Safety Testing of New Drugs

Laboratory predictions and clinical performance

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Introduction

D. R. LAURENCE, A. E. M. McLEAN and M. WEATHERALL

When a doctor prescribes a drug, he should be aware of the specific effects he hopes to achieve and of the unwanted effects which will occur if the dose is too high or the patient reacts abnormally. He will probably take for granted that the drug will not cause any of a very large number of possible unwanted effects in the individual before him, and he will nearly always be right in this respect. However, in the last fifty years, many new drugs have been introduced. Among them a few have caused grave alarm by producing serious unwanted and unexpected effects. Some of these have remained in use, but others have been withdrawn with losses to therapeutics and to their manufacturers. An enormous amount of labour has been expended on devising tests *in vitro* and experiments on living animals to make quite sure that there will be no more serious alarms or accidents. But the results of this labour have been sadly disappointing. The number of valuable new drugs introduced has declined (May *et al.* 1983), but the proportion which have caused alarm has probably changed very little. However, the procedures intended to confer greater safety have become enshrined in the legal requirements of many countries, although their efficacy is questionable. The purpose of this work is to examine the procedures which were adopted for a number of undoubtedly useful new drugs marketed more than seven years ago, and to see how far subsequent experience in the clinical use of the drugs has reflected benefits from the testing procedures adopted.

New drugs are valuable for the cure or alleviation of previously incurable diseases, and as part of the stepwise improvement of existing remedies of all kinds. The discovery of new drugs can happen in various ways, ranging from chance observation to planned research. But the full development and manufacture of potential new remedies needs resources which only the pharmaceutical industry provides. In the course of its activities in the last century or so, the industry has had many successes and some disasters, of which the thalidomide tragedy is the most notable. The disasters have led to

increasing attempts by the industry to detect potential dangers of new remedies before they are made widely available (Report, 1964) and to very substantial growth of government regulations in most of the developed nations of the world (Report, 1966). The regulations are now seen in many quarters to have become excessive, to the extent of hampering the introduction of valuable drugs without conferring the hoped-for benefits of greater safety (Report, 1976; Lasagna, 1979; Weatherall, 1982).

Among the reasons for this situation is the lack of standing of toxicology as an experimental science relevant to man. It is obviously dangerous and impractical to administer new chemical entities to humans with no knowledge of their pharmacological and toxicological properties, and the necessary information is obtained by experiments in animals. Such information is, broadly considered, of great value, but variations in the reactions of different species, and of individuals within a species limit the predictive reliability of such evidence. Many toxicologists work entirely in laboratories remote from the practice of medicine and do not have experience of real clinical problems. Extrapolation from data obtained in animals is liable to be naive in such conditions. As long as favourable properties are being studied, the worst that can go wrong is that a substance, promising in animals, does not work so well in man, and little is lost by its trial. But if a substance has unfavourable properties in animals, there is a *prima facie* incentive to caution which may lead to abandonment of a good remedy before its benefits are properly assessed and its risks understood.

The methods of assessing toxicity in animals are largely empirical and unvalidated (Report, 1978). It has become common practice to use large numbers of animals in each experiment, so they are costly. Most of the experiments are done in industrial laboratories and the information obtained has considerable commercial value; it is submitted in confidence to regulatory authorities, and much of it is usually not made public. For both these reasons, the ordinary criterion of scientific reliability (that the results are reproducible in other laboratories) does not operate. The details are not widely known, and the cost of further experiments on the same lines is prohibitive even if there are good academic or commercial reasons for doing them. When the methodology of conventional test procedures is discussed it becomes evident that quite small differences in procedure can have large effects on the outcome, and so on the inferences which may be drawn from them. It is urgently necessary to know whether the tests as in fact conducted have sufficient predictive value to be justifiable, or whether they are a colossal waste of resources to no good purpose.

In order to make such judgements, a number of case histories have been assembled with details of the experiments done in animals, the predictions made from them before the substance tested was administered to man, and

the actual outcome both in terms of therapeutic benefit and of ill-effects whether predicted or unforeseen. These case histories have been provided by the courtesy of four pharmaceutical companies—Imperial Chemical Industries plc, Sandoz Ltd, Smith, Kline and French Laboratories Ltd. and the Wellcome Foundation Ltd. Their publication is a substantial departure from normal practice, and we are deeply grateful to the companies for their generous collaboration. This sequence of reports, and possibly more to follow, will provide a source of "case-law", invaluable in planning future studies and in deciding whether any particular procedure has, in fact, ever conferred some worthwhile protection which justified its cost.

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2

Bethanidine

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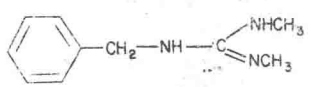
I. Summary

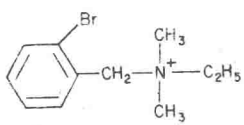
Bethanidine (N-benzyl-N′N″-dimethylguanidine sulphate) is an adrenergic-neurone blocking agent introduced into the U.K. and other countries in 1964 for the treatment of hypertension. It was first synthesized in 1960 and received clinical trials from 1961 onwards, while preclinical studies were continuing. These studies consisted of acute pharmacological observations in cats, dogs and monkeys, and longer experiments with daily doses to cats and monkeys for two or six months, also to rats for up to twelve months. The acute experiments showed no important effects other than those due to adrenergic blockade. The longer experiments did not show any tissue damage attributable to the drug. No ill effects were observed on the foetuses of pregnant rats and rabbits except after doses sufficient to cause maternal deaths. Impaired fertility was shown to be due to failure of ejaculation caused by sympathetic blockade. No carcinogenic properties were found. In brief pharmacokinetic studies, the drug was found to have a selective affinity for adrenergic neurones. Some interactions were noted with catecholamines and with drugs affecting amine uptake and storage mechanisms. The blood pressure of renal hypertensive rats was reduced by the drug. Clinical use of the drug in the U.K. reached a maximum about ten years after it was introduced, with about 400,000 prescriptions issued per year. Reported adverse reactions were infrequent and most could be related to impaired autonomic function. The case history exemplifies the standards initially used by U.K. regulatory authorities. Some of the preclinical experiments were essential in selecting one compound among many candidates for trial, and the predictive value of the experiments, as far as it was tested, was good. The longer term experiments were reassuring but remain of unproven value as evidence.

II. Introduction

During the 1950s the control of hypertension depended on the use of ganglion blocking agents. The treatment was unsatisfactory because the drugs produced parasympathetic as well as sympathetic blockade and so caused many unwanted effects. A considerable advance was made by the first introduction to medical practice of a specific adrenergic neurone blocking agent, namely, bretylium, which was synthesized and developed at the U.K. laboratories of the Wellcome Research Foundation (Boura *et al.*, 1959a, b; Boura and Green, 1959) and marketed in the United Kingdom in 1959. It was effective in clinical trials and early clinical use, but its use declined rapidly because tolerance, which had been anticipated from studies in cats (Boura *et al.*, 1959b), developed to an extent which made it inadequately effective (Dollery *et al.*, 1960; Turner, 1960; Johnston *et al.*, 1962). Also, a number of subjects developed parotid pain during treatment (Laurence and Rosenheim, 1960: Turner, 1960). At about this time guanethidine was discovered (Maxwell *et al.*, 1959, 1960) and became acknowledged to be a better drug for blocking adrenergic neurones in clinical practice. Meanwhile, the line of research which led to bretylium had been extended, and of the many compounds examined in animals (for review see Copp, 1964) five were investigated in man. Three of these are referred to in the literature (Boura, *et al.*, 1960a, 1961; Montuschi and Pickens, 1962; Boura and Green, 1963). The one which proved best, N-benzyl-N′N″-dimethylguanidine sulphate, was named bethanidine (Montuschi and Pickens, 1962; Smirk, 1963; Prichard *et al.*, 1968) and introduced into general use in the U.K. in 1964. This was the time at which



Bethanidine



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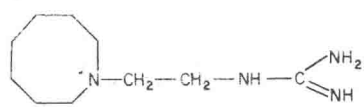

Guanethidine

a voluntary system of control of new drugs in the U.K. (the Committee on Safety of Drugs or "Dunlop Committee") was beginning to operate, and the submission on bethanidine was among the earliest to be considered. No precedent existed, and the submission is therefore interesting as a model of the standards which were regarded as acceptable at that time. Bethanidine was marketed in many other countries, but the submission, like many other submissions, particularly on cardiovascular drugs (Wardell, 1974), was delayed by the American F.D.A. An application made by A. H. Robins and Co. in 1975 was approved only in 1981. The drug has not so far (1983) been marketed in the United States. It has remained in clinical use in other countries and, as discussed later, has a negligible record of adverse effects other than those attributable to its main pharmacological action.

III. Preclinical and toxicological data

The laboratory data accumulated on bethanidine between the time of its discovery and the time of marketing were initially the subject of detailed internal Company reports. The first two reports, of November 1960, were provided to the original clinical investigators, and were the basis for the first trials of the compound in man. Further information was made available as it accumulated, and the complete set formed the basis of the submission to the Committee on Safety of Drugs, and the justification for initial marketing. The main pharmacological findings were subsequently published (Boura and Green, 1963; Green and Robson, 1964, 1965; for reviews see Boura and Green, 1981; Green, 1982; Maxwell and Wastila, 1977).

The summaries of the first two reports were worded as follows:

"*Summary of important biological properties*

The main action of this compound is to block adrenergic nerve mechanisms. Its effects are similar to those of guanethidine and bretylium, with both of which 467C60* has some chemical similarity. It is however more potent than either of these agents and appears to be fully absorbed from the alimentary tract. Its duration of action is similar to that of guanethidine. In acute tests in animals there is a wide margin between the dose which blocks the adrenergic mechanism and that producing toxic effects. The main toxic action appears to be paralysis of the respiration. Chronic tests have revealed no cumulative toxic action. Animals given daily injections of the drug develop some tolerance as to guanethidine and bretylium and likewise this is associated with the "denervated" smooth muscles becoming hypersensitive to noradrenaline and adrenaline".

*467C60 was the code number used at that time for bethanidine.

and

"*Summary of chronic toxicity studies*
The drug has been given daily to rats, cats and monkeys for periods of up to 2 months. The amounts given were at least 20 times the amounts causing a substantial depression of adrenergic nerve function in cats. No toxic action was apparent in rats given 10 or 50 mg/kg orally. Subcutaneous doses of 50 mg/kg retarded the growth in a a group of 5 cats, one animal dying after 2 weeks. In no instance was there evidence of tissue damage but some infiltration into the adrenal medulla was apparent in 2 animals; as such changes could not be found in rats or monkeys the significance of this finding is doubtful. There was an indication that daily administration of 50 mg/kg orally was toxic in 2 monkeys but 4 others were apparently unaffected. None of 4 monkeys given 10 mg/kg have shown adverse response during 2 months—they are being kept on the drug".

It is instructive to note the recommendations for dosage for clinical trials, which were included in the initial pharmacological report and ran as follows:

"*Recommended Dosage for Clinical Trials*
On the grounds that 467C60 sulphate is about 3 times as active as guanethidine in cats by injection and orally, we would expect the dose that will impair sympathetic activity in man to be 10–20 mg. This is also the expected effective dose from consideration of the amounts which caused some impairment of sympathetic function in cats, and dogs—these doses approximate to 0.3 mg/kg which is equivalent to 18 mg in a 60 kg man. We suggest however that no more than 5 mg should be given on the first occasion. The oral dose is likely to be similar to the injected dose.
If the drug is to be given intravenously there seems no contraindication to the amount administered being increased at intervals of 30 min. The main toxic action in cats is on breathing and this occurs with doses of about 10 mg/kg i.v. (600 mg/60 kg) and over 100 mg/kg when the drug is given subcutaneously or orally. The respiratory paralysis with an intravenous dose of 10 mg/kg in cats lasts only a few minutes and cats have been recovered from 15 mg/kg by application of artificial respiration. No necrotic action was shown by a 10 mg/ml solution when injected intradermally in guinea-pigs".

The estimates of potency and duration of action were based on studies of the relaxation of the nictitating membranes in cats caused by single subcutaneous doses. Studies of cumulative effects and of the depletion of the adrenergic transmitter had not been made at the time. It is now recognized that daily doses of guanethidine are highly cumulative in animals and rapidly cause substantial depletion of tissue amines by comparison with bethanidine (for review see Maxwell and Wastila, 1977). This probably accounts for the recovery from sympathetic blockade following a course of treatment, with bethanidine being much more rapid than after guanethidine treatment in man as in laboratory animals. In all other respects the statements provided accurate predictions relevant to the action, potency and safety of the drug in man.

At this time (1961–63) no requirement existed for official authorization of clinical trials, and these proceeded satisfactorily and promptly. More prolonged studies of toxicity in animals were proceeding at the same time, and reports on them were available at the time when the Committee on the Safety of Drugs was preparing to operate with the voluntary co-operation of the pharmaceutical industry. Summaries of two of these reports, as submitted to and used by the Committee read as follows:

A. Chronic Toxicity Studies

"No contraindication to the use of bethanidine for controlling hypertension in man by blocking adrenergic nerves has been found in the experiments described in this report. Large doses of bethanidine have been given daily to rats for 12 months and to dogs, cats and monkeys for up to 6 months. The drug was given orally except in the cats, for which the subcutaneous route was chosen. Records were kept of growth. Haematological and clinical biochemistry examinations were carried out at intervals. At the termination of the experiment the main organs were weighed and a detailed histological study made.

Doses of up to 10 mg/kg, equivalent to about ten times the amounts required to depress sympathetic nerve function, showed no toxic effect whatsoever in any of the species used.

In the rats a lethal effect occurred occasionally soon after drug administration when the dosage was 50 mg/kg but a few animals survived 500 mg/kg daily for 12 months. No other untoward effects were observed except retardation of growth with the 500 mg/kg doses. A few organ weights showed some minor deviations from control values and in the 500 mg/kg group erythrocyte counts were slightly reduced.

No untoward effects occurred in dogs given 20–25 mg/kg bethanidine daily but growth was retarded by 80–100 mg/kg. A few minor variations in organ weights were observed. The blood picture suggested that a slight non-progressive haemodilution occurred after giving drug for a few weeks.

Subcutaneous doses of 50 mg/kg in cats were well tolerated for a period of 6 months.

All monkeys given 10 mg/kg bethanidine daily by stomach tube for 6 months and 4 of 6 given 50 mg/kg daily for 2 months were unaffected. In the group on the higher dose, one animal began to lose weight after a month and another died unexpectedly after 7 weeks. No satisfactory explanation of their illnesses were found. The examination of the tissues did not show any structural change thought to be due to drug action.

At least some of the minor organ weight variations may be attributable to adrenergic neurone blockade and some of the variations were not consistent between species".

B. Studies in Pregnant Animals

"Daily administration of bethanidine to pregnant rats and rabbits produced no harmful effect on foetal development even when the drug was given at doses greatly exceeding those used for treating hypertension in man. When the dosage was sufficient to cause some maternal deaths and a marked reduction in weight

gain, at a level of 100–200 mg/kg by stomach tube, a small but significant increase in embryonic deaths was found in rats. No contraindication to the use of bethanidine during pregnancy is suggested by these experiments".

C. Further Studies

When male and female rats receiving approximately 50 mg/kg bethanidine daily in their diet were mated after receiving drug for 6 to 9 weeks markedly reduced fertility was found. Comment on this preliminary experiment ran as follows.

"The experiment suggests that the impairment of fertility caused by bethanidine may be due to failure of the males to impregnate the females with adequate spermatozoa. This possibility is in keeping with reports that ejaculation is suppressed by the drug in man. Like other adrenergic neurone blocking agents bethanidine apparently blocks the sympathetic mechanism on which ejaculation depends. Confirmation that this is the cause of the reduced fertility in rats on bethanidine is being sought by withdrawing the males from drug treatment. The experiment also shows that lactation is fully satisfactory in bethanidine treated animals and that the growth and development of their litters is not impaired".

A further brief report ran as follows:

"*Assessment of Carcinogenicity*
Experiments described [in an earlier report] in which large doses of bethanidine have been given daily for a year in rats and for 6 months in dogs, cats and monkeys did not reveal any carcinogenic properties.
The possibility of bethanidine having a carcinogenic action in animals is being explored further by continuing drug administration for 2 years in rats and 80 weeks in mice".

The "large doses" referred to are those described above in the paragraph headed "Chronic Toxicity", i.e. up to 500 mg/kg daily by stomach tube in rats, up to 80–100 mg/kg on five days per week orally in dogs, up to 50 mg/kg daily subcutaneously in cats and up to 10 mg/kg daily by stomach tube in monkeys.

D. Pharmacokinetics

Observations on the distribution and excretion by cats of ^{14}C-labelled bethanidine had already been published in 1962 by Boura *et al.*, and formed part of the submission to the Committee on Safety of Drugs. Estimations were reported on the quantity of ^{14}C in twenty organs and tissues of two cats, and in nerves and ganglia from eighteen sites, together with details of urinary excretion after subcutaneous and after oral administration, each in two cats. A search for metabolites showed little evidence of chemical change *in vivo*. The selective affinity of the drug for adrenergic neurones was demonstrated.

This affinity accounted for its specificity of action, as had previously been found for bretylium (Boura *et al.*, 1960b; Green, 1960).

On this basis, bethanidine was agreed by the regulatory authority in the United Kingdom as being safe for marketing, and its use began in various countries. Toxicological studies continued, and a number of out-standing points were clarified as follows.

E. Continuation of Reproduction Studies

As already mentioned the finding that the fertility of rats was impaired when both sexes were receiving bethanidine led to investigation of the effect of withdrawing the drug from the males only. Impairment of fertility did not persist, thus supporting the earlier supposition that the only significant adverse effect of bethanidine on reproductive processes is the expected impairment of the ejaculatory mechanism which is dependent upon sympathetic innervation.

The study of foetal development in rabbits was extended by dissection of two formalin fixed foetuses from each of 11 litters from does treated with bethanidine sulphate (150 mg/kg per day orally from days 8–16 of pregnancy) and from 2 litters of control animals run at the same time. "No abnormalities were found."

F. French Visa

In order to obtain a "visa" for marketing in France, it was necessary to satisfy the demand for a direct comparison of the toxicity of pulverized bethanidine ("Esbatal") tablets with the pure drug by daily administration for three months in young rats. The doses of each were 10 or 50 mg/kg of active ingredient per day. No effects were discovered on weight gain, survival or behaviour. No haematological or histopathological change attributable to drug action was found.

G. Continuation of Carcinogenicity Studies

The two studies mentioned above were carried out primarily with the objective of discovering whether malignant changes might arise as a consequence of treatment with the drug. In one the compound was given to young rats at a dose of 20 mg/kg per day for two years. In the other, 50 mg/kg of bethanidine was given daily to mice for 80 weeks. Neither study revealed an adverse effect attributable to the drug. The organs examined after completion of dosing in both studies were kidney, liver, lung, spleen, heart, brain, pituitary, adrenal, thyroid, thymus, ovary, uterus, prostate, seminal vesicle,