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Research Article

Stochastic Process Pharmacodynamics: Dose Timing in Neonatal Gentamicin Therapy as an Example

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ABSTRACT. We consider dosing regimens designed to cure patients by eradicating colony forming units (CFU) such as bacteria. In the field of "population" pharmaco-kinetics/dynamics (PK/PD), inter-individual variability (IIV) of patients is estimated using model parameter statistical distributions. We consider a more probabilistic approach to IIV called stochastic process theory, motivated by the fact that tumor treatment planning uses both approaches. Stochastic process PD can supply additional insights and suggest different dosing regimens due to its emphasis on the probability of complete CFU eradication and its predictions on "pure chance" fluctuations of CFU number per patient when treatment has reduced this integer to less than ~100. To exemplify the contrast between stochastic process PD models and standard deterministic PD models, which track only *average* CFU number, we analyze, neglecting immune responses, neonatal intravenous gentamicin dosing regimens directed against *Escherichia coli*. Our stochastic calculations predict that the first dose is crucial for CFU eradication. For example, a single 6 mg/kg dose is predicted to have a higher eradication probability than four daily 4 mg/kg doses. We conclude: (1) neonatal gentamicin dosing regimens with larger first doses but smaller total doses deserve investigation; (2) in general, if standard PK/PD models predict average CFU number drops substantially below 100, the models should be modified to incorporate stochastic effects more accurately, and will then usually make more favorable, or less unfavorable, predictions for front boosting ("hit hard early"). Various caveats against over-interpreting the calculations are given.

KEY WORDS: anti-bacterial dosing regimens; front boosting; PK/PD eradication probability; small-number stochastic fluctuations; stochastic birth-death cell population dynamics.

INTRODUCTION

Scope

Many medical treatments aim to cure a disease by eradicating, with or without the aid of the patient's immune system, cells, such as bacteria, that are colony forming units (CFU), rather than aiming to control the disease by indefinitely prolonged therapy. This paper gives examples where mathematical modeling predicts that random fluctuations in CFU number per patient are important during protracted anti-bacterial drug treatments aiming to cure immunocompromised patients.

Therapies often use dosing regimens where the total dose is split into fractions separated by extended time intervals. Figure 1 shows a schematic cycle of 9 daily fractions, with the first dose larger than the others ("boosted"). In our specific examples the first several days turned out to be the most important period.

PK/PD Modeling

Most PK/PD models of antibiotic action [reviewed, e.g., in (1–3)] are *deterministic*: the main equations concern *averages*, e.g., the average number of CFU such as bacteria. Time dependent averages are typically computed by integrating coupled nonlinear first order ordinary differential equations [reviewed, e.g., in (4)]. Inter-individual variability (IIV) is usually addressed by assuming that some of the parameters in the differential equations are described by appropriate statistical distributions for a patient population [reviewed in (5)].

PK/PD modeling has analyzed gentamicin treatment directed against neonatal *Escherichia coli*. Neonates are immunocompromised (6) and *E. coli* has become the leading cause of U.S. fatalities due to early-onset neonatal sepsis; one population-based estimate (7) is that in 2005–2008 yearly incidence was about 840 cases (95% CI 710–980), with mortality of 210 (150–290) corresponding to ~0.05 deaths per 1,000 live births; premature neonates had significantly higher risks.

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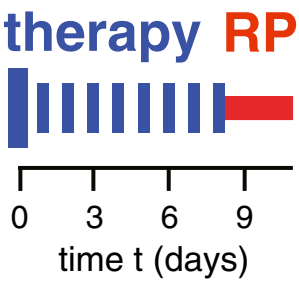

Fig. 1. Intermittent dosing. Shown is a hypothetical cycle of 9 dose fractions, one every 24 h. The first dose fraction is boosted. During a recovery period (*RP*), the drug clears and short-term patient toxicity effects ameliorate

One PK/PD approach to modeling gentamicin treatment of *E. coli* in neonates is given in (8,9). The approach takes into account drug-induced "adaptive resistance," which is reversible within days [reviewed in (4,10)]; it does not consider irreversible resistance caused, e.g., by bacterial mutations (11,12). It predicts standard intravenous injection dosing regimens may sometimes drive the average CFU number per patient to <1. This prediction indicates that corrections should be made to take into account random small-number fluctuations (13), as studied in the theory of stochastic processes [also called random processes; reviewed, e.g., in (14,15)].

Tumor treatment planning, especially in the case of radiotherapy, uses many different stochastic process calculations, relevant on different time scales, from femtoseconds to years, as reviewed in (16–19) and in the online AAPS Supplement section S.4.1. The influence on IIV of stochastic fluctuations at the single cell level is considered (16,18); an approach similar to the statistical methods of population PK/PD is also used to analyze IIV [reviewed, e.g., in (20)].

Preview

Stochastic process calculations of tumor control probability by radiotherapeutic eradication of cancer cells [reviewed in (16,17,19)] suggested to us analogous analyses of drug dosing regimens: the main novelty in the present paper is transferring a mathematical stochastic process theory technique from radiobiological tumor treatment planning to a PK/PD model of neonatal gentamicin treatment. Differences due to the fact that drugs remain in the body during a period of hours or more had to be taken into account, as did the fact that typically a substantial fraction of bacteria are CFU whereas only a small minority of tumor cells are CFU.

Some of our results suggest using large first doses. However our approach is preliminary and has a number of limitations. For example, patient immune system action is neglected. This and other important limitations are itemized at length in the "Discussion" section.

In the stochastic-process approach to PD introduced here some IIV is attributed to "pure chance" or, as is almost equivalent in practice, to biological differences below the limit of resolution of currently feasible observations. Despite the many powerful new methods that are becoming available for observing both individual patient and population characteristics (21,22) there are still PK/PD scenarios where probabilistic calculations are indicated. A conceptual example of at present essentially unobservable differences would be the difference between failure *vs.* success of binary fission for one specific bacterium in a given patient, whose analysis involves considering fission failure probability, i.e., using a stochastic model. In discussing systems PD modeling [reviewed, e.g., in (23)], it was pointed out that "A notable success … has been the recognition that noise plays an important role in … creating cell-to-cell variability" (24). In situations close to cure, where there are only a few CFU in a patient, such stochastic cell-to-cell variability can presumably sometimes lead to much bigger differences and IIV, such as cure *vs.* relapse.

MATHEMATICAL AND COMPUTATIONAL METHODS

Deterministic and Stochastic Neonatal Gentamicin Pk/Pd Modeling

Our calculations extend two papers (8,9) on PK/PD modeling of neonatal gentamicin treatments. Figure 2 is a condensed summary. Complete details on all the calculations are given in the online AAPS Supplement, section S3. In Fig. 2, and throughout, we often use the notation of the two published papers.

The papers considered four gestation ages (GA). We shall give results for a highly premature neonate with GA=25 weeks and a full term one with GA=40 weeks. Many different dosing regimens, with individual doses given as 5-min infusions, will be considered; however, in view of both current thinking (4) and results in the present paper, regimens involving inter-dose intervals of at least 24 h are emphasized.

We always assume treatment starts at birth, taken to be time t=0. For parameters, we use the central values from (9) and from Table III, "combined data," in (8). Using central values gives the predicted individualized treatment response of a hypothetical "typical" patient, not the predicted treatment response of a heterogeneous population. For the purposes of our exploratory analysis of the difference between deterministic PD and stochastic process PD, this simplification is arguably the most informative approach.

As usual, the minimum inhibitory concentration (MIC) is taken to be 2 mg/L (9). Though not directly relevant to the question of how deterministic and stochastic estimates differ, toxicity will be discussed below by the method [reviewed in (4,25)] of comparing the (deterministically calculated) *in vivo* free drug concentration in the central compartment to MIC.

The rest of this Methods section describes: (a) a CFU eradication probability function that will be emphasized

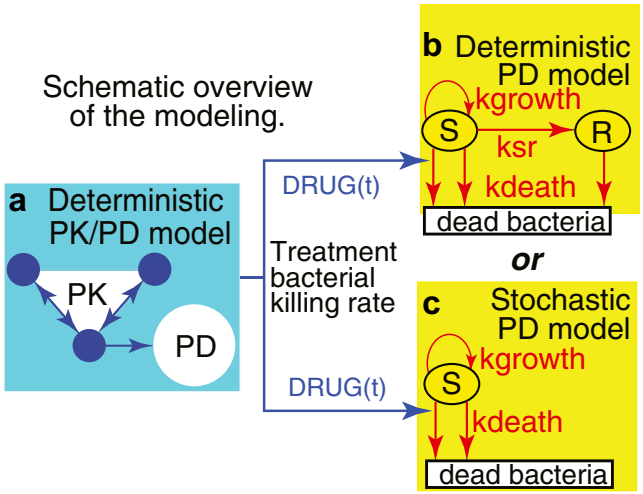

Fig. 2. Abbreviated synopsis of the modeling. **a** As input for deterministic or stochastic PD models of CFU growth and death one needs a rate for the killing of cycling bacteria by gentamicin. This rate, the function DRUG(t), was estimated by using published PK/PD mathematical models, consisting of a standard 3-compartment PK model (*small circles*), and then a deterministic PD calculation (*large circle*) accounting for the fact that CFU develop reversible, transient, "adaptive" gentamicin drug resistance in response to dosing. DRUG(t) is a non-linear function of the free drug concentration calculated from the PK model. **b** Additional PD calculations are needed to estimate, using the published deterministic model, the total number, $s(t)$, of bacteria per patient in the compartment S for cycling CFU, and the number $r(t)$ of possible "resting" (quiescent) bacteria in compartment R, which is initially empty, i.e., $r(0)=0$. *Arrows* indicate bacterial cell population dynamical processes and their labels are transition rates. For example, ksr, which is time and density dependent, is the rate at which cycling bacteria become quiescent. According to the model, ksr becomes substantial only if the total number of bacteria grows to about 100 times the number present at birth; until then the decrease of cycling bacteria due to onset of quiescence is predicted to be negligible. **c** The present paper emphasizes a stochastic mathematical model of the eradication probability $E(t)$ that $s(t)=0$. In the stochastic calculation: (i) The number s is considered to jump from integer to integer as time increases. For reasons explained in the "Results" section, it is possible to use a simplified model which has no R compartment. (ii) Jump probabilities are determined by rates: for treatment killing (*downward arrow at left*) with per-cell jump probability DRUG(t) h^{-1}; for background killing (*other downward arrow*) with per-cell jump probability kdeath h^{-1}; and for bacterial fission with per-cell jump probability kgrowth h^{-1}. (iii) $E(t)$ is the probability that at some time between 0 and t, s has jumped from 1 to 0, whereupon, according to the model, it remains 0. Thus, $E(t)$ always increases as time increases. None of the calculations attempt to take into account irreversible gentamicin resistance or neonate immune system assistance to gentamicin treatment

throughout, (b) the stochastic process methods used, and (c) a description of how open-source computational PK/PD modeling is here implemented.

Eradication Probability

In Fig. 2b, c, there are no arrows into compartment S for bacterial CFU other than the arrow which also emanates from S. Correspondingly the models predict that if the number $s(t)$ of CFU in S ever becomes zero, it remains at zero (eradication). The initial value at $t=0$ is estimated from published data (9) at $s(0)\sim10^9$ CFU per neonate, with the specific value depending on weight at birth.

The deterministic PD calculation Fig. 2b predicts only the average of $s(t)$ and predicts that $s(t)=0$ never occurs. Thus the calculation does not directly predict eradication probabilities. For example if the calculated average of $s(t)$ is 2, that might mean all patients harbor two CFU so no eradication has [yet] occurred; but it might alternatively mean that in 99% of the patients eradication has occurred and the remaining "unlucky" 1% harbor 200 bacteria each. In such situations an estimate of the eradication probability as exp(-average), where average is 2 in our example, is sometimes used. However, seminal papers by S. Tucker and coworkers on tumor treatment planning, e.g., (26), emphasized that this estimate implicitly assumes Poisson statistics, which can be quite inaccurate in situations where CFU birth and death rates are large and time dependent. Moreover the estimate refers to a single time rather than being a gradually increasing eradication probability; for example if the average is evaluated at the bottom of an $s(t)$ trough, it seems unrealistic to ignore the question of how long the CFU population lingers at or near that trough. Subsequent analyses [reviewed in (19)] have reinforced these objections.

In general, an advantage of stochastic models over deterministic models is that the former give systematic numerical estimates of eradication probabilities (27). Using the model summarized in Fig. 2c and given in full in the online AAPS supplement section S3, we calculated an eradication probability $E(t)$ that $s(t)=0$ for various dosing regimens.

Independent-CFU: Kendall's Stochastic Model

Review of Deterministic Methods and Results for Independent-CFU Dynamics

In Fig. 2c, the CFU in S undergo independent (also sometimes called "non-interacting" or "exponential") population dynamics. Stochastic exponential growth/decay models generalize deterministic ones, so we start by reviewing the latter. Denote average CFU number by $m(t)$; thus, $m(t)$ is analogous to $s(t)$ in Fig. 2b. The deterministic equation of exponential population dynamics (growth or decay) is $m(t)=m(0)\exp[\mu t]$, where $m(0)>0$. Here μ is a constant, called the Malthusian parameter and interpreted as the difference between per-CFU birth rate and per-CFU death rate (both always assumed non-negative in the present paper), with μ thus positive or negative according to whether birth or death rate is larger.

More generally, the Malthusian rate could be a function $\mu(t)$ of the time, driven, e.g., by time dependent drug

concentrations. Then the relevant ordinary differential equation and its solution are the following:

$$(\mathrm{A})\ \ \mathrm{dm/dt} = \mu(t)m \Rightarrow (B)\ \ m(t) = m(0)\exp\left[\int_0^t \mu(\tau)d\tau\right] > 0. \quad (1)$$

We are mainly interested in situations where, due to a large death rate caused by drug treatment, $\mu(\tau) \ll 0$ for most times τ. Then the integral in Eq. 1 is negative, which implies CFU population decrease, i.e., $m(t) \ll m(0)$.

An essential assumption implicit in Eq. (1) is that different CFU are independent of each other. In our case, the assumption is reasonable because we are considering scenarios where the CFU density is so small stochastic small-number fluctuations are important. Formally speaking, with $\mu(t)$ independent of $m(t)$ the differential equation (1A) is linear; knowing the time dependence for a single CFU initially, i.e., knowing the solution with $m(0)=1$, determines the time dependence of the average for any initial value $m(0)$ simply by multiplication. In contrast, inter-CFU interactions at high densities are described by nonlinear equations. A simple example is the logistic differential equation for sigmoidal growth (28), where the solution for $m(0) \gg 1$ is qualitatively different from, rather than just a multiplicative rescaling of, the solution for $m(0)=1$.

Stochastic Process Methods

The stochastic process model corresponding to Eq. (1) is one of only a few that can be analyzed with explicit analytic equations, rather than requiring extensive Monte-Carlo simulations. It is a birth-death stochastic process model for a population of independent identically distributed cells, first investigated in detail by Kendall (29). The per-cell rates of the model can be time-dependent but must be state independent; the full definition of the model, and its mathematical properties, are given in many standard texts [e.g., as example 4.8 by Tan (15)] and are reviewed in our online AAPS Supplement, section S2. In contrast to the deterministic model in Eq. (1), the stochastic counterpart model requires knowing the birth and death rates $b(t)$ and $d(t)$ individually, not merely their difference $\mu(t)=b(t)-d(t)$; the (per-CFU) birth rate $b(t)$ is interpreted intuitively (14) by saying that the chance that 1 CFU divides into 2 CFU within a very short time interval dt centered at t is $b(t)dt$; a corresponding interpretation holds for the death rate $d(t)$.

Sections S2 and S4 of the online AAPS Supplement derive the equations needed to calculate the eradication probability $E(t)$ of this stochastic model; they include intuitive comments about how stochastics work in this comparatively simple case. The overall conclusion there is that stochastic predictions, relative to deterministic ones, tend to favor front boosting over back boosting.

Computer Modeling

Technicalities of interest primarily to other computational/mathematical modelers are relegated to the supplement. However mathematical and computational PK/PD papers should, in our opinion, contain enough information that any reader with a moderate knowledge of modern computational methods can duplicate every one of the results. We have therefore included in section 3 of the online AAPS Supplement copies of, and links to, our customized software, written in the fully open-source computer language R.

RESULTS

Preview

Except where explicitly stated to the contrary, all dosing regimens discussed have at least 24 h between doses. We first calculated the time-dependence of average CFU number using deterministic PK/PD models for a 10-day period. The results suggested focusing on the first 85 h when comparing stochastic and deterministic PD models. Carrying out the comparison gave numerical estimates of the eradication probability $E(t)$. The results suggested that, in a stochastic calculation that neglects irreversible resistance and immune system action, a large first dose, and CFU population dynamics during the first 36 h, are crucial for the predicted eradication probability: $E(t)$ increasing to >0.5 was calculated to occur quite early or not at all. Finally, a statistically highly significant ($p \ll 0.001$) correlation was found between the final eradication probability and a simpler index obtained directly from the dosing regimen without invoking any models or adjustable parameters.

Deterministic PK/PD Predictions for the First 10 Days

Figure 3, obtained by using the deterministic PK/PD model outlined in Fig. 2a, b for one of the dosing cycles analyzed in (9), indicated that, for this specific dosing regimen: (a) we should simplify by focusing on the first 85 h of treatment since predicted average total CFU number at 85 h becomes and remains so high that stochastic small-number corrections to the deterministic model are no longer needed; and (b) when using a stochastic calculation to find $E(t)$ the simplified independent-CFU (i.e., ksr=0) model of Fig. 2c is adequate, because $E(t)$ grows significantly only at early times when the total CFU number in the central compartment is estimated to be many logs smaller than the number which would lead to substantial deviations from a ksr=0 model.

The First Few Days

Figure 4 shows, for the first 85 h of one dosing regimen chosen to be reasonably typical, results (panels

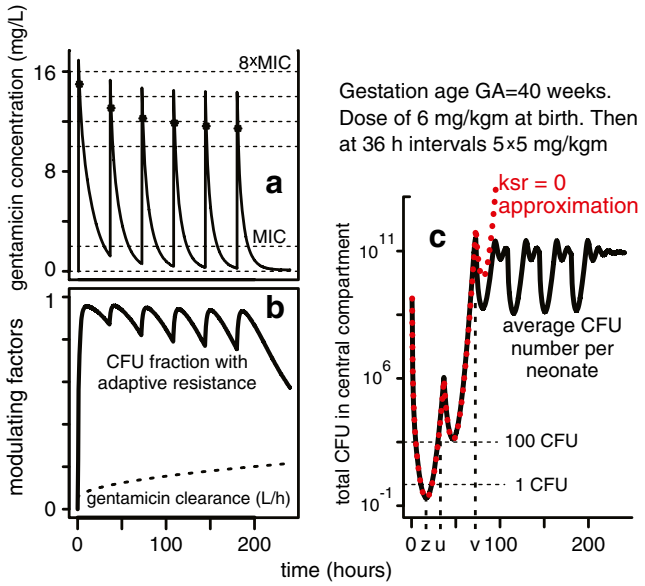

Fig. 3. Deterministic PK/PD estimates for a 10 day period: an example. **a** As in the references, *asterisks* denote calculated concentrations 1 h after each dose (simulated as a 5-min infusion). For the dosing regimen in this example, peak and trough concentrations are predicted to decrease. **b** Most bacteria are predicted to acquire reversible adaptive drug resistance within 10 h after the first dose. In addition, the drug clearance gradually increases during the 10-day period. Both factors tend to make later doses less effective than early ones. **c** This panel illustrates some key properties. First, deterministic PK/PD models sometimes predict that the average total CFU number in S becomes less than 1. Here, the minimum (at time z) is only about 0.255 CFU per patient. Second, we here have u<36 h for the time u where the predicted CFU number is less than 100 for the last time. Third, a simplified model, in which the rate ksr=0, is very nearly exact until a time greater than u when the predicted CFU number has grown very large. Such properties facilitate the comparison of stochastic to deterministic PD predictions (see text)

a–d) of deterministic PK/PD modeling and (panels e and f) of the stochastic PD model summarized in Fig. 2c. It is seen (panels e and f) that the stochastic PD model predicts, for the extinction probability $E(t)$, that $E(t)$ increases most rapidly at early times (when adaptive resistance is still small) and reaches nearly its final value E_F at ~15 h for the premature neonate or <30 h for the full-term neonate.

We concluded that, to the extent that these properties hold for other dosing regimens: (a) the first dose is the key one as far as obtaining a large final eradication probability E_F; (b) doses later than about 36 h after the first dose are usually almost useless as regards increasing the predicted E_F.

Tables and Statistics for the First 75 h

We therefore investigated to what extent Figs. 3 and 4 are typical by considering a number of 72 h dosing regimens with equal intervals between doses; each regimen was followed by a 3-h recovery period. Results for 24- and 36-h intervals between doses are shown in Tables I and II. In each row we state the estimated E_F, specifically the eradication probability $E(75)$ that the central compartment contains no bacterial CFU at 75 h; we add calculated free drug concentrations, often considered as indicators of effectiveness and/or toxicity (4,30,31), with troughs>MIC usually deprecated. It is seen that comparing GA=40 weeks with GA=25 weeks for a given dosing regimen: (a) $E(75)$ for GA=40 weeks is larger (e.g., rows Table I rows 13 and 14 *vs.* Table II rows 6 and 7); (b) for eradication probabilities >0.5 there are more troughs<MIC, especially after several days. In this sense the term neonate is easier to treat than the premature neonate (whose central compartment volume per kg is larger). Tables I and II are selected excerpts from a 32-row larger table, given in section S3 of the online AAPS Supplement, each row of which contains results for both GA=25 and GA=40.

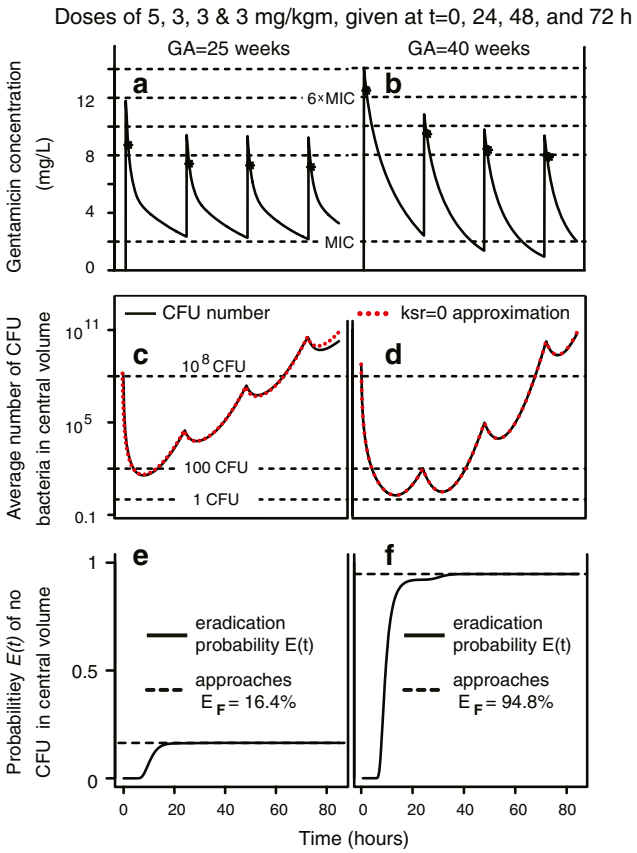

Fig. 4. Deterministic and stochastic PK/PD estimates for an 85 h period: an example. **a** and **b** show deterministically calculated averages wholly analogous to **a** of Fig. 3. The main difference is that here average concentration remains above MIC for all times (**a**) or all times less than 48 h (**b**). **c** and **d** are wholly analogous to **c** of Fig. 3. **e**, **f** The curves are the stochastically calculated eradication probabilities $E(t)$. In **e**, $E(t)$ has reached almost its final value, E_F ~16%, by <20 h. In **f,** the second dose, administered at 24 h, does give a visible increase in $E(t)$ but the last two doses do not

Table I. Eradication probabilities and concentration troughs/peaks. GA=40 weeks

	doses mg/mL	int[a]	E_F	concentration at time t (e.g. 7.01 mg/L at time 0.1 hours in row 1)[c]								
				t=0.1	23.99	24.1	35.99	36.1	47.99	48.1	71.99	72.1
1	2.5,7,0,0	24	0.06	7.01	1.21	20.81	6.26	6.21	2.41	2.39	0.36	0.36
2	3,6,0,0	24	0.27	8.42	1.45	18.24	5.54	5.50	2.14	2.12	0.33	0.33
3	4,0,0,0		0.60	11.22	1.93	1.92	0.81	0.81	0.34	0.34	0.08	0.08
4	4,4,4	36	0.60	11.22	1.93	1.92	0.81	12.01	3.35	3.32	0.46	11.65
5	4,7,7	36	0.60	11.22	1.93	1.92	0.81	20.41	5.61	5.57	0.75	20.33
6	4,4,0,0	24	0.68	11.22	1.93	13.12	4.10	4.07	1.59	1.58	0.26	0.26
7	4,4,4,0	24	0.68	11.22	1.93	13.12	4.10	4.07	1.59	12.78	1.21	1.20
8	4,4,5,4	24	0.69	11.22	1.93	13.12	4.10	4.07	1.59	15.58	1.45	12.63
9	4,4,7,4	24	0.91	11.22	1.93	13.12	4.10	4.07	1.59	21.17	1.92	13.10
10	4,5,4,4	24	0.92	11.22	1.93	15.92	4.92	4.89	1.91	13.09	1.25	12.44
11	4,7,0,0	24	1.00	11.22	1.93	21.52	6.56	6.52	2.53	2.51	0.39	0.39
12	4,7,4,4	24	1.00	11.22	1.93	21.52	6.56	6.52	2.53	13.71	1.34	12.52
13	4.5x4[b]	24	0.97	12.62	2.17	14.76	4.61	4.58	1.79	14.37	1.36	13.94
14	5,0,0,0		0.92	14.03	2.41	2.40	1.02	1.01	0.42	0.42	0.10	0.10
15	5,6,6	36	0.92	14.03	2.41	2.40	1.02	17.81	4.94	4.90	0.68	17.46
16	5,4,0,0	24	0.99	14.03	2.41	13.60	4.30	4.27	1.68	1.67	0.28	0.28
17	5,4,4,4	24	0.99	14.03	2.41	13.60	4.30	4.27	1.68	12.86	1.23	12.41
18	6,0,0,0		0.99	16.83	2.89	2.88	1.22	1.21	0.51	0.50	0.12	0.12
19	6,4,4	36	0.99	16.83	2.89	2.88	1.22	12.41	3.52	3.49	0.50	11.69
20	6,3,0,0	24	1.00	16.83	2.89	11.28	3.69	3.66	1.45	1.44	0.25	0.25
21	6,4,0,0	24	1.00	16.83	2.89	14.08	4.51	4.48	1.76	1.75	0.30	0.30
22	7,0,0,0		1.00	19.64	3.38	3.35	1.42	1.41	0.59	0.59	0.14	0.14
23	7,4,0	36	1.00	19.64	3.38	3.35	1.42	12.61	3.60	3.58	0.52	0.52
24	7,4,0,0	24	1.00	19.64	3.38	14.56	4.71	4.68	1.85	1.83	0.32	0.31

[a] Interval between doses, e.g. in row 1, 2.5 mg/kg at birth, then 7 mg/kg after 24 h
[b] Four doses, each 4.5 mg/kg
[c] Concentration troughs>MIC (=2 mg/L), and peaks >6MIC, are highlighted blue or yellow respectively to facilitate comments on toxicity in the "Discussion" section

Lexicographic Ordering

The tables are "*lexicographically ordered*" as follows. If the first dose in row x is smaller than the first dose in row y, row x is located above row y. Ties which remain after applying this criterion are broken by using dose intervals: rows having no second dose are above those having intervals of 36 h, which in turn are above those having intervals of 24 h. Ties which still remain are broken by using the size of the second dose, then by using the size of the third dose, and finally by using the size of the fourth dose. Lexicographic order indicates the degree of front boosting. It is seen that lexicographic order correlates with $E(75)$. For example in Table II the front boosted regimen row 20 has a substantially larger $E(75)$ than the corresponding back boosted, lexicographically earlier, regimen, row 2.

In order to quantify the correlation, we computed Spearman's rank correlation coefficient "rho" for the sum of both $E(75)$ values (GA=25 and GA=40 weeks) using lexicographic order as predictor; the result was rho=0.96, with $p<10^{-15}$ for the alternate hypothesis that the correlation of $E(75)$ with lexicographic order was due to chance. For vividness, the input to this rank correlation coefficient calculation is shown in Fig. 5.

Additional Results

For completeness, Table III gives predictions of our mathematical model (Fig. 2) for a twice daily dosing regimen, compared to a single dose that gives a similar $E(75)$ value. It is seen that during the first 3 days the predicted concentration for the twice daily regimen never drops below MIC (2 mg/L) and never gets as high as 5×MIC.

Table II. Eradication probabilty and concentration troughs/peaks. GA=25 weeks

	doses mg/kg	int[a]	E_F	concentration at time t (e.g. 9.3 mg/mL at time 0.1 hours in row 1)[c]								
				t=0.1	23.99	24.1	35.99	36.1	47.99	48.1	71.99	72.1
1	4,0,0,0		0.01	9.30	1.87	1.87	1.22	1.22	0.81	0.81	0.40	0.40
2	4,7,7	36	0.01	9.30	1.87	1.87	1.22	17.49	5.36	5.34	2.14	18.40
3	4,6,4,4	24	0.03	9.30	1.87	15.82	5.22	5.20	3.31	12.59	2.96	12.25
4	4,7,0,0	24	0.04	9.30	1.87	18.14	5.89	5.87	3.72	3.71	1.59	1.59
5	4,7,4,4	24	0.79	9.30	1.87	18.14	5.89	5.87	3.72	13.01	3.13	12.42
6	4.5x4[b]	24	0.05	10.47	2.11	12.56	4.37	4.36	2.78	13.23	2.95	13.40
7	5,0,0,0		0.16	11.63	2.34	2.34	1.53	1.52	1.01	1.01	0.50	0.50
8	5,6,6	36	0.16	11.63	2.34	2.34	1.53	15.47	4.91	4.90	2.00	15.93
9	5,4,0,0	24	0.17	11.63	2.34	11.63	4.19	4.18	2.68	2.67	1.18	1.18
10	5,4,4,4	24	0.17	11.63	2.34	11.63	4.19	4.18	2.68	11.96	2.72	12.01
11	5,5,5,5	24	0.67	11.63	2.34	13.96	4.86	4.84	3.09	14.70	3.28	14.88
12	6,0,0,0		0.53	13.96	2.81	2.80	1.83	1.83	1.22	1.21	0.60	0.60
13	6,4,4	36	0.53	13.96	2.81	2.80	1.83	11.12	3.82	3.80	1.60	10.89
14	6,6,6	36	0.53	13.96	2.81	2.80	1.83	15.77	5.12	5.10	2.10	16.03
15	6,4,0,0	24	0.66	13.96	2.81	12.10	4.50	4.48	2.88	2.87	1.28	1.28
16	6,4,4,0	24	0.70	13.96	2.81	12.10	4.50	4.48	2.88	12.17	2.82	2.81
17	6,4,4,4	24	0.70	13.96	2.81	12.10	4.50	4.48	2.88	12.17	2.82	12.11
18	7,0,0,0		0.83	16.28	3.28	3.27	2.14	2.13	1.42	1.42	0.70	0.70
19	7,4,0	36	0.83	16.28	3.28	3.27	2.14	11.43	4.02	4.01	1.70	1.69
20	7,4,0,0	24	0.97	16.28	3.28	12.57	4.81	4.79	3.08	3.07	1.39	1.38
21	7,4,4,0	24	0.99	16.28	3.28	12.57	4.81	4.79	3.08	12.37	2.92	2.92
22	7,4,4,4	24	0.99	16.28	3.28	12.57	4.81	4.79	3.08	12.37	2.92	12.21

[a] Intervals between doses, in hours; e.g. row 2 shows 4 mg/kg given at birth, then 2×7 mg/kg at 36 h intervals
[b] Four doses, each 4.5 mg/kg
[c] Concentration troughs greater than MIC (=2 mg/L), and peaks >6MIC, are highlighted blue or yellow respectively to facilitate comments on toxicity in the Discussion section

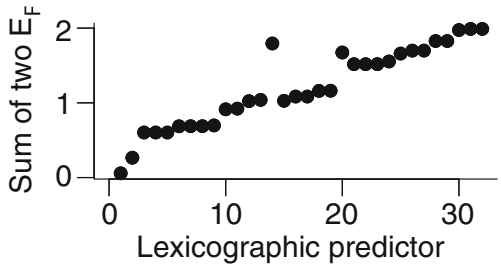

Fig. 5. Rank correlation input. The vertical axis gives the sum of the 2 eradication probabilities $E(75)$ for the 25-week and the 40-week neonates, as predicted using the mathematical model of Figs. 2a, c. The horizontal axis is the row number of the 32-row table in the supplement. The x axis is merely ordinal: stretching it non-linearly would give a graph with the same information

One adjustable constant in the published model of Fig. 2a is Koff, the rate at which adaptively resistant bacteria return to sensitivity when no longer subjected to gentamicin, with ln2/Koff the corresponding half-life, taken as 50 h in (9) and above. The value of the half-life in patients is not well known, but may often be smaller (32). Therefore, to check robustness of the results, we redid our calculations using half-life 25 h instead of 50 h. The main conclusions were not changed. In particular, the Spearman coefficient rho was 0.84, with $p=4\times10^{-7}$. However, as expected, the model-estimated final eradication probability E_F is higher. Details are in online AAPS Supplement section S3.3. One of the more notable examples is the following: for GA=40 and an initial 3 mg/kg dose followed 24 h later by a 6-mg/kg dose, E_F assuming a half-life of 50 h is 27%; but when assuming a 25-h half-life, it increases to E_F=90%.

Table III. Additional dosing regimens for GA=40 weeks

doses		concentration (mg/L) at time t (hours)												
mg/kg	E(75)	t=0.1	11.99	12.1	23.99	24.1	35.99	36.1	47.99	48.1	59.99	60.1	71.99	72.1
4	0.60	11.19	4.32	4.28	1.93	1.91	0.81	0.81	0.34	0.34	0.15	0.15	0.08	0.08
7x2.2[a]	0.59	6.16	2.37[b]	8.507	3.08	9.19	3.08	9.19	2.86	8.97	2.60	8.71	2.37	8.48

[a] 7 dose fractions of 2.2 mg/kg 12 h apart

DISCUSSION

Our results concern a new approach to anti-bacterial PK/PD modeling, attempting to estimate an eradication probability $E(t)$ by stochastic PD modeling *in silico*. This "Discussion" section will first list some limitations of our calculations, which imply that the results cannot, at least at present, serve as prospective estimates of cure rates. Then we discuss the results in more detail and make suggestions.

Some Limitations of the Analysis, Especially as Regards $E(t)$ and E_F

Among the limitations of our analysis are the following.

- Correlating E_F with effectiveness observed *in vitro*, preclinically, or clinically was not attempted.
- Our results only apply to individualized treatment of a hypothetical neonate that has parameters typical of neonates with the same GA and birth-weight. The statistical methods of population PK/PD have to be added to the stochastic process calculations unless detailed information on each neonate is available and is used to design individualized treatments. For example, in general any predicted advantage of front boosting will apply only to a fraction of the patient population—there will be some patients for whom both front boosted and corresponding back boosted regimens are predicted to be effective and some for whom neither is predicted to be effective. Any predicted advantage of front boosting is therefore "fuzzed out" when applied to many different individuals in a heterogeneous population.
- Our equations merely graft one stochastic process PD calculation onto an otherwise deterministic PK/PD calculation. To really estimate eradication, a systematically stochastic approach is needed for all aspects. For example, suppose one sample treated *in vitro* has CFU counts below the limit of detection at a preassigned final time. In a deterministic treatment the question of whether all CFU in such a sample are eradicated need not be considered (9) but in a comprehensively stochastic approach that would be a key question.
- Our model has many of the same problems as other current *in silico* PK/PD models (33), e.g. an unfortunate combination of over-simplification with over-parameterization. The changes in predicted E_F values when changing the parameter Koff (see the "Results" section) are one example of how over-parameterization can lead to substantial uncertainties.
- In particular, the model may not be detailed enough biologically to connect cure to CFU number. For example, even if there are no remaining CFU in the central compartment, CFU could be "hiding" at other locations in the body and subsequently repopulate the central compartment. The deterministic PD model we are modifying does not address this possibility, and does not need to address it because effectiveness is judged by clinical trials rather than predicted mathematically. But in our stochastic argument, the possibility is important.
- Similarly, and perhaps most importantly, in its present form the model is applicable only to immunoincompetent patients. Neglecting immune system dynamics may be less acceptable in a fully stochastic calculation than in the corresponding deterministic approximation. A more extensive calculation, which models the coupled effect of immune reactions and treatment driving total CFU number down to such small values that stochastic fluctuations become important, will eventually be needed.

Comments on E_F

Despite the caveats listed above, we suggest there are reasons to consider E_F as a possible effectiveness index, which deserves experimental and clinical investigation. One reason is that E_F considers a relevant biological scenario (Fig. 2a, c), however oversimplified. Another is that the eradication probability $E(t)$ calculated from the model increases and approaches its limiting value E_F gradually, which seems plausible. Corresponding deterministic estimates (discussed in the "Eradication probability" subsection of Methods) refer to just one time, such as the end of treatment or the time when the predicted average CFU number is at its minimum, not to the entire time course of a treatment regimen. Finally, the fact that, at least for neonatal gentamicin treatments, E_F calculated from a model correlates strongly with a lexicographic ordering, which is so simple that it is automatically available in the clinic, indicates a potential practical advantage.

Regimens with Large First Dose but Small Total Dose: Toxicity

The most interesting rows in Tables I and II have, compared to most current practice, a larger first dose but

smaller total dose. The most extreme examples consist of a single dose of 5–7 mg/kg at birth and then no further treatment.

Gentamicin overdosing is dangerous, especially as regards nephrotoxicity and irreversible vestibular ototoxicity (4,25,34). Clinical guidelines for neonates vary (4,25,30,31,35,36). Guidelines often specify that free drug concentration in the central compartment should include frequent or prolonged troughs<MIC (=2 mg/L) during the first week, which our extreme examples (e.g. Table II row 18) clearly satisfy. Prolonged or repeated concentrations above a certain value (6×MIC is one estimate) are also usually deprecated. However it has been argued that a single high peak which decays rapidly may perhaps not be too dangerous, due to saturation effects in drug uptake for both ear and kidney (4,25,36). Comparing with Tables I and II indicates that the high initial doses needed to achieve high predicted values of E_F, especially doses of 6 mg/kg or more which are used only rarely for neonates (36), are as yet neither unambiguously safe nor unambiguously ruled out by toxicity constraints.

Front Loading

If mathematically predicted concentration troughs<MIC (36) and E_F>50% are taken as goals (see caveats above), there are implications for front boosting. Examples of model-predicted front boosting advantage include many cases like the contrast between the single 5 mg/kg dose in Table I row 14 and higher-total-dose regimens (rows 2, 8–11) which lead to smaller E_F, despite also having at least one dose at least as large as 5 mg/kg and having higher concentration troughs. The intuitive reasons for this predicted difference include the development of adaptive resistance and increasing clearance (Fig. 3b) and also the general tendency of stochastics to favor early dosing (AAPS Supplement, sections S2 and S4).

Conclusions and Recommendations

For neonatal gentamicin treatments directed at *E. coli*, we suggest that the safety and effectiveness of larger first doses coupled with smaller total doses than usual in current practice deserve further investigation *in silico* and *in vitro*, which could in turn eventually lead to preclinical and clinical trials. More generally, if standard PK/PD models predict that the average CFU number ever drops substantially below ~100 per patient, the models should be modified to incorporate stochastic effects systematically, which will then usually give more favorable, or less unfavorable, predictions for front boosting ("hit hard early").

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