with a quadratic error minimization being selected for the optimization criterion. For each model, the coefficient of determination R^2 , Akaike information criterion (AIC) and corrected Akaike information criterion (AICc) was also calculated. The last two are used as an estimator of the relative quality of the models.

Table 4. Identified parameters for both drugs

D1				Pig	D2			
C_0 , $\mu\text{g/ml}$	C_∞ , $\mu\text{g/ml}$	β , h	k, 1/h		C_0 , $\mu\text{g/ml}$	C_∞ , $\mu\text{g/ml}$	β , h	k, 1/h
1,813	0,092	-1,515	0,266	1	2,008	0,120	-1,657	0,206
1,753	0,092	-1,757	0,344	2	2,109	0,103	0,402	0,261
1,866	0,092	-1,394	0,239	3	1,932	0,085	0,288	0,278
1,863	0,092	-1,454	0,180	4	2,083	0,094	1,708	0,274
1,690	0,060	-1,928	0,286	5	2,215	0,124	0,266	0,250
1,892	0,092	-1,090	0,196	6	2,180	0,112	0,502	0,258
1,885	0,092	-1,879	0,209	7	1,942	0,083	1,089	0,304
1,817	0,092	-1,879	0,216	8	1,837	0,077	1,501	0,315
1,803	0,119	-2,029	0,258	9	1,911	0,071	1,324	0,354
1,849	0,092	-1,636	0,292	10	2,316	0,130	0,294	0,250
1,823	0,092	-1,656	0,249	average	2,058	0,098	0,819	0,283
0,063	0,014	0,293	0,050	SD	0,162	0,021	0,583	0,035
-1,095	-0,627	0,639	0,482	skewness	0,198	0,373	0,484	1,159
0,896	4,689	-0,211	-0,256	kurtosis	-1,223	-1,248	-1,795	0,738

The identification of the two kinetic curves based on average values determined the following equations:

Concentration model of D1:

$$C1(t) = 1.731.e^{-0.240(t-1.656)} + 0.092 \quad (11)$$

Coefficient of determination $R^2 = 0.9822$; AIC = -45.92; AICc = -36.67

Concentration of D2:

$$C2(t) = 1.960.e^{-0.279(t+0.819)} + 0.098 \quad (12)$$

Coefficient of determination $R^2 = 0.9851$; AIC = -46.51; AICc = -37.36

The graphs of the two models are in Figure 3.

Figure 3. Graphics of the mathematical models

3.3. Dynamic parameters of drugs

For each pig, the dynamic parameters of the process were calculated. The data for D1 are in Table 5 and for D2 in Table 6.

Table 5. Dynamic parameters of drug D1

Pig	C _s , μg/ml	t _{1/2} , h	T _s , h	S _r , μg.h/ml	AUC, μg.h/ml	MRT, μg.h ² /ml	E, μg ² .h/ml ²	P, μg ² /ml ²
1	0,178	2,421	11,254	18,399	5,835	4,091	0,847	0,075
2	0,175	1,874	8,714	13,746	4,359	3,177	0,626	0,072
3	0,181	2,694	12,529	21,116	6,696	4,542	0,981	0,078
4	0,180	3,574	16,615	27,959	8,866	6,023	1,292	0,078
5	0,142	2,256	10,489	16,248	5,152	3,715	0,559	0,053
6	0,182	3,279	15,253	26,080	8,271	5,523	1,217	0,080
7	0,182	3,085	14,341	24,419	7,744	5,197	1,138	0,079
8	0,179	2,987	13,888	22,754	7,216	5,048	1,048	0,076
9	0,203	2,501	11,629	18,610	5,902	4,336	1,034	0,089
10	0,180	2,206	10,256	17,115	5,428	3,722	0,793	0,077
average	0,178	2,688	12,497	20,645	6,547	4,538	0,954	0,076
SD	0,015	0,534	2,485	4,601	1,459	0,901	0,244	0,009
skewness	-1,433	0,215	0,215	0,197	0,197	0,179	-0,354	-1,686
kurtosis	5,232	-0,843	-0,846	-1,030	-1,030	-0,881	-0,886	4,980

Table 5. Dynamic parameters of drug D2

Pig	C _s , μg/ml	t _{1/2} , h	T _s , h	S _r , μg.h/ml	AUC, μg.h/ml	MRT, μg.h ² /ml	E, μg ² .h/ml ²	P, μg ² /ml ²
1	0,214	3,129	14,550	26,105	8,279	5,368	1,492	0,103
2	0,207	2,467	11,275	21,665	6,871	4,153	1,154	0,102
3	0,178	2,319	10,784	18,916	5,999	3,876	0,845	0,078
4	0,194	2,349	10,925	20,638	6,545	3,933	1,010	0,092
5	0,229	2,576	11,978	23,799	7,547	4,392	1,430	0,119
6	0,216	2,501	11,629	22,841	7,243	4,231	1,272	0,109
7	0,176	2,122	9,867	17,428	5,527	3,537	0,764	0,077
8	0,165	2,046	9,513	15,904	5,044	3,405	0,652	0,069
9	0,163	1,819	8,458	14,784	4,688	3,004	0,589	0,070
10	0,239	2,576	11,978	24,874	7,888	4,392	1,565	0,131
average	0,198	2,309	11,096	20,695	6,563	3,880	1,077	0,095
SD	0,027	0,262	1,663	3,854	1,222	0,479	0,358	0,021
skewness	0,069	-0,820	0,559	-0,207	-0,207	-0,716	0,007	0,257
kurtosis	-1,424	-0,294	1,379	-1,223	-1,223	-0,437	-1,614	-1,118

4. DISCUSSION

In Table 7 are the calculated significance levels for the concentration measured during the experiment. Comparison of the plasma concentrations of the two antibiotics did not indicate a statistically significant difference in the pharmacokinetic curves. The process of elimination proceeds uniformly.

Table 7. p-level of the plasma concentration by hours

Time, h	2	3	4	6	8	12	24	48	72
p-level	0,587	0,426	0,890	0,770	0,905	0,504	0,930	1,000	1,000

Table 8. Identified parameters: p-level

Parameter	C_0	C_∞	β	k
p-level	0,00053	0,47851	0,00000	0,10948

The evaluation of the parameters of the mathematical model of the two drugs showed a statistically significant difference for the initial value C_0 and the correction parameter β (Table 8). These two parameters reflect the concentration of the drug at the initial time. The difference can be explained by the operational differences of the laboratory assistants, as well as by slight deviations in the timing of sampling [9].

Table 9. Level of significance for dynamics parameters

Parameter	C_s	$t_{1/2}$	t_s	S_r	AUC	MRT	E	P
p-level	0,05983	0,07096	0,15568	0,97895	0,97895	0,16539	0,37818	0,01706

A statistically significant difference is established only for the power of the processes. The final conclusion that can be drawn from the conducted study is that there is no difference in the pharmacokinetic of the two forms of tilmicosin.

5. CONCLUSION

In the present study, two production forms of the antibiotic tilmicosin have been compared. A new approach to assessing the pharmacokinetic curves is applied - identification of a mathematical model. With his help, the dynamic parameters of the two drugs were calculated. A statistically significant difference was found only in the parameter "power" of the pharmacokinetic curve. The final conclusion is that the two forms of tilmicosin have the same effect on the experimental animals.

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