Concentration-effect relations of anti-asthma medications. Studies on inflammation markers
Derks, M.G.M.

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Chapter 2

Combined drug action; introduction to kinetic-dynamic modelling

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Over the last decade there has been significant progress in understanding the kinetics of drug action in vivo. However in comparison with pharmacokinetics we are at the stage where the "standard approach" is still debated. Mathematical models based on classical receptor theory have been used to describe the relationships between drug concentrations and effects in in vitro experiments using isolated tissues and organs. Such pharmacodynamic models derived from the law of mass action have also been shown to be useful tools to describe concentration-effect relationships in vivo.\(^1\)\(^2\). The emphasis on pharmacodynamics today is on concentration rather than dose as a predictor of effect. Understanding the kinetics of drug actions presents a major challenge due to the multifaceted nature of this enterprise. (table 1)

Table 1.

<table>
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<tr>
<th>STEPS IN PK/PD MODELLING</th>
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<tr>
<td>Identification of effects representative for therapeutic or adverse actions</td>
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<td>Precise and reproducible effect quantification</td>
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<td>Pharmacokinetics of parent compound and active metabolites and enantiomers</td>
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<td>Mathematical model design</td>
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The first requirement for these studies of drug action involves the identification of those effects that can represent the therapeutic or adverse actions of a drug. Subsequently, these effects must be quantified in a precise and reproducible manner and the pharmacokinetics of the drug and its active metabolites must be accurately described. Finally, realistic models to link pharmacokinetics and pharmacodynamics must be developed which identify the site of action and can describe and predict the time course of drug action under physiological and pathological conditions.\(^3\).

In table 2 the three most important PK/PD modelling approaches for linking the
pharmacokinetic behaviour of a drug to its effects are shown. Characteristics favouring a physiologically based model approach are: rapid drug distribution to site of action, slowly developing or declining effect, inhibition or stimulation of response controlling factors and indications for an indirectly reversible mechanism of action. Because of the multitude of molecular, biochemical and physiological steps between the drug-receptor interaction and measurable effects in vivo, it should be realised that also physiological models are only models and therefore give a simplified description of reality and that for the design of these models often many assumptions have to be made. Even if the above characteristics apply, in most cases also an effect compartment model can be used to describe the effect-time curve. If a large number of subjects is studied but per individual only limited data become available, population based models still can provide very valuable information.

For the two studies that will be presented in this paper in some more detail, effect compartment models were used. In principle, an effect compartment model consists of three parts: a pharmacokinetic model, a pharmacodynamic model and a link model, the latter of course providing the characteristic element. The basic concept behind this link model is that the rate of drug movement towards the effect site can be estimated from the time course of drug effect. This idea was already used by Serge in 1968 and by Forrester in 1974 and Hull in 1979, but it was the work of Sheiner and Holford who described the effect compartment model in detail that really moved clinical pharmacology from the era of pharmacokinetics into pharmacodynamics.

Table 2.

<table>
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<th>PK/PD MODELLING APPROACHES</th>
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<td>Effect compartment models: direct effects with delay</td>
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<td>Physiologically based models: indirect effects</td>
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<td>Population parameter estimation</td>
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Chapter 2

A hypothetical effect compartment is postulated to which such an infinite small amount of drug is transferred that it does not influence the overall pharmacokinetic behaviour of the compound. Drug removal from the effect compartment to the outside, and not to plasma, is controlled by the rate constant $k_{e0}$ and it is this rate constant that describes the delay between plasma concentrations and effects. Equations were derived from well established pharmacokinetic principles to estimate the effect compartment concentration $C_e$ for a one-compartment model as well as for multi-compartment models. It is of paramount importance that it is then realised that $C_e$ represents the plasma concentration that would correlate with the effect site concentration under steady state conditions. This is the reason why the effect compartment approach can replace the archetypal but cumbersome multiple dose-effect studies. Various frequently used pharmacodynamic models are listed in table 3.

Table 3.

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<th>DYNAMIC MODELS</th>
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<td>&gt; Linear models</td>
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<td>&gt; Simple $E_{\text{max}}$ models</td>
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<td>&gt; Sigmoid $E_{\text{max}}$ models</td>
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<td>&gt; Baseline effect models</td>
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<td>&gt; Models for non competitive agonism or antagonism</td>
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Of course linear models can be used over a rather large segment of the concentration response curve. However only a slope parameter is obtained and information about potency and efficacy is not acquired. Simple $E_{\text{max}}$ models are directly derived from the law of mass action and give us the pharmacodynamic parameters $EC_{50}$ and $E_{\text{max}}$ which under certain circumstances can indeed tell us something about respectively potency and efficacy. In sigmoid $E_{\text{max}}$ models an
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exponent is added to all concentration terms and this has an important effect on the steepness of the concentration response relationship. Hill was the first who introduced such a sigmoidicity factor for his description of the binding of oxygen to hemoglobin and the term Hill equation is therefore also used for these models. Baseline effect models are used to take into account non-drug related variability of the chosen effect parameter. Important causes of such variability could be diurnal variations and changes in the effects of endogenous agonists or antagonists. Combined administrations and the application of models for competitive or non-competitive agonism or antagonism have been shown to be of help for the clarification of mechanisms of action.

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The use of mathematical models in combination with the application of classical receptor theory has greatly enhanced the study of pharmacodynamics in vivo. Although it is realised that the mechanistic meaning of the Hill equation or other $E_{\text{max}}$ models can be diluted by post-receptor events, they remain models of receptor interactions with specific meanings attached to the parameters $EC_{50}$ and $E_{\text{max}}$ related to, respectively, potency and intrinsic activity. Quite early it was realised that kinetic/dynamic modelling studies could have important potential for drug development. In 1992 Carl Peck, at that time responsible for drug policies of the FDA, chaired a conference on the opportunities for integration of pharmacokinetics, pharmacodynamics and toxicokinetics in rational drug development. It has been argued that in early phases of development reliable predictions of all factors involved in the eventually occurring drug action can be made and that this will then direct subsequent research much faster to relevant unanswered questions than is possible with more traditional approaches. Preclinical PK/PD information would create possibilities for better defining of safe doses in phase I. With PK/PD modelling in early human studies better defining of effective doses in phase II and the design of concentration controlled trials in phase III would be promoted.

The academic and medical applications can be summarised by saying that PK/PD modelling enhances our understanding of drug action in vivo. Kinetic/dynamic studies hold great promise for discerning possible causes of dynamic variability such as age, sex, disease, ethnic differences, circadian variation, etc. In the future it may be feasible to create databases of pharmacodynamic parameters similar to those widely available for pharmacokinetics. Other applications are the study of
interactions with endogenous compounds and of dynamic drug-drug interactions with agonistic and antagonistic models. With similar models also the activity of metabolites and/or enantiomers can be defined. Last but not least, validated information about receptor selectivity, potency and efficacy in in vivo situations which is obtained with pharmacokinetic/pharmacodynamic modelling approaches will make more rational comparisons between drugs possible.

Now, two examples of studies will be presented in which different time-courses of effects are linked to the time course of drug concentrations with respectively a non-competitive agonist model and with a competitive agonist model. Formoterol is a long-acting β₂-adrenoceptor agonist that is closely related to terbutaline. The development of a selective and accurate HPLC assay with electrochemical detection for measurement of picogram concentrations of formoterol in plasma enabled us to perform pharmacokinetic/pharmacodynamic research in humans with this compound. We described hypokalemic and eosinopenic effects of formoterol after oral administration in healthy subjects. The formoterol plasma concentration-dependent eosinopenic effect most likely represented a redistribution of the eosinophils and could probably be a surrogate marker for anti-inflammatory activity. Plasma potassium can be used as an alternative parameter for FEV₁ values.

A significant lowering of eosinophils and a hypokalemic effect has also been described after oral administration of theophylline in humans. The use of β₂-adrenoceptor agonists as well as the use of theophylline in the treatment of mild to moderate asthmatic patients has been under much debate lately and a need for more detailed pharmacokinetic/pharmacodynamic research with these drugs seems indicated. As formoterol and theophylline increase cyclic AMP most probably via different mechanisms we set out to test the hypothesis that these drugs interact in a non-competitive way. In this study in healthy subjects hypokalemic and eosinopenic activities were used for effect measurements. Healthy subjects were investigated on three different days, all separated by one week. On the first day, a single oral dose of 144 μg formoterol, on the second day a single oral dose of 375 mg theophylline and on the third day oral doses of 144 μg formoterol as well as 375 mg theophylline were administrated. Over a period of 7 hours, 10 blood samples were taken for measurements of formoterol or theophylline plasma concentrations, plasma potassium and peripheral eosinophil
A tri-exponential equation describing a two-compartment open model with first order absorption was employed to describe formoterol and theophylline pharmacokinetics in all subjects for the single administration as well for the combination of formoterol and theophylline. To account for the delay observed between formoterol or theophylline peak plasma concentrations and the hypokalemic and eosinopenic nadir an effect compartment model was used. The following integrated sigmoid $E_{max}$ model was used to describe the effects after single drug administrations:

$$E = E_0 - \frac{(E_0 - E_{max}) \times C_e^n}{EC_{50} + C_e^n}$$

In this equation $E_0$ is the baseline potassium or eosinophil level, $EC_{50}$ is the $C_e$ that corresponds with 50% of the effect interval between $E_0$ and $E_{max}$ (maximal achievable effect) and $n$ is the factor expressing the steepness of the concentration-effect relationship. For $C_e$ the equation for the effect compartment concentration for a two-compartment model was substituted, using the previously estimated pharmacokinetic parameters of formoterol and theophylline as constants.

To describe the observed effects after the administration of the drug combination the following model for non-competitive agonism derived from the equation given by Ariëns and Simonis was used:

$$E_{\text{comb}} = E_{\text{comb}}^0 \left( \frac{(E_0^\text{comb} - E_{max_A}^\text{comb}) \times C_{eA}^n}{EC_{50A}^n + C_{eA}^n} + \frac{(E_0^\text{comb} - E_{max_B}^\text{comb}) \times C_{eB}^n}{EC_{50B}^n + C_{eB}^n} \right)$$

$$= \left( \frac{(E_0^\text{comb} - E_{max_A}^\text{comb}) \times C_{eA}^n}{EC_{50A}^n + C_{eA}^n} + \frac{(E_0^\text{comb} - E_{max_B}^\text{comb}) \times C_{eB}^n}{EC_{50B}^n + C_{eB}^n} \right)$$

$$= \left( \frac{(E_0^\text{comb} - E_{max_A}^\text{comb}) \times C_{eA}^n}{EC_{50A}^n + C_{eA}^n} + \frac{(E_0^\text{comb} - E_{max_B}^\text{comb}) \times C_{eB}^n}{EC_{50B}^n + C_{eB}^n} \right)$$
In this equation $E_{\text{comb}}$ is the effect of the combined formoterol and theophylline administration, $E_{0\text{comb}}$ is the baseline of the combined effect and $EM$ is the hypothetical maximum achievable effect of the receptor effector system. $EM$, $E_{0\text{comb}}$, $E_{\text{max,}A}$ and $E_{\text{max,B}}$ were estimated by the nonlinear regression program PC-NONLIN while for the other parameters the values as obtained for the single drug administrations were used.

No statistically significant differences were observed between the pharmacokinetic parameters of the single administrations and the combined administration of formoterol and theophylline, making kinetic interactions of any importance unlikely. After administration of the combination the observed effects were more pronounced than after single drug administration. However, the effects calculated by simple addition were higher than the observed effects. The hypokalemic and eosinopenic effects induced by the combined administration of formoterol and theophylline could be described in a very satisfactory way with the above equation, supporting our hypothesis of non-competitive interaction.

In another study with formoterol, the main objective was to investigate if systemic anti-inflammatory effects of this $\beta_2$-adrenoceptor agonist could be shown in healthy subjects after a single inhaled dose via a Metered Dose Inhaler. As possible anti-inflammatory effect parameters eosinophilic granulocyte blood counts and the size of the early cutaneous reaction to intradermal injection of an allergen were used. The hypokalemic effect was also studied in order to find a concentration-response relation after inhalation. The experiment had a double-blind placebo controlled crossover design.

Healthy male students without clinical evidence of allergy were tested after informed consent had been obtained for a positive skin reaction to grass, house dust mite or cat allergen, injected intradermally in a dose of 30 BU/ml. In this way 7 subjects could be recruited for the study. The allergen dose was further titrated in a log way to find the dose that gave a cross-section of the flare of approximately 5 cm. This dose was then used on the experimental days. On the days of the experiment a first blank blood sample was obtained and two skin tests were done. Then 10 puffs of either 12 ug of formoterol or placebo were inhaled. Consecutively during the following eight hours ten more blood samples for analysis of formoterol and eosinophil and potassium levels were taken and nine
skin tests were done. The skin tests were performed in duplicate, with five pairs of skin tests on each forearm. Fifteen minutes following the allergen injection, the outline of the wheal and flare was delineated with a marker and copied to adhesive tape. The wheal and flare reaction was then quantified by weighing the pieces that were cut out of the paper on which the outlines were photocopied. Because the observed two peaks of the concentration-time curve were postulated to be caused by absorption via both the gastrointestinal tract and the lung, the pharmacokinetic behaviour was described by the summation of two standard tri-exponential equations for a two-compartment model with first order absorption with the same distribution and elimination rate constants, but different absorption rate constants. In the second equation a lag-time had to be incorporated. Thus the first observed peak concentration which was already observed after 15 minutes was exclusively made up of the first absorbed fraction of the dose, the second peak was a summation of concentrations resulting from absorption via two different routes. After oral dosing formoterol peak concentrations are observed after 1 hour. Therefore it must be assumed that the first serum peak after 15 minutes was a result of absorption via the lungs and that the second peak was a result of absorption via the gastrointestinal tract. It appeared that 70% of the formoterol that reached the systemic circulation was absorbed rapidly via the lungs while the remainder was absorbed more slowly and after a lagtime of an hour via the gastrointestinal tract. Because of the existence of time delays between the observed two peaks of formoterol plasma concentrations and respectively the observed two peaks of the responses, a PK/PD model with an hypothetical effect compartment to account for this time delay was used for both kinetic equations. And as the time plots of the formoterol concentrations and the time plots of the effects showed that the time between the first concentration peak and the first effect peak was different from the delay between the second pair of peaks, different rate constants for the elimination of formoterol from the hypothetical effect compartment, $K_{e01}$ and $K_{e02}$, were needed. Other differences in the two $C_e$ equations were of course the absorption rate constants and the lag-time for the second curve. Also the doses had to be different, $Fr_1$ times dose being the fraction of formoterol that entered the systemic circulation via a first absorption route and $1 - Fr_1$ times dose the fraction of the dose that appeared in the systemic circulation via a second absorption route.
The observed effects were related to the effect compartment model with the use of a sigmoid $E_{\text{max}}$ model for competitive agonism, which was also derived from an equation of Ariëns and Simonis. The size of the wheal and flare on the placebo day clearly showed a linear increase in time. Therefore, correction of the size of the wheal had to be made for this diurnal rhythm by multiplying time with the estimated slope ($sl$) of the placebo day, resulting in the following rather complicated $E_{\text{max}}$ equation:

$$E = E_0 - \left( \frac{(E_0 - E_{\text{max}}) \cdot C_{e_1}^n}{EC_{50_1}^n + \left( \frac{EC_{50_1}^n \cdot C_{e_2}^n}{EC_{e_1}^n} \right)} + \frac{(E_0 - E_{\text{max}}) \cdot C_{e_2}^n}{EC_{50_2}^n + \left( \frac{EC_{50_2}^n \cdot C_{e_1}^n}{EC_{e_2}^n} \right)} \right) + C_{e_2}^n$$

The eosinophil counts and potassium values on the placebo day showed little variation over the day and the effects of formoterol on these values could be described with the equation without a slope parameter. The maximal attainable effect for the eosinopenia and the inhibition of the wheal was complete disappearance of respectively the number of eosinophils and of the wheal. For both the size of wheal and flare and for the eosinophil count, the maximum obtainable effect ($E_{\text{max}}$) was therefore fixed at 0. The $E_{\text{max}}$ for the decline of plasma potassium was estimated. All effects also showed biphasic patterns, but the biphasic plasma formoterol concentration-time course was not identically to the biphasic response pattern and the sizes of response were approximately reciprocal to the respective areas under the curve of the two absorbed fractions. To obtain acceptable fits two different values of $EC_{50}$ appeared to be necessary for all studied effects, one for each absorbed fraction of the dose. Thus, $EC_{50}$ values for each of the two absorbed fractions of the dose were found that differed for all three effects with a factor of 3.1.

Formoterol absorbed via the alimentary tract appears to be on an average 3.1 times more potent than absorbed via the pulmonary route. A possible explanation for this substantial difference in potency could be found in enantiomer ratio changes, depending on the route via which formoterol enters the body. We had found in another experiment with inhaled formoterol that over a period of 24 hours the ratio of the $RR$ and $SS$ enantiomers in urine steadily and consistently
increased, from 0.49 in the first urine samples to 0.95 in the urine samples collected over the last time period. Probably both differential systemic appearance of the two enantiomers as well as different elimination half-lives are responsible for the observed changes in ratio. We can conclude that by using a PK/PD modelling approach we found indications that after inhalation of formoterol preferentially the inactive enantiomer (SS) reaches the systemic circulation, an observation that could have important clinical ramifications.

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