J.Sci.I.R.Iran

Vol. 2, No. 3, 4
Summer & Autumn, 1991

A MATHEMATICAL MODEL TO DESCRIBE THE SYNERGISTIC EFFECT BETWEEN SULFAMETHOXAZOLE AND TRIMETHOPRIM

E. Forouhi

Faculty of Medicine, Guilan University of Medical Science, Rasht, Islamic Republic of Iran

Abstract

Based on folic acid biosynthetic pathway a mathematical model was made for bacterial growth to describe the synergistic effect between sulfamethoxazole and trimethoprim. The results obtained show a strong synergism at growth inhibiting concentrations of these two drugs. Two cyclic pathways included in the model are responsible for this synergism. The roles of these pathways in producing synergism are discussed.

Introduction

When used together sulfamethoxazole and trimethoprim exhibit a strong synergism. The simultaneous action of these drugs results in an effect far greater than could be expected from the simple addition of individual effects. This is often quoted as the best known example of antimicrobial synergism by sequential inhibition in a common biochemical pathway [1]. Although sulfamethoxazole and trimethoprim act as inhibitors on two steps in the tetrahydrofolate formation pathway, there has been some uncertainty on theoretical grounds in that the sequential inhibition of a linear pathway could produce a truly synergistic effect [2]. Therefore it has been suggested that the observed synergism occurs as a result of the multiple inhibition of dihydrofolate reductase by these two inhibitors [3]. However, the concentration of sulfamethoxazole used in this study was much greater than that needed to produce synergism in experiments with growing organisms. Since there is no evidence for such intracellular concentration of sulfamethoxazole, multiple enzyme inhibition can hardly be considered an alternative to sequential inhibition [4,5]. Other workers pointed to the role of cyclic pathways in producing synergism [2,4]. In this work focussing on the most important steps and proper cyclic pathways in tetrahydrofolate formation a mathematical model is developed for the bacterial growth in the presence of the two inhibitors, in an attempt to cast light on the mechanism of synergism between these two antibacterial agents.

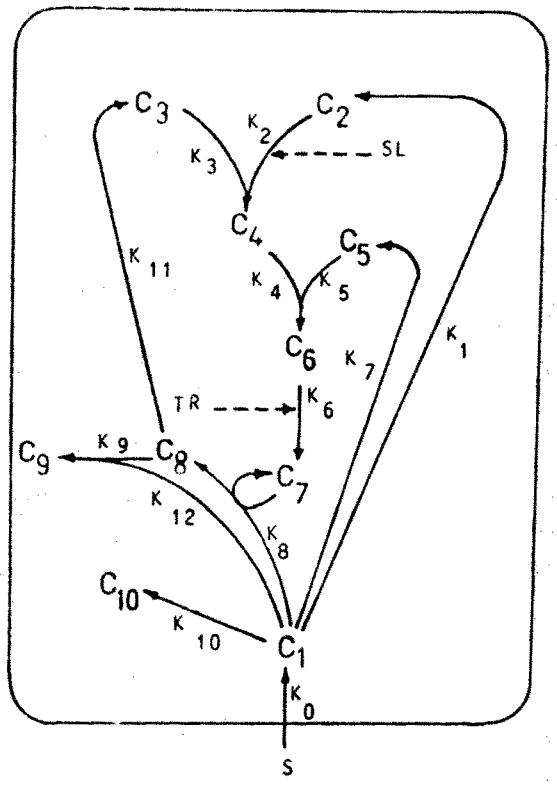
Keywords: Sulfa methoxazole, Trimethoprim

Mathematical model

Fig. 1 shows schematically the structured model based on folate metabolism. Dihydropteroate (C_4) is synthesized from one molecule each of pteridine (C_3) and p-aminobenzoic acid (C_2). The enzyme catalyzing this reaction is dihydropteroate synthetase and sulfamethoxazole (SL) is the competitive inhibitor of this enzyme. Glutamic acid (C_5) is then added to dihydropteroate to form dihydrofolate (C_6). This is reduced by dihydrofolate reductase to tetrahydrofolate. Trimethoprim (TR) is the competitive inhibitor of dihydrofolate reductase.

Tetrahydrofolate is the carrier of single carbon fragments used in biosynthesis of nucleotides including guanosine triphosphate (C_8). Guanosine triphosphate is the precursor in pteridine biosynthesis [6]. Interference with any stage of tetrahydrofolate synthesis will affect the cell's ability to synthesize DNA, RNA (genetic materials (C_9)) and proteins (including enzymes) and

inhibit growth [6]. Glutamate is synthesized from an intermediate of tricarboxylic acid cycle and therefore is produced indirectly from the glucose (C_1) which may be assumed to be the growth limiting substrate (S). P-aminobenzoic acid is obtained from Shikmic acid which is known to be formed from the intermediate of carbohydrate metabolism [7]. Therefore p-aminobenzoic acid may also be assumed to be produced indirectly from the glucose.



C_1=Substrate within the cell (Glucose)
C_2=P-Aminobenzoic acid
C_3=Pteridine
C_4=Dihydropteroate
C_5=L-Glutmate
C_6=Dihydrofolate
C_7=Tetrahydrofolate
C_8=Guanosine triphosphate
C_9=Genetic materials (DNA & RNA)
C_{10}=The other biosynthetic materials
S= Substrate in external environment (Glucose)
SL= Sulfamethoxazole
IR=Trimethoprim

Fig.1. Schematic diagram of a structured model for bacterial growth based on tetrahydrofolate metabolism.

Assuming that the density of cells P_c is time invariant writing a material balance on component i, finally gives result to the following equation (8).

$$\frac{dC_i}{dt} = r_i - \mu C_i \qquad (1)$$

Where C_i and r_i are the intracellular concentration and rate of accumulation of component i, and μ is the specific growth rate of bacteria. r_i is equal to the difference of rates of the reactions producing and consuming the component i . The rate of bimolecular reaction is assumed to be second order and mono-molecular reaction is asumed to follow the first order kinetics. Dihydropteroate synthetase and dihydrofolate reductase are subject to inhibition by sulfamethoxazole and trimethoprim respectively. The concentration of the enzymes catalyzing the reactions is proportional to the concentration of genetic materials. Tetrahydrofolate is actually a cosubstrate. Considering these points and writing equation (1) for each component the model can be constructed. The equations are of the following form.

$$\frac{dC_2}{dt} = K_1.C_1.C_9 - \frac{K2}{1+\frac{SL}{K_s}}.C_2.C_3.C_9 - \mu C_2 \qquad (2)$$

$$\frac{dC_3}{dt} = K_{11}.C_8.C_9 - \frac{K_3}{1+\frac{SL}{K_s}}.C_2.C_3.C_9 - \mu.C_3 \qquad (3)$$

$$\frac{dC_4}{dt} = \frac{K_2+K_3}{1+\frac{SL}{K_s}}.C_2.C_3.C_9 - K_4.C_4.C_5.C_9 - \mu.C_4 \qquad (4)$$

$$\frac{dC_5}{dt} = K_7.C_1.C_9 - K_5.C_4.C_5.C_9 - \mu C_5 \qquad (5)$$

$$\frac{dC_6}{dt} = (K_4+K_5).C_4.C_5.C_9 - \frac{K_6}{1+\frac{TR}{K_t}}.C_6.C_9 - \mu.C_6 \qquad (6)$$

$$\frac{dC_7}{dt} = \frac{K_6}{1+\frac{TR}{K_t}}.C_6.C_9 - \mu C_7 \qquad (7)$$

$$\frac{dC_8}{dt} = K_8.C_1.C_7.C_9 - K_{11}.C_8.C_9 - K_9.C_1.C_8.C_9 - \mu C_8 \qquad (8)$$

$$\frac{dC_9}{dt} = (K_9+K_{12}).C_1.C_8.C_9 - \mu C_9 \qquad (9)$$

$$\frac{dC_{10}}{dt} = K_{10}.C_1.C_9 - \mu C_{10} \quad (10)$$

$$\frac{dC_1}{dt} = \mu.\rho_c - (K_1+K_7+K_{10}+K_8.C_7+K_{12}.C_8)C_1.C_9 - \mu.C_1 \quad (11)$$

$$\frac{dS}{dt} = -\Upsilon.\ \mu.X \quad (12)$$

$$\frac{dX}{dt} = \mu.X - K_d.x \quad (13)$$

$$\mu = \frac{K_o.S.C_9}{\rho_c} \quad (14)$$

Where K_i, i = 0,1,2,.....,12 are rate constants, K_s and K_t are inhibition constants . Υ is reciprocal of yield coefficient and ρ_c is cell density. S and X are the concentrations of substrate and cell respectively. K_d is the death rate constant of bacteria.

The model consists of a set of 12 ordinary differential equations with time, t, as the independent variables and dependent variables as introduced in Fig. 1. Using a microcomputer the Runge-Kutta method was adopted to solve the set of differential equations.

The arbitrary values of the parameters used in the model are listed in Table 1.

Table 1. Parameters values used in the model

$K_0 = 0.15$	$K_6 = 2.0$	$K_{12} = 0.05$
$K_1 = 0.002$	$K_7 = 0.002$	$K_d = 0.01$
$K_2 = 0.5$	$K_8 = 1.8$	$K_s = 1.0$
$K_3 = 0.6$	$K_9 = 0.1$	$K_t = 1.0$
$K_4 = 1.0$	$K_{10} = 0.5$	$\Upsilon = 2.0$
$K_5 = 0.3$	$K_{11} = 0.003$	

As in any numerical attack on a set of differential equations, the solution begins at the known points (starting points) on the curves and uses the equations for the derivatives (equations in the model) at those points to locate the adjacent points. The program for the computation will begin reading the starting value of S,X and C_i, i= 1,2.....10, and the value of h, where h is the time interval between two adjacent points. The computation thereafter is relatively straightforward. The starting values are substituted in the formulas given in the Runge-Kutta method to compute the new values of S,X and C_i, i= 1,2,...10 at the time t+h.

[The new values are printed. The same formulas are then applied to these new values of S,X and C_i, i=1,2...10 .]

The process is continued until as many points on the curves have been computed as desired.

Results and Discussion

Fig. 2 shows the substrate consumption and the growth curve in the absence of antibacterial agents as predicted by the model. As can be seen the model reproduces the batch growth dynamic quite well. The model prediction of growth in the absence and presence of the antibacterial agents is shown in Fig. 3. The growth is postponed when the drugs are present. This is what actually is expected to happen, because sulfamethoxazole and trimethoprim are bacteriostatic agents. Figures 4 to 6 show the concentration of one inhibitor for a specified level of growth at various concentrations of the other inhibitor as predicted by the model. The concave toward axes represent the synergistic effect between two inhibitors. The more concave is the curve, the stronger is the synergism, because much less than half of the inhibitory concentration of each drug is necessary to achieve the same effect. A very strong synergism is seen at high concentration of the two agents.

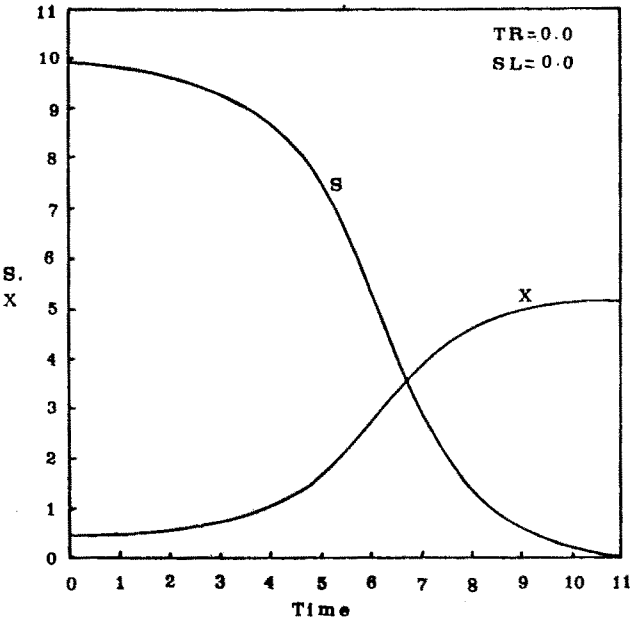


Fig. 2. The substrate consumption and the growth curves in the absence of antibacterial agents as predicted by the model. All the units are arbitrary.

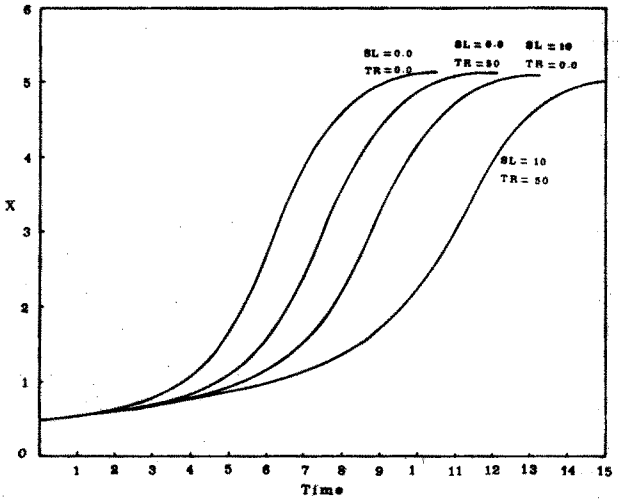


Fig.3. Growth curves in the presence and absence of antibacterial agents predicted by the model. All the units are arbitrary.

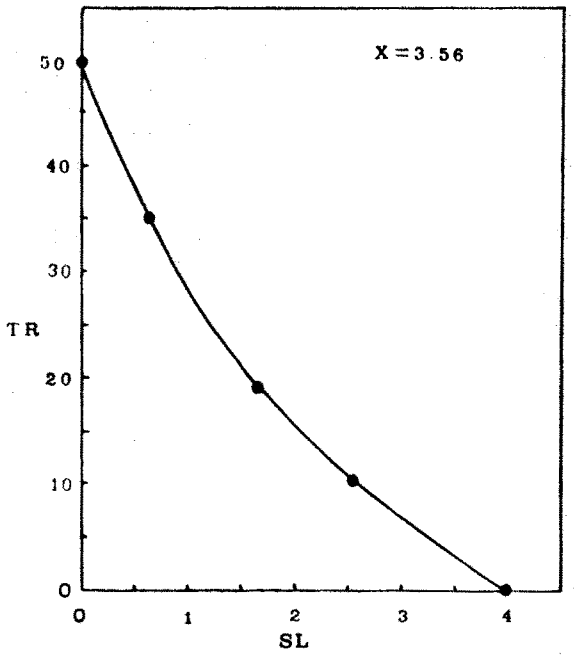


Fig.4. Concentration of trimethoprim (TR) for growth level of 3.59 at various concentrations of sulfamethoxazole (SL).

•The points predicted by the model. All the units are arbitary.

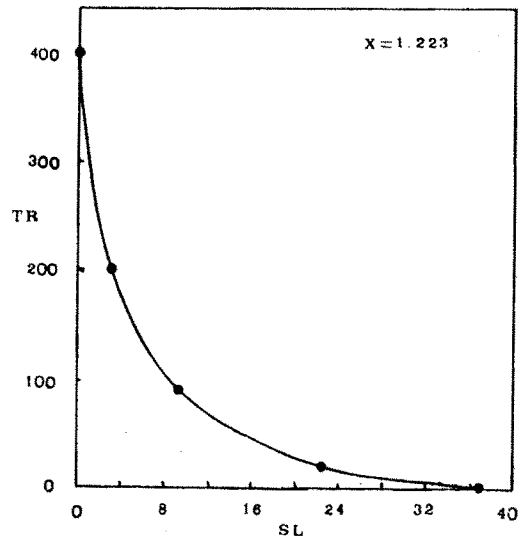


Fig.5. Concentration of trimethoprim (TR) for growth level of 1.223 at various concentrations of sulfamethoxazole (SL).

•The points predicted by the model. All the units are arbitrary.

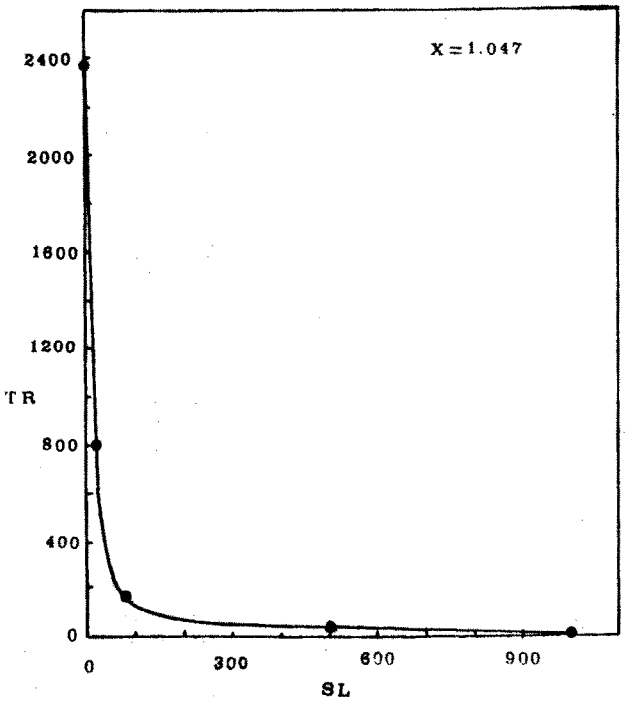


Fig.6. Concentration of trimethoprim (TR) for growth level of 1.047 at various concentrations of sulfamethoxazole (SL).

•The points predicted by the model. All the units are arbitrary.

The uncertainty about the effect of sequential inhibition in producing synergism led one author [3] to propose multiple enzyme inhibition of dihydrofolate reductase by sulfamethoxazole and trimethoprim. However, as has been noted by other workers [4,5] dihydrofolate reductase inhibition by sulfamethoxazole can not be of significance since bilogical effects have been noted at much less concentration of sulfamethoxazole than has been used in the study [3] of enzyme inhibition. To support the sequential inhibition mechanism the idea of involvement of some cyclic pathway has been put forward [3]. According to this idea, dihydrofolate reductase is acting in a cycle in which its

end product, tetrahydrofolate, is recycled to dihydrofolate, its substrate, through conversion to thymine by thymidylate synthetase. It has been claimed that sequential inhibition in such a system should be capable of producing synergism if the sulfamethoxazole reduces the quantity of intermediates available. But this may not be so, because sulfamethoxazole is acting outside the cycle in which trimethoprim is effective and this sort of cycle does not seem to be what was actually meant by Webb [2] who gives tricarboxylic acid cycle as an example in which the synergistic effect between two inhibitors, both acting in the cycle, may be observed. But there is an effective cycle in tetrahydrofolate metabolism. The end product of dihydrofolate reductase reaction, i.e. tetrahydrofolate is a coenzyme in production of guanosine triphosphate which is a precursor of pteridine biosynthesis. Pteridine itself is one of the substrate of hydropteroate synthetase which is subject to inhibition by sulfamethoxazole. Therefore, through this cycle the effect of trimethoprim is finally reflected in the reaction inhibited by sulfamethoxazole. (Fig.1.)

There is another cycle which is equally important. As has been mentioned above, any disturbance in tetrahydrofolate synthesis will affect the cell's ability to synthesize DNA, RNA and proteins including the enzyme of tetrahydrofolate biosynthetic pathway themselves. This cycle has been included in the model and is partly responsible for the synergism shown by the model. No mention of the roles these pathways have in producing synergism has been made by other workers.

Conclusion

The model presented shows a strong synergism between sulfamethoxazole and trimthoprim at growth inhibiting concentrations. There are two cyclic pathways which play important roles in producing this synergism. Through these cyclic pathways, folic acid affects the level of a substrate and the enzymes of its own biosynthetic pathway.

Acknowledgement

Financial support for this project from Guilan University of Medical Sciences is gratefully acknowledged.

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