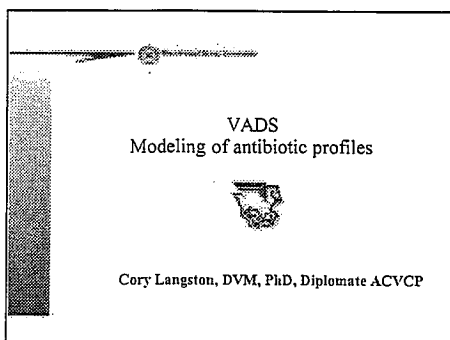



VADS MODELING OF ANTIBIOTIC PROFILES

Cory Langston, DVM, PhD, Diplomate ACVCP




First off, this is really like Mike said, how we are making the clock, and the good part of this is, although I have tried to impress when I teach students about the importance of kinetics and everything, the actual veterinarian won't have to know any of this. You'll be coming in and there will be those of us that have worked through these kinetic regimes. So, hopefully we have derived the most appropriate regimen for your particular needs.



Basic assumption

- For antimicrobials there is a basic relationship between pharmacokinetics and pharmacodynamics relative to efficacy.
 - Time-dependent killing
 - Beta-lactams
 - Concentration-dependent killing
 - Aminoglycosides
 - AUC
 - Fluoroquinolones

Basically my involvement is with the modeling of the pharmacokinetics, to come up with the best dosage regime to address particular pathogens. And, it is based on the concept that there is a relationship between pharmacokinetics and pharmacodynamics. And I won't go through these again because Dr. Apley did this in the previous session, but I am going to be using ampicillin as an example, so just note that for the beta-lactams it is time-dependent killing. So we are trying to keep the trough concentration above the MIC for the longest period we can - for Gram (-) ideally 90 - 100% above MIC, perhaps less for Gram (+).



Goals

- Develop pharmacokinetic models that best describe the disposition of each antibiotic formulation.
- Apply that model to achieve steady-state plasma time-concentration predictions
- Determine the variability of the steady-state concentrations / kinetic parameters to derive a subsequent population distribution.

Now, what my particular goals are, are wanting to develop a pharmacokinetic model that best describes the disposition of each antibiotic. Now realize that nearly always the actual stuff in the literature, we are not generating new information, though that may be something that is a spin-off from the things we need to do, but we are going back and trying to review the literature, and these are nearly always single dose studies. Well, in certain cases, that's fine, with micotil you could argue that you could go off a single dose study and that sort of thing, But for a lot of the products, particularly the older products, we need to know what the actual steady-state concentrations are. So the second thing that I am going to be doing is once I establish that model, I come in and use it to produce an estimate of steady-state

concentrations, which are really the important thing for a lot of these in terms of trying to correlate with the facts. Now the last thing we are going to do, is when I have generated the preferred regime for the mean or the average number of animals, we are going to try to apply some of those variability characteristics that we know to a population distribution. And that is what Dr. Fajt will be talking about later on, is our approach to that, and some of the problems that we've encountered in that regard.



Develop pharmacokinetic models that best describe the disposition of each antibiotic formulation.

- Intensive literature searches
 - Raw time-concentration data provided.
 - Complete kinetic parameters for individual animals provided.
 - Complete models with mean parameters and their variability estimates provided.
 - Mean time-concentration data provided with estimates of variability.
 - Incomplete models with mean parameters and estimate of variability.

Like Mike said, we are doing extensive literature searches in this regard, and I have just listed here some of the things that we have encountered, kind of from a 'best case' scenario down to a 'worst case' scenario. And the 'best case' scenario really is the one where you are going to have all of the data included in the paper. That is, you are going to have your raw time concentrations data, or at least the kinetic parameters for the individual animals included. This makes it really nice for me because all I have to do is simply plug in the data and away I go with the model. More commonly, we might get a complete model with mean parameters, in essence, the variability, not the individual animal kinetic parameters, but just the mean and standard deviation for the elimination rate and that sort of thing. Unfortunately, we often times don't get those, we rarely

get this at all, we might get this, but more commonly we wind up with this. We wind up with mean time concentration data, perhaps provided with estimates of variability and often time's incomplete models with some mean parameters, and estimates of variability.



Literature evaluation
Procaine Pen G as an example

- Obtaining information from the literature.
 - Of 21 references describing pen G disposition in cattle.
 - > 1 - complete kinetic analysis with individual parameters
 - > 1 - partial analysis
 - > 2 - C_{max}, T_{max}, half-life or graph, no data
 - > 2 - mean data only
 - > 1 - half-life & graph
 - > 3 - local therapies
 - > 11 - Na, K, or benzathene formulations

Just as an example, I went through penicillin G literature here, and when we go to obtaining it from the literature, I wound up reviewing about 21 articles. And out of 21 articles, we had only one paper that actually had a complete kinetic analysis, not with raw numbers, but at least with individual parameters. We had one that had a partial analysis, 2 that reported C_{max} and T_{max} and the half-life, or a graph, but no data presented. Two with mean data only, and no standard deviations included. One with just a half-life and a graph. Three local therapies, and eleven that were totally different formulations. So out of the 21 articles I went through for procaine pen, we are going to wind up with being able to use anywhere from 2 to 4 perhaps, that will actually give us some idea of what was going on.



Literature evaluation

- No assay validation provided.
- A great deal of information and effort has been wasted due to poor analyses, poor design, and circumspect analytic techniques.

In addition, one of the things I'd like to point out is that there is almost no assay validation that occurs in these papers. And this, I think, is really a shortcoming in terms of the journals and the review process and perhaps also in our graduate programs. How can you have faith in the numbers if you don't know that the assay is correctly done? So, you've got a lot of information out there, but unfortunately, there really is a great deal of information that has been wasted, and a lot of time has been wasted in that regard, and it's really a shame. And that may be again something that comes out of this, is some suggestions if you are going to do this, at least do these things.



Apply that model to achieve steady-state plasma time-concentration predictions

- For ampicillin trihydrate we are encountering two types of data from the literature.
 - Mean ± SD of time-concentration data
 - Mean ± SD of some kinetic parameters

I am going to go ahead and show you what we have done with ampicillin trihydrate, we have two pieces of literature; one where we looked at mean plus or minus standard deviation of time-concentration data, and the other with mean standard deviation of some kinetic parameters.



Analysis of mean ± SD of time-concentration data

- Modeling of mean time-concentration data.
- Superpositioning of mean time-concentration data.
 - Advantages
 - simple conceptually
 - makes no assumptions of an underlying model
 - Disadvantages
 - first-order process must always exist
 - literature fails to denote necessary data requirements

So when we are dealing with, again, with mean time-concentration data, basically here we have a paper that reported the concentration mean and its standard deviation, but no kinetic analysis - no half-life, no nothing. What can you do with it? Well, you can certainly come in and model it, which I have done, but truthfully I am very much more in favor of superpositioning this sort of data, and the reason is that it is simple conceptually, but mainly it doesn't make any assumption about any underlying models. Is it a flip-flop kinetic model, is it one-compartment, two-compartment, those sorts of things.

Dose Number	Time (hr)	Plasma drug concentration (mcg/ml)						Total
		Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	
1	0	0						0.0
1	1	21.0						21.0
2	2	22.3						22.3
3	3	15.8						15.8
2	4	16.9	0					16.9
3	5	15.4	0					15.4
6	6	12.0	22.3					34.3
7	7	10.1	19.8					29.9
3	8	8.5	16.0	0				24.5
9	9	7.15	14.3	21.0				42.45
10	10	7.01	12	22.5				41.51
11	11	5.95	10.1	19.8				35.85
4	12	4.25	8.5	16.9	0			29.7
13	13	3.58	7.15	14.3	21.0			46.03
14	14	3.01	6.01	12.0	22.3			43.3
14	14	3.45	6.01	10.1	19.8			39.4
5	16	2.15	4.25	8.5	16.9	0		31.8
17	17	1.70	3.58	7.15	14.3	21.0		47.73
18	18	1.51	3.01	6.01	12.0	22.3		44.8
19	19	1.27	2.45	4.95	10.1	19.8		38.5
6	20	1.07	1.70	3.45	6.9	14.3	0	33.7
21	21	0.9	1.27	2.58	7.15	14.3	21.0	48.7
22	22	0.75	1.51	3.01	6.01	12.0	22.3	45.6
23	23	0.64	1.27	2.53	5.05	10.1	19.8	38.4
24	24	0.53	1.07	2.33	4.25	8.5	16.9	33.4

A couple of things that I want to point out relative to superpositioning, and I'll explain it just very briefly. You do have to present it as a first order process, but that is also one of the things we are talking about when you look at these paper reviews. We are going through and looking for different doses to see if they are producing proportional increases or decreases in the plasma concentrations. Because when you lose first order absorption, it makes it very difficult to model. One of the things point out to anyone in graduate training is really that the literature doesn't address some of the problems and how to go about doing superpositioning, because the textbooks, this is from a textbook, and it shows the standard way of doing superpositioning, where you measure a sample every hour and you get this from one dose. And then you pick your dosing interval, and in this case, was 4 hours. So every 4 hours, 4, 8 and 12, you simply overlay, or overlay that single dose. So this column is the same as this one, but it begins at 4 hours. And again, this column is the same as the first dose, but it begins at 8 hours. And then you simply sum across to get your superposition data.



Nuances of superpositioning

0	0.000	0	0.000
0.5	1.535	0.5	1.535
1	1.534	1	1.534
2	1.393	2	1.363
3	1.179	3	1.177
4	1.009	4	1.007
5	0.831	5	0.831
6	0.776	6	0.931
10	0.544	10	0.544
15	0.449	15	0.776
18	0.382	18	0.382
24	0.000	24	0.000
25	0.000	25	0.000
26	0.000	26	0.000
27	0.000	27	0.000
28	0.000	28	0.000
29	0.000	29	0.000
30	0.000	30	0.000
31	0.000	31	0.000
32	0.000	32	0.000
33	0.000	33	0.000
34	0.000	34	0.000
35	0.000	35	0.000
36	0.000	36	0.000
37	0.000	37	0.000
38	0.000	38	0.000
39	0.000	39	0.000
40	0.000	40	0.000
41	0.000	41	0.000
42	0.000	42	0.000
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93	0.000	93	0.000
94	0.000	94	0.000
95	0.000	95	0.000
96	0.000	96	0.000
97	0.000	97	0.000
98	0.000	98	0.000
99	0.000	99	0.000
100	0.000	100	0.000

Well, the problems comes in when no one samples every hour, you couldn't afford it from the standpoint of your assay, much less the logistics of doing it. So, you may start out sampling fairly early and can just check your paper fairly often, and then you start skipping to every other hour, and then you are out there skipping every three hours, so on and so forth. So that when you need to superposition, what happens here? You need a 13-hour sample to add to this one right here, but you don't know it because you didn't measure it. So, what you have to wind up doing is you have to extrapolate concentrations. And what I have done is written a little program that basically does linear regression to the two points that we have, with an intermediate point.

0	0.000	0	0.000
0.5	1.535	0.5	1.535
1	1.534	1	1.534
2	1.393	2	1.363
3	1.179	3	1.177
4	1.009	4	1.007
5	0.831	5	0.831
6	0.776	6	0.931
10	0.544	10	0.544
15	0.449	15	0.776
18	0.382	18	0.382
24	0.000	24	0.000
25	0.000	25	0.000
26	0.000	26	0.000
27	0.000	27	0.000
28	0.000	28	0.000
29	0.000	29	0.000
30	0.000	30	0.000
31	0.000	31	0.000
32	0.000	32	0.000
33	0.000	33	0.000
34	0.000	34	0.000
35	0.000	35	0.000
36	0.000	36	0.000
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96	0.000	96	0.000
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98	0.000	98	0.000
99	0.000	99	0.000
100	0.000	100	0.000



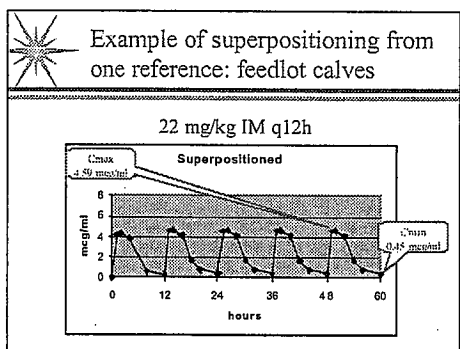
For superpositioning to work

- Either
 - must sample at equal intervals throughout study
 - or
 - predict intervening concentrations
- Must sample for at least 2x the proposed dosing interval.

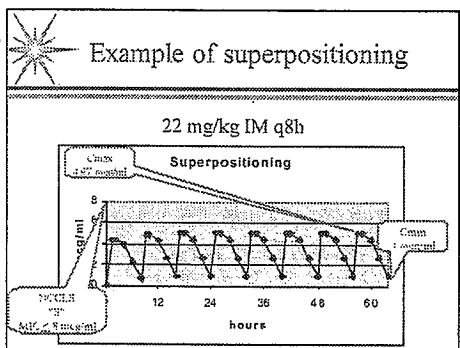
The other thing is what do you do when you're dosing, in this case, every twelve hours, and you are out here needing samples on out at 24 hours and you haven't done anything to sample those times? What you have to have, you either have to be able to sample at least twice whatever dosing interval you are going to be proposing, or you extrapolate. In that latter instance, what we are doing is we are performing regression on the terminal data points, and using those to predict those paths, the last actual sample time. It is not ideal, but it is really the only way I have found to deal with this sort of data from the literature.

Example of Superpositioning

So, just to give you a very brief idea of how simple this is, which is the beauty of it, is simply if I have a time-concentration profile, which I do. And this is again, a real one, and we say that this was actually dosed at 7-½ mg/kg. And then this one, say we want to increase that to 22, we want 12 hour dosing interval and you can pick however many times you want to do it, and simply hit 'calculate'. It tells you that it needs a zero sample, which at time zero is going to be zero. So there we have our superposition data, and if we plot that out, we see the effect of superpositioning where in fact you accumulate until you do reach steady state. So that is one of the approaches, and that is what we are probably going to go with. And one of the reasons for doing this is to get ideas, so I am open to other suggestions in that regard.



Relative to the specific example, this is from one reference that was done in feedlot calves and when I adjusted the dose superpositioned for 22 mg/kg, which is a pretty decent dose of polyflex IM every 12 hours, we wound up with a Cmax of roughly 4.5 and a Cmin or trough of about 0.5.



I went ahead for the heck of it and did it for 8 hours, realized probably in food animal; you're lucky if you are going to do it once a day, and twice a day... I just wanted to see what would happen on 8 hours. But notice your Cmin is down here, we have increased it now to 1mcg/ml. However, NCCLS sensitive is lesser than or equal to 8, and this goes back to what Mike was saying, is if you simply get an organism coming back to you as sensitive, and your goal is to have that trough concentration at 8 or above, folks, there's no way it is going to happen with ampicillin trihydrate. And we may be not in a position to tweak and say what specific formulation or dosage regime is going to differentiate between a half a mcg/ml or a mcg/ml, but I think we are going to rule out a lot things, because you ask a lot of vet students, what about

ampicillin? Well, that's a broad-spectrum antibiotic, works good against Gram (-), I'm going to use polyflexin on it. Now it might work if you have an MIC and your MIC is showing it down to a half mcg/ml, it may be for pasteurilla, or maybe it would be; but for E. coli, not very likely.



Only partial kinetic parameters supplied: adult dairy cows

- No time-concentration data supplied

Parameter	Extravas.	IV
Dose (mg/kg)	27.5	145
C_p (nmol/g)	1.97	1.39
λ	0.0613 / hr	0.019 / hr
$t_{1/2}$ (min)	1266	348
k_a	4.33 / hr	2.53 / hr
T_{max}	1.30 hr	0.26 hr
C_{max}	27 mcg/ml	1.15 mcg/ml
M	0.248 L/kg	0.021 L/kg
AUC	21.89 hour*ug/ml	0.035 hour*ug/ml

Now, the other way we have done it, here's another example done in adult dairy cows, where we had this information that we applied to it, it was a fairly complete kinetic model. And they did a crossover so that it got ampicillin sodium where they gave us the volume of distribution in an area-under-the-curve.

So things are looking pretty good, except they didn't give us the fraction absorbed and they didn't give us the area-under-the-curve for the ampicillin trihydrate.



Only partial kinetic parameters supplied

- Literature provided
 - λ_z
 - k_a
 - V_d (area?)
 - Dose
- Missing for simulation
 - F to determine dose entering central circulation

Well, model, you have to have your rate constants, your volume of distribution, but you have to know how much of the dose actually goes into the central circulation. So here is, and I'm glad professor Corey isn't in here because he'll probably shiver when he sees me do all of this.



Only partial kinetic parameters supplied

- For an extra-vascular dose,
 - $AUC = (C_p/\beta) - (C_p/k_a)$
 - $= (1.97 / 0.0613) - (1.97 / 4.33)$
 - $= 31.68 \text{ hour} \cdot \mu\text{g/mL}$
- $F = (AUC_{non-iv} / \text{Dose}_{non-iv}) + (AUC_{iv} / \text{Dose}_{iv})$
- $= (31.68 / 10.12) + (21.89 / 27.5)$
- $= 0.53$

Since they gave us the area-under-the-curve for the IV type, how could they get an extravascular dose for the trihydrate form? There is a formula method you can go about, so they gave us a C_p0 , or Y intercept, so I took that and divided it by the elimination rate or the terminal portion, the K_a , and derived an area-under-the-curve, and again fit it into the fraction absorbed, to derive an F of 0.53.

Perform WinNonLin Simulation

- Model 3; 1-comp extravascular, 1st-order, no lag

$$C(T) = D \cdot K_{01} / V \cdot (K_{01} - K_{10}) \cdot (\exp(-K_{10} \cdot T) - \exp(-K_{01} \cdot T))$$

I then plugged that into a WinNonLin simulation where it is dosed, it goes into a central compartment and is eliminated. K01 is the absorption rate from the depo site of the injection K10 is the elimination rate constant.

WinNonLin Simulation Single-dose

Simulation of single-dose ampicillin trihydrate

Dosage: 22 mg/kg

Parameter	units	estimate
V	mL/kg	0.348
K01	1/hour	0.0619
K10	1/hour	0.53

Secondary Parameters

Parameter	units	estimate
AUC	hour*ug/ml	24.767
K01-HL	hour	11.307
K10-HL	hour	0.761
CL	mL/hour/kg	0.781
Tmax	hour	1.005
Cmax	ug/mL	2.004

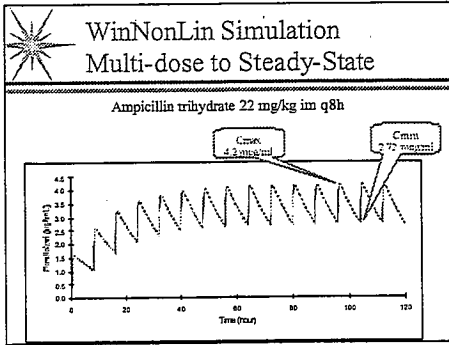
Annotations: "This represents the Vd of 0.348 times an F of 0.53" and "Reported Cmax 2.14 (ug/ml) Reported Tmax 1.3 hr"

And here we see some of the resulting things, again these are fixed, this is the absorption rate from the paper, this is the elimination rate from the paper, WinNonLin takes a volume of distribution divided by the bioavailability to 0.348 from the paper times the 0.53 that I derived gives me 184. And then it produces the simulation. How well this actually fits you can argue, but at least when it predicted its secondary parameters, it did predict a Cmax of 2.04 and the reported Cmax was 2.14, and the Tmax was predicted at 1 hour and the actual Tmax was 1.3. So at least it matches somewhat in that regard.

WinNonLin Simulation Multi-dose to Steady-State

Ampicillin trihydrate 22 mg/kg im q12h

And when you look at those particular concentrations again, we've increased this so now we have a trough concentration of 1.5.

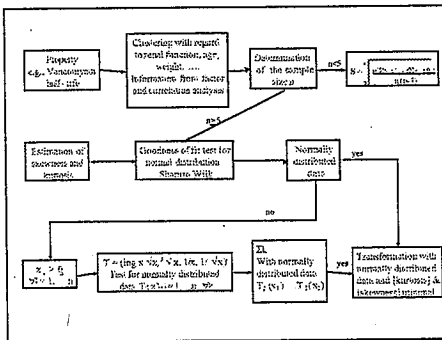


And when you look at those particular concentrations again, we've increased this so now we have a trough concentration of 1.5.

Picking the most appropriate result

- Expert panel review
- "Statistical Analysis of Heterogeneous Pharmacokinetic Data from the Literature". Zellner D, Frankewitsch, Simon S, and Keller F. *Eur J Clin Chem Clin Biochem*, 1996, 34, 585-589.

Now those are different, and the question becomes, 'How do we decide which is the most appropriate?' It could be that it is a difference of dealing with the feedlot animal and the adult dairy cow. This is going to be one of the parts that is going to be perplexing to us. At the very least, we are going to have to rely a lot on expert panel reviews, we are going to have not only clinical pharmacologists involved, but other areas in the various animal industry that are recognized experts looking at this. So this is not going to be just kinetics, we may rule out certain things with the kinetics, but it is going to be looking at clinical problems, the MIC values, MIC 90, hopefully value, those sorts of things.



Now, I'm going to end this up with my part with just a mention of what I would really like to do, and that is really kind of approach it from more of a statistical viewpoint of how to handle this. More or less a meta-analysis kinetic value. But you won't find much on, probably the best paper I found was this particular article from a gentleman looking at human data in Germany. And here is a portion of the flow chart and algorithm for dealing with heterogeneous kinetic data from the literature. And this is only a portion of it. Basically if I put the whole algorithm it would extend from ceiling to basement. So, I just wanted to make a point. What you do is start with the individual animal kinetic parameters, and if it is a very small number, you work off, in essence, median values or

geometric medians. And if it is larger, you start looking for goodness of fit, whether it is normally distributed. This goes on to test for outliers, and you can also divide this by certain characteristics, such as the example here is age, weight, renal function. So you get subpopulations. So if you can do this, then maybe we can come up with a more statistically valid approach for saying, maybe OK, for adult dairy cattle you need this dosage, whereas in feedlot steers you need this dosage, etc, etc. The key thing though, is getting those individual parameters. So what we are trying to do at this particular point is go back into the literature and contact those primary authors and see if they will supply us with the original data so that we can go ahead and reanalyze it, and hopefully follow it from this perspective.