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VALIDATION OF OPTIMAL AMPICILLIN/SULBACTAM RATIO IN DOSAGE FORMS USING IN-VITRO DYNAMIC MODEL

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ABSTRACT

To validate the optimal ampicillin/sulbactam ratio in dosage forms the antimicrobial action kinetics of the combinations against ampicillin-resistant strain of E. coli (MIC=250 μg/ml) under simulated clinical conditions in an in-vitro dynamic model was studied. The in-vitro dynamic model simulating the drug kinetic profiles described by the two-compartment pharmacokinetic model (serum drug kinetics after intravenous injection) and the one-compartment pharmacokinetic model (drug profiles in serum and tissue fluid after oral and intravenous administration respectively) was used. The profiles realized after receiving of 0.5 g of ampicillin and 0.125, 0.25, 0.5, 1.0 g of sulbactam were reproduced. Changes in the viable count in the dynamic model were estimated microcalorimetrically with BioActivity Monitor LKB

2277-202. The use of the recently developed parameter of antimicrobial effect duration T_E (the time from the moment of drug administration till the moment when the bacterial count reaches again its initial level) provided determination of relationship between effect and ampicillin/sulbactam ratios. It is demonstrated that the variation of ampicillin/sulbactam ratio from 4:1 to 1:1 was accompanied by marked enhancement in the antimicrobial effect. Further increase of sulbactam content in the formulation not resulted in more pronounced effect. We conclude that validation of optimal ratio in chemotherapeutic combinations using in-vitro dynamic models is a promising approach to development of modern drug formulations.

INTRODUCTION

To-day combined antibiotic therapy is not an exception but a rule. Along with using various combinations of antibiotics their combined use with inhibitors of bacterial enzymes inactivating antibiotics is ever increasing. Of special interest are combinations of ß-lactam antibiotics with ß-lactamase inhibitors. At present fixed combinations of clavulanic acid, an ß-lactamase inhibitor, with ß-lactam antibiotics such as amoxycillin (Augmentin) and ticarcillin (Timentin) are very useful (1,2). At the same time, validation of optimal ratios of antibiotics and inhibitors in combined dosage forms is a problem. The thing is that the choice of such ratios should include not only estimation of the drug effect on microorganism by MIC and MBC but also differences in pharmacokinetics of the components. Such information on the effect of the pharmacokinetic

factor on efficacy of antibiotic and ß-lactamase inhibitor combinations is provided by dynamic models which enable to study the antimicrobial action kinetics in vitro at the background of varying drug concentrations in strict conformity with the drug pharmacokinetic profiles in humans. The general principle of in-vitro reproduction of pharmacokinetic profiles comes to controlled dilution or concentrating drug solutions being in contact with microorganisms (3,4). Dynamic models were success in studying the effect of combinations of clavulanic acid with amoxycillin (5) or ticarcillin (5,6).

This communication presents data on using the in-vitro dynamic model for validation the optimal ratio of ampicillin, an antibiotic belonging to penicillins, and sulbactam, a ß-lactamase inhibitor, in a combined dosage forms under conditions simulating the pharmacokinetic profiles observed in blood and tissue fluid of humans after intravenous injection and in blood after oral administration of ampicillin and sulbactam in various ratios.

MATERIALS AND METHODS

Drugs

The drugs used in the study were sodium ampicillin (940 µg/mg) and sodium sulbactam (930 µg/mg).

Bacterial Strain

Ampicillin-resistant strain Escherichia coli R 46 (ampicillin MIC = 250 µg/ml) as the test microorganism was used.

Medium

Mueller-Hinton broth (pH 7.2-7.4) supplemented with Mg^{++} and Ca^{++} according to NCCLS standart (1983) was used as the nutrient and diluent medium.

Pharmacokinetic Profiles Simulation

Pharmacokinetics of both the drugs within the therapeutic doses was linear and obeyed the two-compartment pharmacokinetic model (serum levels after intravenous injection) and one-compartment pharmacokinetic model with first-order absorption (tissue fluid and serum levels after intravenous and oral administration respectively) (7,8). In this connection for simulating pharmacokinetic profiles of ampicillin and sulbactam in serum after intravenous administration the dynamic model I was used and for simulating pharmacokinetic profiles in tissue fluid after intravenous injection and in serum after oral use the dynamic model II with subcompartment 0 imitating the administration site was used (9). The average values of the drug pharmacokinetic parameters and respective values of the dynamic model parameters are presented in Table 1.

Dynamic Model I (without subcompartment 0)

When the liquid volume in the main vessel is given the value of V_1 and is equal to it, the amount of the drugs A_1° required for addition to the vessel (vessel I, Figure 1a) to provide the initial maximum concentration C_1° (t=0) is estimated by the equation:

$$A_1^\circ = C_1^\circ V_1 \ . \qquad /1/$$

In this case the profile of the drug concentration changing in vessel I as a result of the drug solution dilution with fresh nutrient medium supplied by pump P

Table 1. Pharmacokinetic Parameters of Ampicillin and Sulbactam and Corresponding Dynamic Model Parameters

Parameters	Oral Administration Serum	Intravenous Injection Serum	Intravenous Injection Tissue Fluid
k_a (min^{-1})	0.0414	-	0.0855
F (ml/min)	0.115	-	0.077
V_o (ml)	2.77	-	0.9
k_{el} (min^{-1})	0.0115	-	0.0077
F (ml/min)	0.115	-	0.077
V_1 (ml)	10.0	-	10.0
α' (min^{-1})	-	0.0345	-
$F_{\alpha'}$ (ml/min)	-	0.345	-
V_1 (ml)	-	10.0	-
ß (min^{-1})	-	0.0115	-
$F_ß$ (ml/min)	-	0.115	-
V_1 (ml)	-	10.0	-
T_o (min)	17	-	-
T_{max} (min)	60	0	30

Abbreviations: k_a - first-order absorption rate constant,

F - flow rate,

V_o - subcompartment 0 (vessel 0) volume,

k_{el} - elimination rate constant,

V_1 - main compartment (vessel I) volume,

α' - apparent exponent value described the first region of the drug concentration-time curve,

ß - exponent described the terminal region of the drug concentration-time curve,

$F_{\alpha'}$ and $F_ß$ - corresponding values of flow rates,

T_o - lag-time reflected time shift between administration of a drug and its appearance in sampling compartment,

T_{max} - time at which the maximal concentration is achieved.

at the flow rate F is described by the equation:

$$C_1(t) = C_1^{\circ} e^{-(F/V_1)t} . \qquad /2/$$

The role of the elimination rate constant in equation /2/ is defined by the ratio of the dynamic model parameters F and V_1. To simplify simulation of the complicated biexponential profile formalized by the pharmacokinetic two-compartment model (serum levels of ampicillin and sulbactam after single intravenous injection) the principle of piecewise-linear approximation of the pharmacokinetic profile (10) was applied and the biexponential profile was divided into two fragments. Each of these two fragments was described by the monoexponent.

The flow rate during the "α-phase" ($F_{\alpha'}$ within the first 45 minutes) was evaluated by the equation:

$$F_{\alpha'} = \alpha' V_1 , \qquad /3/$$

where α' is apparent exponent value described the first region of the drug concentration-time curve ("α-phase") and during the "ß-phase" (F_{β} for the terminal region of the drug concentration-time curve) by the equation:

$$F_{\beta} = \beta V_1 , \qquad /4/$$

where ß is the least exponent of the pharmacokinetic equation.

Since the values of parameters α' and ß of ampicillin and sulbactam are close, simultaneous simulation of the drug required profiles was achieved at similar flow rates. At the same time the initial concentrations of ampicillin and sulbactam in all the cases were different because of differences in distribution volumes and bioavailability of the drugs.

Dynamic Model II (with subcompartment 0)

For simulating the pharmacokinetic profiles described by the pharmacokinetic one-compartment model with first-order absorption (pharmacokinetic profiles of ampicillin and sulbactam in tissue fluid after intravenous injection and in serum after oral use), the drugs were added not to vessel I but to subcompartment 0 (vessel 0, Figure 1b). In this case changing of the drug levels in vessel I was described by the equation:

$$C_1(t) = B\,(e^{-(F/V_1)t} - e^{-(F/V_o)t}), \qquad /5/$$

where

$$B = \frac{A_o^\circ (F/V_o)}{V_1(F/V_o - F/V_1)} = \frac{A_o^\circ}{V_1 - V_o} \qquad /6/$$

is the preexponential factor.

By analogy with the previous model the part of the absorption constant k_a was that of the drug ratio F/V_o and the part of the elimination rate constant k_{el} was that of F/V_1.

When the liquid volume in vessel I is given the value of V_1 and is equal to it, the flow rate F of pump P is defined by the relationship:

$$F = k_{el}V_1 \qquad /7/$$

and the vessel volume V_o (subcompartment 0) is measured by the equation:

$$V_o = F/k_a \,. \qquad /8/$$

According to equation /6/ the amount A_o° required for addition to vessel 0 can be calculated by the relationship:

$$A_o^\circ = B(V_1 - V_o) \qquad /9/$$

The pharmacokinetic parameters of ampicillin and sulbactam and the corresponding parameters of the dynamic models are presented in Table 1.

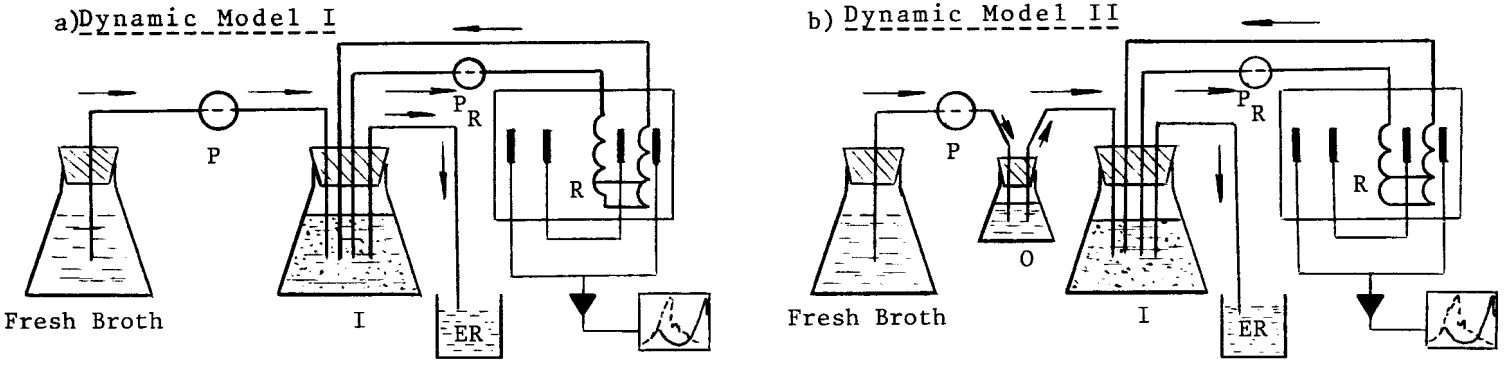

Figure 1. Design of the In-Vitro Dynamic Models.

I - Main compartment (vessel I),

O - Subcompartment (vessel O),

P, P_R - Peristaltic pumps,

R - Registration unit (microcalorimeter),

ER - Elimination reservoir.

The arrows indicate flow directions.

Antimicrobial Effect Registration

After filling the dynamic model with sterile broth and incubating it at 37°C a drop of 18-hour culture was added to vessel I. The beginning of the logarithmic growth phase was detected by the heat production rate (dQ/dt). When the rate reached 62 μW/ml ($1.9 \cdot 10^6$ CFU/ml for Escherichia coli R 46) the drugs were added to vessel I (or vessel 0) and the peristaltic pump P (Minipuls 2, Gilson) was turned on to provide the flow rate F and to maintain the conditions of the drugs level changing in vessel I. An additional pump supplied the medium to the microcalorimeter from vessel I. The microbial count was determined with the BioActivity Monitor LKB 2277-202 operating in the flow mode at 37°C in the registration range of 0-300 μW. The bacteria containing medium was supplied from vessel I at a rate of 20 ml/h with pump P_R (Microperpex 2132, LKB). The effective volume of the registration cylinder was 0.55 ml. The calibration procedure and operation sequence are described in detail elsewhere (11). Since the heat output rate dQ/dt versus CFU/ml dependence is linear (over the range of 10^4-10^8 CFU/ml) it is possible to calculate CFU/ml from dQ/dt (12,13).

The experimental values of dQ/dt were corrected for the dilution factor taking account of the simultaneous cell washing out during changing of the drug concentrations (14).

RESULTS AND DISCUSSION

To determine the optimal ampicillin/sulbactam ratios in the combined dosage forms, the dose of sulbactam was varied from 0.125 to 1.0 g while the dose of ampicillin remained unchaged at the level of the thera-

peutic one (0.5 g). The ratios of the drug doses and respective values of the drug maximal concentrations in the zones of the imitated infectious process are presents in Table 2.

The Table shows that the ratios of the ampicillin and sulbactam maximal concentrations in biological fluids did not correspond to the ratio provided by the used doses of the drugs. Thus, with using the equivalent doses of ampicillin and sulbactam the ratios of the maximal concentrations after intravenous injection were equal to 1:1.5 for serum and 1:2 for tissue fluid. After the drug oral administration the ratio was 1:1.25 for serum. This peculiarities reflecting differences in bioavailability of ampicillin and sulbactam were taken into account in simulation of the drug pharmacokinetics in the in-vitro dynamic models. The modeled profiles of changes in the ampicillin and sulbactam concentrations in the biological fluids at varying ratios of the dosage form components and the administration routes are presented in Figure 2.

Figure 3 presents thermograms for the time course of the count of the bacterial strain in the dynamic models in the absence and presence of ampicillin/sulbactam combinations. As Figure 3 shows, the drugs decreases the heat output rate as a result of microbial growth inhibition and kill while in the absence of the drugs the rate grows steadily. When the drug levels in the dynamic model decreases the heat output rate begins to increase again thus reflecting the resumption of cell proliferation. It is to be noted that the regrowth curves and those of microbial growth in the absence of ampicillin and sulbactam show similar behavior. This means that microbial generation times remain unchanged after incubation in the drug combination containing medium.

Table 2. Ratios of the Doses (D) and the Maximal Concentrations (C_{max}) of Ampicillin (Amp) and Sulbactam (Sulb) for Intravenous and Oral Administration

Drugs	D, g	D_{Amp}/D_{Sulb}	C_{max}, μg/ml	$C_{maxAmp}/C_{maxSulb}$	B*, μg/ml
Oral administration, Serum					
Amp	0.5	-	8.0	-	19.12
Sulb	0.125	4:1	2.5	3.2:1	5.98
Sulb	0.25	2:1	5.0	1.6:1	11.95
Sulb	0.5	1:1	10.0	1:1.25	23.90
Sulb	1.0	1:2	20.0	1:2.5	47.80
Intravenous Injection, Serum					
Amp	0.5	-	24.0	-	-
Sulb	0.125	4:1	9.0	2.7:1	-
Sulb	0.25	2:1	18.0	1.5:1	-
Sulb	0.5	1:1	36.0	1:1.5	-
Sulb	1.0	1:2	72.0	1:3	-
Intravenous Injection, Tissue Fluid					
Amp	0.5	-	10.0	-	13.90
Sulb	0.125	4:1	5.0	2:1	6.95
Sulb	0.25	2:1	10.0	1:1	13.90
Sulb	0.5	1:1	20.0	1:2	27.80
Sulb	1.0	1:2	40.0	1:4	55.60

* - preexponential factor, see equation /6/.

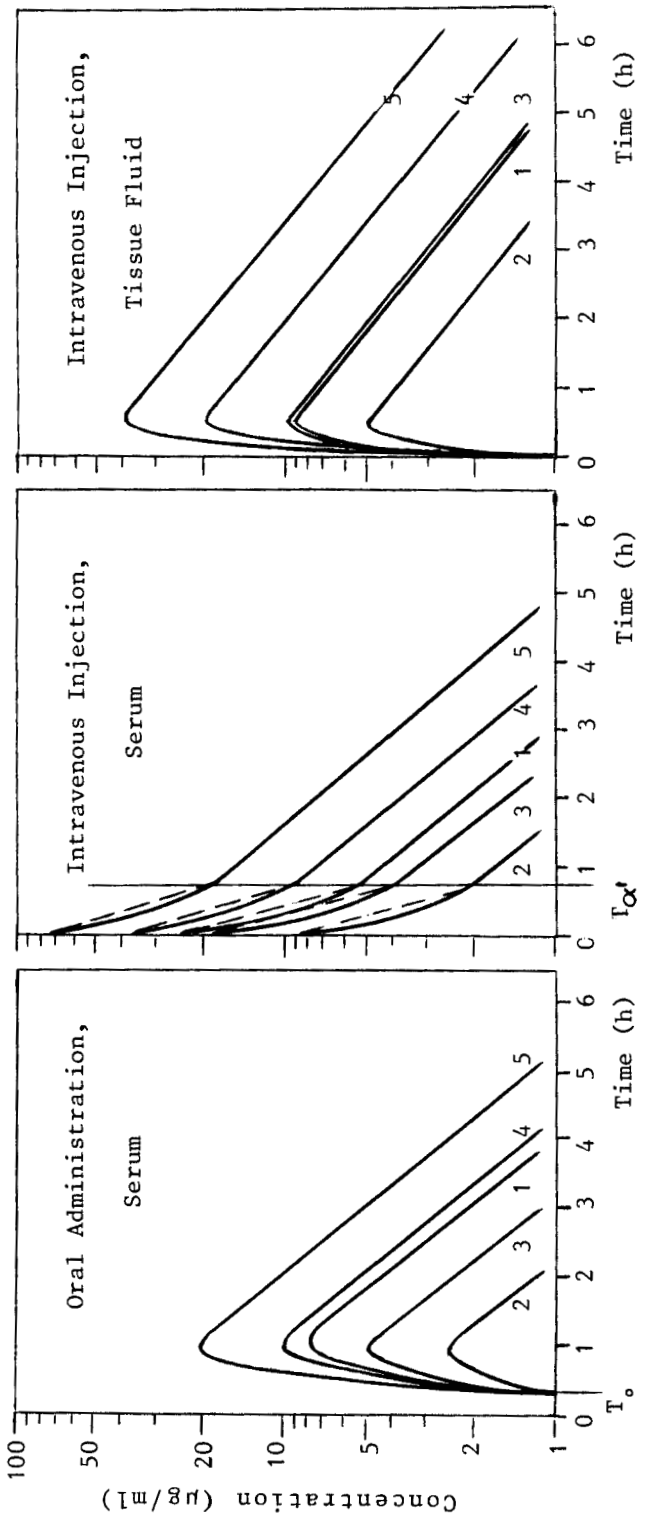

Figure 2. Simulated Pharmacokinetic Profiles of Ampicillin and Sulbactam. 1 - 0.5 g of Ampicillin, 2 - 0.125 g of Sulbactam, 3 - 0.25 g of Sulbactam, 4 - 0.5 g of Sulbactam, 5 - 1.0 g of Sulbactam. T_0 - lag-time reflected time shift between administration of a drug and its appearance in sampling compartment, $T_{\alpha'}$ - duration of "α-phase".

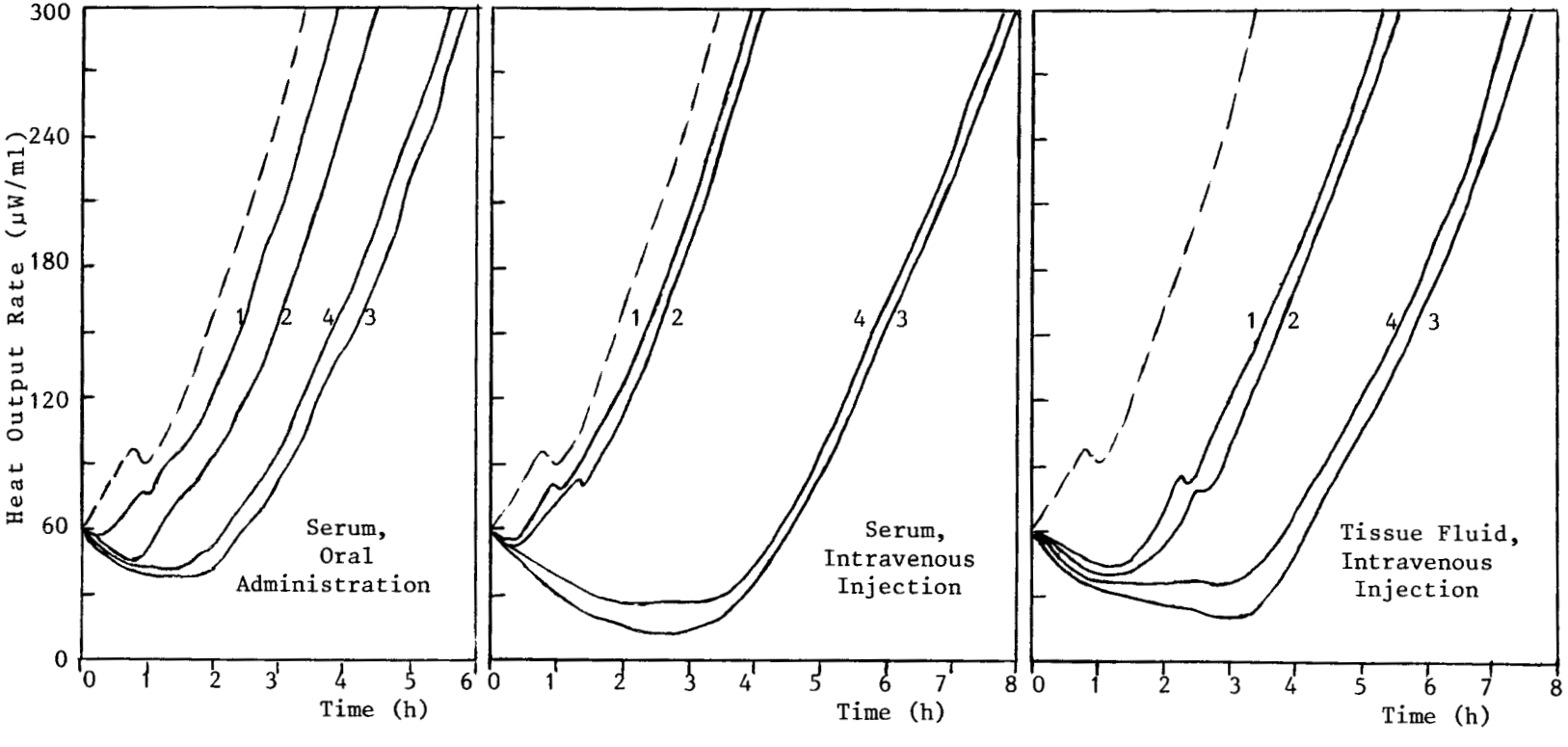

Figure 3. Thermograms of Escherichia coli R 46 During Simulation of Different Ampicillin/Sulbactam Pharmacokinetic Profiles. 1 - 0.5 g of Ampicillin plus 0.125 g of Sulbactam, 2 - 0.5 g of Ampicillin plus 0.25 g of Sulbactam, 3 - 0.5 g of Ampicillin plus 0.5 g of Sulbactam, 4 - 0.5 g of Ampicillin plus 1.0 g of Sulbactam. Dashed lines represent thermograms in the absence of the drugs.

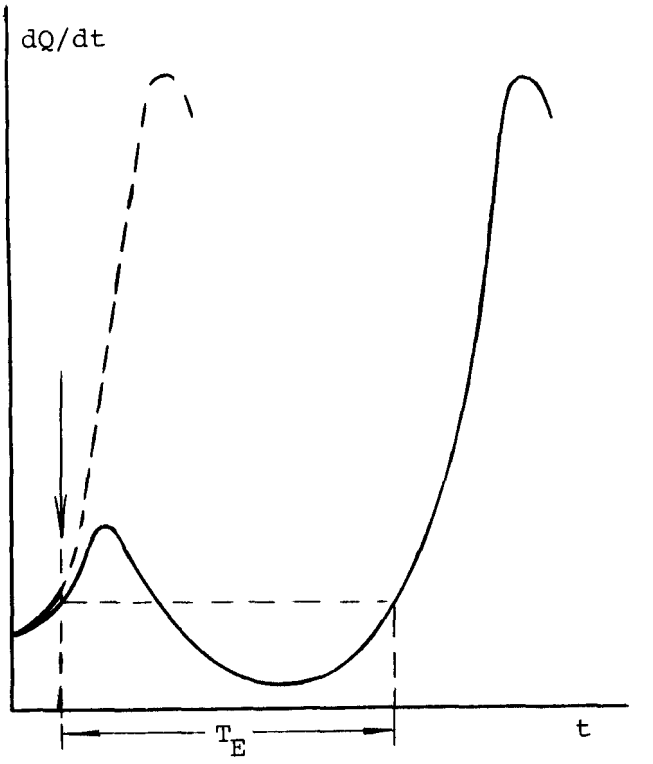

Figure 4. Antimicrobial Effect Quantitation from the Curves for the Time Course of the Heat Output Rate in the Presence of the Drugs (solid line) and in the Control (dashed line). The arrous indicates the moment of drug input. The abscissa and ordinate give time and heat output rate respectively.

Figure 3 shows that time from drug injection till growth resumption generally increases with the increase sulbactam content in combinations, the regrowth curves being shifted to the right. To reveal the ampicillin/ /sulbactam ratio dependence of the antimicrobial effect T_E values were evaluated. This recently developed parameter is defined by the time from the moment of drug administration till the moment when the bacterial

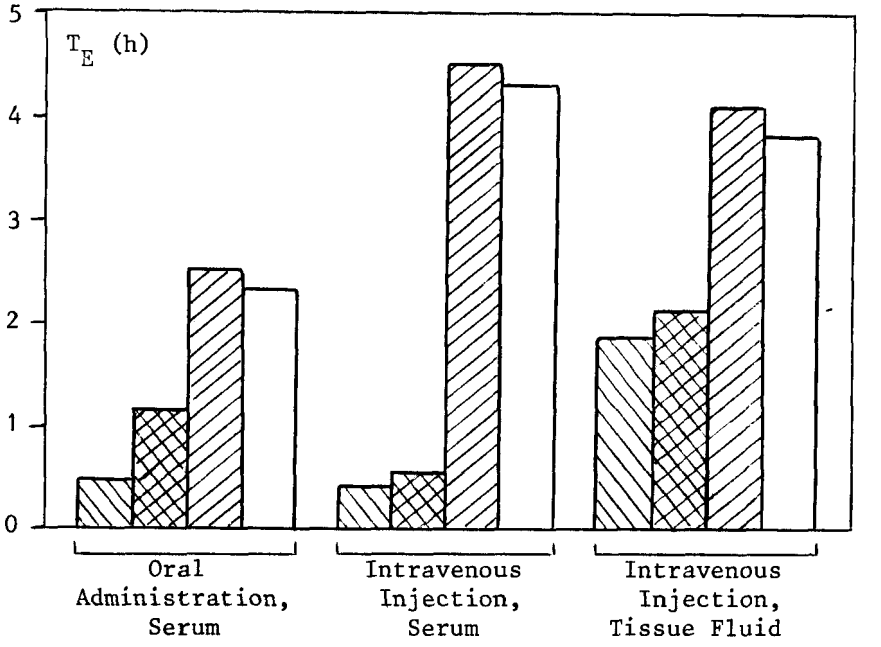

Figure 5. T_E-Values Reflected Antimicrobial Effect of Ampicillin-Sulbactam Combinations on Varying Component Ratio in Dosage Forms.

- 0.5 g of Ampicillin plus 0.125 g of Sulbactam,
- 0.5 g of Ampicillin plus 0.25 g of Sulbactam,
- 0.5 g of Ampicillin plus 0.5 g of Sulbactam,
- 0.5 g of Ampicillin plus 1.0 g of Sulbactam.

count reaches again its initial level (15,16) - see Figure 4.

The T_E values of the different ampicillin/sulbactam ratios obtained by simulating pharmacokinetic profiles in the dynamic models are shown in Figure 5. It is evident from the Figure that the variation of ampicillin/sulbactam ratio in dosage forms from 4:1 to 1:1 was accompanied by an increase in the antimicrobial effect (maximum at the ratio of 1:1). Further incresing

of the sulbactam content in the combination did not lead to increasing of the effect. The antimicrobial effect duration at the ampicillin/sulbactam ratio of 1:1 was the highest after the drug intravenous injection simulation when the infection was localized in serum and tissue fluid i.e. 4.5 and 4 hours respectively. The similarity in the effect level for the cases discussed can be explained by more prolonged preservation of the drugs in tissue fluid as compared to blood (half-lives are equal to 90 and 60 minutes respectively), which compensated the initial more than 2-fold differences in the attained levels of the maximal concentrations in favour of serum. Duration of the antimicrobial effect in simulation of the pharmacokinetic profiles observed after oral use of the ampicillin combination with sulbactam was much lower (T_E equal to 2.5 hours). This was in conformity with lower values of the drug maximal concentrations and its later attaining in comparison to intravenous administration.

The experimental data indicated to possible determining the optimal ratio chemotherapeutics used in combination with an account of the pharmacokinetic factor. In this connection the use of in-vitro dynamic models providing simulation of the required pharmacokinetic profiles and simultaneous estimation of the realized antimicrobial effect is a promising approach to development of modern drug formulations.

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