Dose-response relationships in clinical pharmacology.
L. Lasagna, S. Erill, C.A. Naranjo, editors

DOSE-RESPONSE RELATIONSHIP IN RESPECT OF BIOCHEMICAL EFFECTS OF DRUGS AND THE PROBLEM OF ADAPTIVE RESPONSES

D.G. GRAHAME-SMITH

University Department and MRC Unit of Clinical Pharmacology, Radcliffe Infirmary, Oxford, United Kingdom.

In the scientific analysis of drug therapy in practice, the process can be broken down into four essential parts. They are:

1. The pharmaceutical process. "Is the drug getting into the patient?"
2. The pharmacokinetic process. "Is the drug getting to its site of action?"
3. The pharmacodynamic process. "Is the drug producing the required pharmacological effect?"
4. The therapeutic process. "Is the pharmacological effect being translated into a therapeutic effect?"

The pharmacokinetic process is not strictly within my brief except in so far as it impinges upon the pharmacodynamic effect. It is however, my purpose to deal with the links between pharmacokinetics and pharmacodynamics, and in greater detail, with the pharmacodynamic process itself, and with its translation into a therapeutic effect.

The link between the pharmacokinetic and pharmacodynamic processes is not always simple, as a few examples will show:

a) some drugs combine with their receptors quickly and disassociate from them quickly. For those drugs the pharmacological effects wax and wane in time with the plasma concentration. An example is the use of intravenous sodium nitroprusside in the control of blood pressure, for example during neurosurgery.

b) other drugs combine with their receptors but do not readily disassociate from them, so that despite a falling plasma concentration, the pharmacological effect persists and is not directly related to the plasma concentration. The irreversible monoamine oxidase inhibitors and inhibition of platlet aggregation by Aspirin, are good examples.
c) other drugs combine with their receptors and irrespective of their rates of association or disassociation, set in train a sequence of events which runs on despite a falling plasma concentration. An example of this is the anti-inflammatory effect of corticosteroids.

Much consideration in clinical pharmacology stops short at the stage of the pharmacodynamic process. However, if the patient is to benefit from drug therapy, the pharmacological effect of the drug must result in clinical benefit. This presumes that the detailed nature of the pharmacological effect responsible for the therapeutic action of the drug is known, and this is not always so, as in the case of tricyclic antidepressants in depression. The question we have asked about the therapeutic process: "Is the pharmacological effect being translated into a therapeutic effect?" is in practice very important. For instance, does lowering the blood pressure prevent stroke, does control of the blood glucose in diabetes prevent diabetic neuropathy?

Another factor altering dose-response relationships in drug therapy is the occurrence of pharmacological adaptive responses. Table 1 shows some patterns of adaptation which occur during drug therapy, which either result in tolerance i.e. a shift of the dose response curve to the right, or a situation where pharmacological adaptation may well be part of the therapeutic

process, as is very likely the case with antidepressant drug therapy, and the use of beta-blockers in the treatment of hypertension.

Finally, there are adaptive responses which may result in adverse reactions because of changes of receptor sensitivity to endogenous or exogenous agents. A good example is tardive dyskinesia caused by neuroleptic drugs.

I shall now illustrate these principles with specific examples, concentrating particularly upon the pharmacodynamic complexities determining the "dose-response relationship".

RELATIONSHIPS BETWEEN PLASMA LEVELS, PHARMACODYNAMIC BIOCHEMICAL EFFECTS, AND THERAPEUTIC EFFECTS OF DIGOXIN

The problem of monitoring digoxin therapy (as opposed to toxicity) by measuring plasma digoxin concentrations at a given time after a dose, is a complex one, because little is known about the precise relationships amongst the factors involved in the sequence of events determining the therapeutic outcome.

We aimed to apply a technique which would give an indication of the pharmacological effect of digoxin, which might reflect the pharmacodynamic effect of digoxin on the heart, and relate this pharmacological effect to plasma digoxin concentrations and see if the pharmacological effect bore any relationship to the therapeutic effect of digoxin[1].

Briefly, digoxin inhibits Na^+,K^+-ATPase in many tissues. Digoxin binds to Na^+,K^+-ATPase in red blood cells and is difficult to wash off, such that if a patient takes digoxin, it binds to the red cell Na^+,K-ATPase, and when blood is taken and the RBC washed, digoxin continues to stick to the Na^+,K^+-ATPase, and inhibit the enzyme and the sodium pump. In RBC inhibition of Na^+,K^+-ATPase and the sodium pump, is accompanied by a decreased

uptake of K+. To monitor K+ uptake by red cells ^{86}Rb may be used. If RBC Na^+,K^+-ATPase activity is inhibited in patients taking digoxin, one would therefore expect the red blood cells of such patients to show inhibition of ^{86}Rb uptake and a rise in intracellular [Na^+]. The study which was done by Aronson et al,[1], concerned the relationship between plasma digoxin concentration, RBC ^{86}Rb uptake and the slowing of the ventricular rate in atrial fibrillation in patients being digitalised with digoxin.

The following conclusions were drawn.

1. During digoxin therapy, RBC ^{86}RB uptake is initially inhibited, indicating inhibition of RBC Na^+,K^+-ATPase.
2. The degree of inhibition of ^{86}Rb RBC uptake correlated well with the fall in ventricular rate in atrial fibrillation during digitalisation, a correlation which was slightly better than that between the plasma digoxin level and the fall in ventricular rate.
3. Comparison of those who were in atrial fibrillation throughout the period of observation, with those whose atrial fibrillation converted to sinus rhythm during therapy suggested that in those patients who converted to sinus rhythm the slowing of their ventricular rate whilst still in atrial fibrillation occurred with less inhibition of RBC ^{86}Rb uptake.
It is tempting to speculate that in this group of patients, the heart is more sensitive to the effects of inhibition of Na^+,K^+-ATPase activity and therefore the effects of digoxin.
4. A group of patients was studied with supra-ventricular tachyarrhythmias other than atrial fibrillation, and in these patients there was, (as expected), no correlation between the changes in ventricular rate and RBC ^{86}Rb uptake.

These studies encouraged us to apply further biochemical indices to study and extend the actions of digoxin on red cells in patients taking digoxin for the treatment of heart failure. We extended the techniques by measuring: 2,3.

1) plasma digoxin concentrations; (pharmacokinetic phase).
2) [^{3}H]-digoxin binding to assess digoxin receptor occupation;
3) RBC ^{86}Rb uptake inhibition.
4) RBC-intracellular [Na^{+}] concentrations.

(2,3, and 4 above form the pharmacodynamic phase)

5) systolic time interval (QS2I) in heart failure in sinus rhythm. (Therapeutic effect).

During the first few days of treatment [^{3}H]-digoxin binding and RBC ^{86}Rb uptake fell, and intra-erythrocytic [Na^{+}] concentrations rose. Quite clearly there was occupation of cardiac glycoside receptors, inhibition of RBC $Na^{+},{}^{+}K$-ATPase and the RBC Na^{+} pump.

The change in systolic time intervals correlated with all three red cell measurements, but there was no correlation between the change in systolic time intervals and the plasma digoxin concentration. Intra-erythrocytic [Na^{+}] concentration was the best reflection of the change in systolic time intervals.

Out of this study however, came a strange observation. As the digoxin was continued in patients with heart failure in sinus rhythm, so the inhibitory effect of digoxin on the functional aspects of RBC cell Na^{+},K^{+}-ATPase seemed to lessen; and after 2-3 months the red cell functions were back to their starting point. Further investigation showed that the effects of digoxin on the erythrocyte which occur during the early phases of digoxin therapy, do not persist in the long term. There appears to be a compensatory up-regulation of the

number of receptors on the red blood cell.
These conclusions have been confirmed by **in vivo** studies on disposition of an oral, non-radioactive rubidium chloride load[4,5]. By this technique oral rubidium chloride is administered. The rubidium is absorbed and enters the blood. The plasma level is measured with time and the pharmacokinetic parameters of the disappearance and rubidium from the plasma assessed. At the same time entry of rubidium into the red cells **in vivo** is measured. During acute treatment with digoxin it is plain that the entry of rubidium into the patient's red blood cells **in vivo** after the rubidium load is diminished. Presumably also, its entry into other cells is diminished because rubidium plasma levels after loading are raised as compared with rubidium plasma levels in patients who have not received digoxin. However, during chronic digoxin therapy such changes in rubidium distribution in plasma and RBC were not found.

Subsequently, we have found evidence for an up-regulation of the Na^+,K^+-ATPase mediated sodium pump in lymphocytes and transformed lymphocytes of humans, when they are exposed to hypokalaemic conditions, and to acetylstrophanthidin **in vitro** in cell culture. This is manifested by up-regulation of [^{3}H]-ouabain binding to lymphocyte and lymphoblast membranes and an upregulation of the cellular ^{86}RB pump.[6,7].

Does this tolerance have any clinical implication in regard to the long-term treatment of heart failure in sinus rhythm with digoxin? We have attempted to answer this question in a clinical study[8]. We carried out a randomised double-blind placebo-controlled cross-over study of digoxin withdrawal and re-introduction over two periods of 8 weeks, each after long-term therapy. We studied 44 patients with stable heart failure in

sinus rhythm who had digoxin concentrations > 0.8 ngm/ml. We assessed their progress by clinical criteria, by haemodynamic measures (systolic time intervals and echo cardiography and by pharmacological measurements of erythrocytic sodium pump numbers and activity). After withdrawal of digoxin, clincial deterioration occurred in 25% of the patients. Deterioration was accompanied by changes in systolic time intervals. Deterioration was accompanied by changes in the pharmacological effects of digoxin the erythrocytes, consistent with a loss of effect, and these changes did not occur in those who did not deteriorate. However, we were unable to predict the occurrence of deterioration by any clinical, haemodynamic or pharmacological measurements made before withdrawal.

Our study of this problem led us to suggest the following concerning chronic digoxin therapy in heart failure.

1) if a patient is in a stable condition, and has a plasma digoxin concentration less than 0.8 ngm/ml then digoxin withdrawal is very likely to be safe.

2) if a patient is in a stable condition, has a plasma digoxin concentration of 0.8 ngm/ml or over and is not at great risk of toxicity, digoxin withdrawal is probably not worthwhile since there is a 25% risk of deterioration.

3) if however, there is an increased risk of digitalis toxicity (e.g. in a patient with deteriorating renal function or in one who has difficulty in maintaining potassium balance) then careful withdrawal may be worth attempting.

4) in a few cases e.g. those with a third heart sound, it may be best to continue therapy, even when there is an increased risk of digitalis toxicity. In that case however, it would clearly be important to increase one's vigilance in monitoring therapy in

order to decrease the risk of toxicity.

Finally, before leaving our studies on the pharmacodynamic effects of digoxin, it is worth mentioning the study carried out by Aronson and Ford[9], showing that in 10 patients with digitalis toxicity, of whom only 2 described symptomatic abnormalities, colour vision was impaired compared with that of both control subjects and non-toxic patients who have been taking digoxin for more than 2 months. Colour vision scores correlated well with "log" of plasma digoxin concentrations, and with the biochemical measures of inhibition

DOSE DEPENDENT INHIBITION OF PHOSPHOINOSITIDE METABOLISM IN HUMAN PLATELETS BY ASPIRIN IN VITRO AND IN VIVO

Aspirin is now used extensively in the prophylaxis of vascular thrombotic disease and is known to have a variety of anti-platelet effects. These include the inhibition of aggregation and decreased release of platelet contents. These effects result from the irreversible inhibition of the enzyme cyclo-oxygenase which is responsible for the conversion of arachidonic acid into prostaglandins and thromboxanes.

In the platelet, arachidonic acid is thought to produce the breakdown of inositol phospholipids by phospholipase's C and A_2 when stimulated with agents such as thrombin, collagen, ADP and thromboxane A_2. The initial event after receptor occupation is phospholipase C-mediated breakdown of phosphatidyl inositol 4, 5-bisphosphate (PIP_2), to inositol 1,4,5-triphosphate (IP_3) and diacyl glycerol. These second messenger intermediates may act synergistically to bring about platelet activation. Aspirin substantially, though not totally blocks the breakdown of inositol lipids in response to collagen and low concentrations of

thrombin **in vitro** .

Studies were carried out studies to investigate the **in vitro** concentrations and the **in vivo** doses of Aspirin which could inhibit the collagen-stimulated formation of inositol phosphatesin human platelets[10].

Eight volunteers received at two-weekly intervals, a single dose of 10, 30, 100 or 600 mg of Aspirin. Before the study began, platelets were taken and incubated **in vitro** with a range of concentrations (10NM-100 mM, of Aspirin). The formation of inositol phosphates was measured in [^{3}H]-inositol labelled platelets after incubation with collagen.The **in vitro** IC^{50} for inhibition of response to collagen by Aspirin was approximately 1 μM. The **in vivo** ID^{50} was 40-50 mg. Both **in vitro** and **in vivo** Aspirin inhibited collagen-stimulated inositol phosphate formation in a dose-dependent manner.

The data showed that Aspirin **in vivo** in single small doses produced a dose-related inhibition of collagen induced inositol phosphate production.

THE LACK OF DOSE-RESPONSE RELATIONSHIP IN WARFARIN ANTI-COAGULATION: DISCREPANCIES BETWEEN WARFARIN PLASMA LEVELS AND INCREASED PROTHROMBIN TIME

An extremely good example of the variables that exist between the pharmacokinetic and pharmacodynamic processes, is the anti-coagulant effect of Warfarin. Breckenridge and Orme[11] clearly showed a poor correlation between the plasma level of Warfarin and the increase in prothrombin time.
The variability in **response** must lie in the efficacy of Warfarin to inhibit the synthesis of the clotting factors II (prothrombin) VII, VIII and X.These are Vitamin K requiring syntheses and

therefore the level of Vitamin K must be of importance, also a variable inhibition of Vitamin K epoxide reductase, and thus a variable reduction in the availability of reduced Vitamin K which is essential for the conversion of clotting factor precursors into complete clotting factors before they are released from the liver into the circulation.

This variable step between pharmacokinetics and pharmacodynamic effects in the case of Warfarin, is taken care of by the direct assay of the pharmacodynamic effect via the prothrombin time.

PHARMACOLOGICAL ADAPTIVE RESPONSES AND THEIR INFLUENCE UPON DOSE-RESPONSE RELATIONSHIPS

Generally, studies attempting to relate the plasma levels of neuroleptics to improvement of psychiatric state in schizophrenia, have not shown an impressive simple direct correlation. Mills and Roberts[13], showed that chlorpromazine and several of its metabolites, inhibited **in vitro** the 5-hydroxytryptamine (5HT)-induced aggregation of human platelets. We thought that this inhibition of 5HT-induced aggregation of human platelets might be used as a pharmacodynamic measure of the effect of neuroleptics **in vivo** during the treatment of psychotic illness. Surprisingly however, it was found that platelets from many schizophrenic patients treated with chlorpromazine showed enhanced aggregation to 5HT rather than inhibition[12]. This was confirmed for patients treated with Fluphenazine. There was some suggestion that enhanced aggregation coincided with clinical improvement, and in patients who reverted to normal aggregation whilst on neuroleptic agents, there was an association with an occurrence of schizophrenic

symptoms. However, our attempts to replicate these findings were only partly successful although others did.[14].

Because pharmacologically-induced adaptive responses are often accompanied by up-regulation or down-regulation of specific receptors, we applied radio-ligand binding techniques to define 5HT receptors on the human platelet membrane, particularly those mediating 5HT-induced platelet aggregation. We developed a method for labelling 5-HT_2 receptors on human platelets with [^{3}H]-LSD.[15]. This receptor has many similarities to the $5HT_2$ receptor in the human frontal cortex.

Using [^{3}H]-LSD binding to intact platelets, we compared platelet $5HT_2$ receptor number in groups of schizophrenics and matched controls. There was a significant increase in the B_{max} of [^{3}H]-LSD binding in patients on neuroleptics. (See Table 2). The mean binding affinity was significantly lower in the patients receiving neuroleptic treatment than in controls, but this was not relevant to the increased B_{max}. There was a positive correlation between [^{3}H]-LSD receptor binding number and total neuroleptic dose expressed as Chlorpromazine equivalents.

The finding of increased 5-HT-induced platelet aggregability in patients on long-term phenothiazine and thioxanthine drugs, and the up-regulation of platelet $5HT_2$ binding sites suggests that both receptor and receptor-mediated function are upregulated.

5HT RECEPTOR BINDING AND TRICYCLIC ANTIDEPRESSANT TREATMENT

In the rodent, marked and various changes in central 5HT function occur during the chronic administration of antidepressant drugs[16].This has its counterpart in the enhanced 5HT-mediated increases in plasma prolactin levels in response to

TABLE 1
PHARMACOLOGICAL ADAPTATION OCCURING DURING DRUG THERAPY[17]
INCREASING INEFFECTIVENESS OF THERAPY

Morphine analgaesia (tolerance)
Barbiturate tolerance
Tolerance to indirectly acting sympathomimetics
Organic Nitrate tolerance
Vasodilator therapy in heart failure
Chronic digitalis therapy in heart failure

ADAPTATION AS PART OF THE THERAPEUTIC PROCESS

Antidepressant drug therapy
β-blockers in hypertension

ADAPTIVE RESPONSES CAUSING ADVERSE REACTIONS
Neuroleptics: tardive dyskinesias

TABLE 2
PLATELET [^{3}H]-LSD BINDING CHARACTERISTICS IN PATIENTS ON CHRONIC NEUROLEPTIC THERAPY [12]

Controls	11	B_{max}(fmol/mg protein) (±SEM)	K_D (nm)
All	24	57.7±3.7	0.58±0.03
Male	11	54.6±3.1	0.58±0.05
Female	13	60.3±6.3	0.57±0.04
Neuroleptic treated			
All	29	78.5±4.3**	0.69±0.04*
Male	19	75.0+4.4**	0.68±0.05
Female	10	85.1±9.4*	0.71±0.08

*p<0.05 ** p<0.001

TABLE 3
EFFECT OF TRICYLIC ANTIDEPRESSANT TREATMENT ON PLATELET ^{125}-iodo LSD BINDING IN 11 DEPRESSED PATIENTS[12]

	Before treatment	After treatment
B_{max}(fmol/10^{10} platelets)	396±41	679±46*
Kd (pmol/l)	70±5	87±7

*p<0.005 Values are ±SEM

IV-tryptophan loads observed in patients during antidepressant drug therapy.

Cowen et al[17] investigated whether treatment with a variety of tricyclic antidepressants, would alter LSD binding to the platelets of depressed patients (utilising ^{125}I-iodo LSD). Treatment with tricyclic antidepressants increased the number of ^{125}-I-iodo LSD receptor binding sites without significantly altering the affinity. See Table 3.

In a similar experiment[12], but utilising [^{3}H]-LSD binding to the platelets of normal subjects taking Desipramine for 16 days, the B_{max} of LSD binding rose considerably. In that study, the same normal subjects underwent testing of the prolactin response to tryptophan which is believed to be 5HT mediated. Prolactin response to tryptophan was enhanced and this enhancement correlated with the increase in platelet LSD binding.

These studies emphasise that whilst psychotropic drugs given chronically cause clear neuroadaptive responses in the brains of animals and man,adaptive responses can also be demonstrated in patients platelets. It is not clear at the moment whether these mirror precisely the brain changes or not, and much more work needs to be done to settle this point. Chronic neuroadaptive responses to psychotropic drugs might well be a factor in producing the generally poor correlation found between drug plasma levels and clinical response.

1. Aronson JK, Grahame-Smith DG, Hallis, KF, Hibble A and Wigley FM (1977) Br J Clin Pharmac 4:213-221.

2. Ford AR, Aronson JKA, Grahame-Smith DG and Carver JG (1979) Br J Clin Pharmac.8:125-134.

3. Ford AR, Aronson JKA, Grahame-Smith DG, and Carver JG (1979) Br J Clin Pharmac 8:135-142.

4. Boon NA, Aronson JKA, Hallis KF, White NJ, Raine AEG and Grahame-Smith DG (1984) Clin Sci 66:569-574

5. Boon NA, Pugh SE, Hallis KF, Aronson JKA and Grahame-Smith DG (1986) Br J Clin Pharmac .22:27-30

6. Rapeport WG, Aronson JKA, Grahame-Smith DG and Harper C (1986) Br J Clin Pharmac 22:275-279.

7. Kennedy DG, Bloomfield JG, Aronson JKA, and Grahame-Smith DG (1987) Br J Clin Pharmacol 232:639.

8. Aronson JKA (1986) In:Cardiac glycosides, 1785-1985. Biochemistry-Pharmacology-Clinical Relevance. Eds. Erdmann, E., Greef, K.B. and Schou, J.C. Steinkopff Verlag, Darmstadt, Springer Verlag, New York, pp531-533.

9. Aronson JKA and Ford AR (1980) Art J Med (New Series XLIX),pp273-282.

10. Godfrey PP, Bochner F and Grahame-Smith DG (1986) Br J Clin Pharmac 22:443-447.

11. Breckenridge A and Orme M L'E (1973). In: Biological effects of drugs in relation to their plasma concentrations. Eds. Davies, D.S. and Prichard, B.N.C.. MacMillan Press Ltd. London. pp145-153.

12. Grahame-Smith DG, Geaney DP, Schachter M and Elliott JM (1988) Experientia. 44:2:142-145.

13. Mills DCB and Roberts GCK (1967) Nature. 213:35-38.

14. Hefez A, Oppenheim B, and Youdim MBH (1980) In: Enzymes and Neurotransmitters in Mental Disease. pp77-93. Eds. E. Usdm., K.L. Sourkes and M.B.H. Youdim. John Wiley, New York.

15. Geaney DP, Schachter M, Elliott JM, Grahame-Smith DG (1984) Eur J Pharmac 97:87-93.

16. Grahame-Smith DG (1988) New Concepts in Depression Eds. M. Briley and G. Fillion.. MacMillan Press Ltd., London.2:1-14.

Discussion - Dose-response relationships in respect of the biochemical effects of drugs and the problem of adaptative responses

L. Lasagna

I am always struck by data showing serum digoxin levels in patients with and without signs of digitalis toxicity. The digoxin level situation is truly complicated, not only because of potassium but also because of calcium and the level of autonomic activity. It has always seemed to me that the textbook values that are given for the therapeutic and toxic range are totally ridiculous, and I wonder what you tell your students about serum digoxin levels.

D.G. Grahame-Smith

What I say is that first of all they have to suspect that the patient may be toxic. If there are enough clinical grounds to suspect digitalis toxicity, the serum potassium is normal and the patient is not in very bad renal failure, I will assume that a plasma level of, say, 3 ng/ml indicates digoxin toxicity and I will reduce the dose or stop it for a while.

J. Hernández

Do you think that the beta-blocker withdrawal syndrome and the clonidine withdrawal syndrome are dose-related phenomena? Can they be avoided by dose adjustements?

D.G. Grahame-Smith

Yes. I think that pharmacology would tell you that if you cut down the dosage of clonidine extremely slowly, the withdrawal response will not develop, and I suspect that the same applies to the beta-blockers as well.

M. Orme

Can you comment on the possible changes in pharmacological response from time to time, depending upon factors affecting the actual pharmacodynamic effect of the drug?

D.G. Grahame-Smith

It is obvious that changes in potassium levels may affect the response to digoxin, and changes in oestrogen level may modify the response to apomorphine, but other factors should also be taken into account. Thus, a patient with depression may respond to an antidepressant drug at one time and not at another time. Occasionally, and for reasons that I am not quite clear about the administration of triiodothyronine or lithium enables a patient to respond to a tricyclic antidepressant. In this case, an external factor is priming the response.