PHARMACOLOGICAL SCREENING OF NEW CHEMICAL ENTITIES IN HUMAN SUBJECTS AND METHODS OF DATA HANDLING

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- 1 A system is described for screening new chemical entities in normal human subjects, which is designed to search for novel drug actions at an early stage of drug development.
- 2 In single (subject) blind experiments, doses are increased incrementally from one subject to the next, with immediate feed-back of results from each dose.
- 3 As many physiological variables as possible are recorded, for which a purpose-designed apparatus system was constructed, and connected to a computer.
- 4 To overcome problems of inter-subject variability, the need for sensitivity, and to identify drug-related effects from the mass of data generated, a novel data management system was developed, and physiological profiles of volunteer subjects were built up to provide control data.
- 5 Results from a validation experiment in which isoprenaline was infused intravenously at different rates are presented and discussed.

Introduction

Drug research and development is now such a lengthy process that 5 to 10 years may elapse before reasonable certainty of having developed a therapeutically useful drug is obtained, during which time a very large investment of resources of all kinds is made. A particularly important approach to discovering novel or unexpected types of drug action is to investigate as early as possible selected new chemical entities (NCEs) in the human species (Medico-Pharmaceutical Forum, 1976).

The first opportunity during drug development to look for novel actions in humans occurs when the pharmacological actions of NCEs are investigated in normal subjects for the first time. Although there are types of therapeutic activity for which no predictive evidence can be obtained at this stage, nevertheless an opportunity exists to identify novel actions which might provide the basis for some types of therapeutic effects. Furthermore, some effects might indicate possible adverse effects in humans and grounds for rejecting the compound as early as possible.

The general role of clinical pharmacology in discovering new types of drug actions has been discussed in detail elsewhere (Sullivan & Fell, 1976).

This paper is concerned specifically with the role of early pharmacological studies of NCEs in normal volunteers. The objective of the work has been to develop a screening system designed to provide a means of alerting clinical pharmacologists to the possibility of novel drug actions in humans. For this purpose a new approach has been adopted towards methods of screening NCEs, and a technique for handling the data generated has been developed.

Methods

In order to maximize the chances of detecting a novel drug effect the nature of which is not likely to be foreseeable, as many variables as possible are selected for recording and measurement, subject to the constraints of technical feasibility and to the need to generate enough reliable data for each variable. Five to ten male volunteers take part in the screening of each NCE, the experiment being carried out according to a single (subject) blind design. At this early stage of drug development, it is a constraint

upon the experimental design that in the interests of safety each volunteer takes not more than two doses of a compound. Hence, although a dose-response relationship is sought, this has to be done by sequentially increasing the dose from subject to subject rather than by comparing different doses in the same subject. Each volunteer takes part in two experiments under identical conditions, receiving active compound in one and a matching dummy in the other, in a randomized order. A rapid feed-back of the results of each experiment is obtained before proceeding to the next, so as to exclude foreseeable hazards and to decide upon the dose for the next one.

Each experiment consists of physiological recordings and measurements and the collection of biological samples intermittently over three time periods of about 1 h each, one before, one during and one after administration of the compound. Many observations are carried out with the subject in both the recumbent and upright positions. There are variable rest periods between each of the three recording periods, the duration of each rest depending upon the expected duration of action of the compound.

Whenever possible, the compounds are administered by slow intravenous infusion. This route is preferred particularly on the grounds of safety, because the infusion can be stopped at any time should the need arise, whereas the whole of an oral dose is potentially committed to absorption after it has been ingested. Furthermore, the dose and response can be directly related after intravenous administration.

Written informed consent is obtained from all the subjects who take part in the screening experiments. All are volunteers from the staff of Fisons Limited. Careful attention is paid to safety, and all screening experiments are carried out in accordance with guidelines set out in Principles for the Clinical Evaluation of Drugs (WHO, 1968) and the Report of the Committee to Investigate Medical Experiments on Staff Volunteers (Association of the British Pharmaceutical Industry, 1970). Approval of protocols and control of the work is exercised by an Ethical Review Committee. This is composed of senior medical, scientific and lay members of Fisons staff, and of external independent medical members. The latter comprise a medical member in full time clinical practice, and a Professor of Therapeutics at a local university medical school. A record is kept of all the experiments in which each volunteer participates, to ensure that no volunteer is exposed to a NCE more often than at six monthly intervals, and usually less often than this.

The following data are collected during each of the three time periods comprising the experiment: i) ECG Lead II, from which measurements are made of amplitudes and time frequencies every five minutes.

ii) Pulse rate. iii) Blood pressure, using a random zero sphygmomanometer (Gelman-Hawksley). iv) Skin blood flow is measured in selected areas where it is predominantly controlled by α -adrenergic induced vasoconstriction (pinna of the ear) or by β -adrenergic vasodilator action (over the zygoma) (Phillips, Woolnough, Marinova & Turner, 1972), using photo-electric transducers (Grass). A non-invasive method is used for providing an index of the inotropic action of the heart: the time interval between the onset of the R-wave of the ECG and the arrival of the pulse wave at the radial artery in the wrist is measured, representing an inverse function of cardiac contractility, dp/dt max (Jackson, 1974).

A three channel EEG is recorded from leads O1-CZ, O2-CZ and FP1-O1 using platinum needle electrodes (International 10-20 nomenclature). The results from lead O1-CZ are computer analysed by period analysis (Shapiro & Glasser, 1974), the two other leads being used to check for hemispheric differences and artefacts. During the EEG recording the subject carries out an alerting task, while lying quietly with the eyes closed, which consists of maintaining continuous pressure on a resilient button, release causing a buzzer to sound loudly. He also carries out intermittent self-rating assessments of psychological variables of mood and alertness, and physical wellbeing, using analogue scales (Norris, 1971) presented on a video display unit. Psychometric tests include the perceptual maze test (Elithorn & Telford, 1969) and a complex reaction time test. Other psychometric tests, such as for motor coordination, are included if indicated by the likely nature of the test compound. The subject is encouraged to volunteer any subjective symptoms which seem to be unusual.

The volume of salivary secretion induced by citric acid crystals is measured after holding the crystals in the mouth for 15 s and then collecting saliva for 2 min. Tremor is measured by a piezo-electric accelerometer (Grass) attached to an extended finger and recording movement on a polygraph. Measurement of eye convergence and divergence is carried out using a Maddox Wing (Hannington-Kiff, 1970).

Depth and rate of respiration are indirectly measured using a copper/constantan thermo-couple (Grass), attached to one of the external nares. Lung function measurements of FVC and FEV₁ are carried out using a Vitalograph dry wedge spirometer when the subject is in the upright position.

Blood samples are collected at intervals of about 30 min at the beginning, middle and end of each cycle, for studying the pharmacokinetics and metabolism of the test compound, and for haematology and blood biochemistry tests. Mutagenicity studies are performed on small lymphocytes collected during and one week after the experiment. These are cultured and examined for chromosomal damage.

Figure 1 A block diagram showing the assembly of apparatus modules into an interactive system for recording physiological variables in human subjects.

Measurement of pharmacologically active substances present in blood and urine may also be carried out when appropriate, as also are immunological tests on blood serum and cells. The urine is tested before and after NCE administration for the presence of protein, dextrose and blood, using Multistix method.

Owing to the wide range of variables being recorded simultaneously and also to the consequential data management problems, it has been necessary to construct a purpose designed apparatus system which is connected to a computer. The items of apparatus which are integrated together into this system, by means of a patch panel (Selectroboard), are as follows: Two polygraph recorders (Grass models 78 and 79C), two analogue tape recorders (Phillips Analog 7), a four channel tape recorder (Hewlett Packard 3960A Series), a signal generator and two oscilloscopes (Hewlett Packard), and a Digital PDP8E 12 bit mini-computer containing 16 K words of core and 4.8 million words of disk backing store. A specialized video display unit

is also included in order to present computer controlled psychometric tests to the experimental subject. A block diagram of this system is shown in Figure 1. Although individual modules of apparatus are designed up to accepted standards of safety by manufacturers, when these were integrated together into a system additional earth cables external to mains leads, and a Faraday switch, were included in the mains supply circuitry in order to exclude certain foreseeable hazards (Pocock, 1972).

Analysis of data is carried out in a speculative, non-targeted way intended to detect any changes occurring with time, whether of a previously recognized type or not. For instance, the customary ways in which the ECG is normally analysed for clinical purposes will not necessarily identify a new type of drug action on the heart. Computer programmes were therefore prepared in order to analyse data in alternative ways. Because of the complexity of the experimental protocol, computer programmes were also developed to assist the

Figure 2 An example of the application of Cusum techniques to standardized dose-response curves. The abscissae represent a series of doses x , of increasing magnitude.

- (a) Possible dose-response curves where the ordinate represents the magnitude of the standardized response y .
- (b) Corresponding cumulants.
- (c) Fitted straight line segments.

The ordinates in (b) and (c) represent the cumulative magnitude of the standardized response z . In (c), $g = \tan \alpha$ where g is the gradient of the second segment of the fitted straight line, c being the inflection point of this segment.

experimenter to monitor and control the progress of the experiments, and also to control some of the tests used, such as the psychometric tests.

Since different doses of a NCE are administered to different subjects, and a within-subject dose-response relationship is not established, spontaneous physiological fluctuations are likely to be confused with drug-induced effects. In order to compensate for this a physiological profile is built up for each subject enrolled on a volunteer panel for these experiments, so that the type and range of variability of physiological measurements are known for each individual. The profile is compiled by collecting together all the measurements made during experiments in which that individual takes part, in which either a dummy treatment is given or recordings are made without any treatment. The profile continues to be built up cumulatively for as long as the subject remains on the panel, and it is used

as the baseline control by comparing these collected data with the results obtained when a NCE is screened, the subject thus acting as his own control.

Data management

The purpose of data analysis is to detect small drug-related changes of individual variables, particularly in order to make the experiments as safe as possible by administering the smallest possible number of doses of NCEs and the smallest unit dose sizes. The need for a new approach to data management was determined by the quantity of data generated, by the need for sensitivity, and to overcome inter-subject variability.

The obvious method of analysis would be to fit a dose-response curve to the observations made for each variable and each possible time. For such an approach either a sufficient number of measurements

to permit determination of the shapes of all response curves are required, or a knowledge of these shapes. Since neither of these conditions could be fulfilled under the conditions of the experiment, a different approach was necessary.

Cumulants are well known to be sensitive to small systematic changes, and they are also almost unaffected by unequal spacing of observations, but the usual cusum techniques (Woodward & Goldsmith, 1964) are not directly applicable to the data because they too require a large number of observations. However, as shown from examples in Figure 2a and b, the cumulants of standardized responses increase or decrease monotonically whatever the shape of the true underlying response curve.

It was therefore decided to use the cumulants of standardized data. In view of the limited scale of the experiments, it was considered impractical to wait for the cumulants to deviate to a predetermined extent, as is usual in tests of significance (Wetherill, 1969; Davies & Goldsmith, 1972). Instead it was hoped to identify a developing trend which might not have reached any predetermined level of significance.

The first stage of the analysis of data is begun after four or more doses of a NCE have been given, unless drug effects are gross enough to be readily detectable by inspection of the data before this. The physiological profiles (stored in a computer file) of all the subjects taking part in the experiment are pooled, together with the data from the dummy treatment experiments carried out during the particular study. Pooled estimates of within-subject variation for each variable are then obtained, the variation being expressed in terms of standard deviation.

In the experiments in which the NCE is to be administered, control data are collected during the first time period, prior to administration of the compound. In order to eliminate most of the inter-subject variation, and at the same time provide information about the response of the subject to the NCE, the mean value of the measurement for each variable made during this first control time period is subtracted from each value for the same variable measured during the two remaining time periods of the experiment, during and after administration of the NCE.

These values for the differences (expressed as s.d.) are then used to determine cumulative dose-response relationships for each variable at each particular time of measurement. To each of these, a pair of straight line segments is fitted by computer using a least squares minimisation technique.

The first segment up to point C on Figure 2c, corresponding to the cumulative responses at the lower doses with no effect, must have zero gradient, and the second, corresponding with those later (higher) doses where drug activity has an observable

effect, has either a positive or negative gradient. This simple model is the formalisation of an approach indicated by Chaput de Saintonge & Vere (1974). As the first line segment must pass through the origin and also have zero gradient, the method has the advantage of requiring the determination of just two parameters (these being the gradient and X-intercept of the second line segment). Although it is exact only in the case of a uniform change from the baseline level, this model is a reasonable description within the constraints imposed by low sample sizes for the various non-uniform dose-response changes (Figure 2c). As the emphasis here is on identification of possible trends rather than on significance levels, the approach concentrates attention on the value of the non-zero gradient rather than the variance ratio of fitting and residual sums of squares. This ratio is nevertheless calculated and recorded for possible future reference.

After the two straight line segments have been fitted the response is assessed on the basis of the inflection point C, and the gradient of the second segment, g (Figure 2c). These parameters can be respectively interpreted as the threshold dose for drug activity and the mean response for each active dose. The results are presented variable by variable. For each sample time, the coefficients of the curve fitted to the cumulants and the corresponding sums of squares are printed by the computer, together with a warning indicator if any of the gradients exceeds half a standard deviation in order to draw attention to possible drug effects. If the second segment contains fewer than two points beyond the inflection point C, no warning indicator is printed because such gradients are of little significance. The cumulants of the standardized data are available on a computer file, so that they and the fitted line segments can be plotted if required (Figure 3).

The aim of the second stage of the analysis is to examine the consistency and trend of any variable during the course of the experiment. This links the gradients g for each variable at the various sample times, $t_1, t_2, \dots, t_n$, following drug administration. Where there are less than five sample times no satisfactory procedure has yet been established and the results for each sample time must be assessed as if they were independent variables, on the basis of the parameters of the two-segment curve already fitted. When the number of sample times, n , is greater than five the values of g are used as the measures of drug activity, and their cumulative values are plotted against the sample times. This represents graphically (Figure 4) the predominating trends in both the dose and time dimensions.

Apart from trivial alternatives there are two basic possibilities for the time-response relationship. The first is that the compound is inactive during the initial samples before an active concentration is achieved,

Figure 3 Standard dose-related cumulants during and after isoprenaline infusion, derived from the first stage of data analysis (see text).

- (a) Inverse function of cardiac contractility.
- (b) Length of ECG R-R interval.
- (c) Amplitude of T-wave.
- (d) Depth of respiration.

The ordinates represent the cumulative magnitude of the standardized responses in s.d. The points on the abscissae at each sample time ($t_1, t_2, \dots, t_8$) represent individuals infused with isoprenaline at the following rates: 0.37, 0.75, 1.5, 3.0, 6.0, 12.0 $\text{ng kg}^{-1} \text{min}^{-1}$, plotted in order of magnitude. Sample times $t_1 - t_4$ were at 5, 10, 15 and 20 min after starting the infusion, and $t_5 - t_8$ were at 20, 25, 30 and 35 min after stopping it.

and the second that drug activity is present from the start but ceases for later samples. As the time-course of the trial is relatively short it is unlikely that an active period could be sandwiched between two inactive periods, and the cases of activity or inactivity throughout are just forms of the two basic patterns. On the basis of these two possibilities it is feasible to quantify the graphic trends obtained, by fitting a curve comprising two straight line segments to the cumulants of g already plotted.

There are two constraints on this curve, the first that the initial segment should pass through the

origin, and the second that one or other segment has zero gradient. If the least squares minimization yields a curve whose first segment has zero gradient this corresponds with the first pattern outlined above; if the second linear segment has a zero gradient, this corresponds with the second pattern (see Figure 4 for examples). The non-zero gradient and the time period during which it applies numerically summarize the mean response at those doses and times which demonstrate a consistent trend of drug activity.

Results are printed by the computer for the time-response relationship in the second stage of the

Figure 4 The cumulative values of the gradients plotted against sample times during and after isoprenaline infusion, derived from the second stage of data analysis (see text).

- (a) Inverse function of cardiac contractility.
- (b) Length of ECG R-R interval.
- (c) Amplitude of T-wave.
- (d) Depth of respiration.

Ordinates represent the cumulative values of the gradients at particular times, measured in s.d. The abscissae represent sample times t_1 – t_8 which correspond to Figure 3.

analysis in a similar way as for the first stage, and the graph for each variable is plotted. Warning indicators draw attention to gradients exceeding positive or negative values of 0.5 and 1.0 standard deviation which might suggest consistent, though possibly slight, drug induced effects. These levels were chosen in the light of experience with both real and simulated data.

Results

Experiments were performed to validate the sensitivity of the screening system and the data management. Since the sensitivity of the system is of special interest, doses were used ranging from subthreshold for pharmacological effects up to doses causing significant actions. Intravenous infusions of isoprenaline hydrochloride were given to six subjects in random order in a double-blind crossover

experiment, using physiological saline as the control solution.

Each infusion was continued for 20 min, recordings being made at various sample times before, during and after it. The rates of isoprenaline hydrochloride infusions given to different subjects were 0.37, 0.75, 1.5, 3.0, 6.0 and $12.0 \text{ ng kg}^{-1} \text{ min}^{-1}$. The sample times designated t_1 , t_2 , t_3 and t_4 were at 5, 10, 15 and 20 min after starting the infusion, and t_5 , t_6 , t_7 , and t_8 , were at 20, 25, 30 and 35 min after stopping the infusion.

Some of the results from this experiment are given in Figures 3 and 4. It is evident from Figure 3 that cardiac contractility was increased during the period of isoprenaline infusion, as shown by the decrease in the time interval measurements. Over the same time course, the height of the T-wave was reduced. Both returned to normal after stopping the infusion. The R–R interval (heart rate) underwent only a negligible change during this period. Respiration depth began to decrease during the isoprenaline infusion, and continued to do so after the infusion was stopped until the last sample time, which suggested that a reversion to normal might be starting. A summary of these effects of isoprenaline infusion as a function of time is shown in Figure 4, which puts into clear relationship the time course of the infusion of isoprenaline and the recorded changes of the four parameters.

Discussion

Since any attempt to discover a novel type of drug action implies ignorance about what is being looked for, it was decided to monitor the functioning of all the major systems of the body by measuring simultaneously as wide a range of variables as is technically possible. The screening system described constitutes an alerting system, designed to identify the possibility of novel drug effects, or of acute adverse effects, and no more. In order to establish the validity of possible drug-induced effects, a subsequent double-blind controlled experiment must be carried out. This limited aim is imposed by the design of the experiments, in which it is necessary to identify novel effects, differentiate between drug-induced effects and spontaneous fluctuations in the variables being measured, and establish a dose-response relationship in a small number of subjects who are given different doses of the compound.

The choice of the variables to be measured and the methods of analysing the data have to be kept continually under review, and varied from time to time for different kinds of compounds. It is advantageous to measure a wide range of variables simultaneously, because effects on different bodily

systems and actions with different dose-response relationships can be more readily correlated. It also economizes in the number of doses of NCEs given to volunteers, and also in the time and resources allocated to the investigation of each NCE. It is never possible to measure everything that may be thought desirable, and since certain procedures may interfere with one another priorities may have to be allocated; for instance respiratory function tests affect cardiovascular measurements, and changes of posture are likely to disguise drug action on peripheral blood flow. Furthermore some tests, such as psychometric tests, are time consuming to carry out and care has to be taken to ensure that a disproportionate amount of time is not devoted to them. The type of apparatus selected is determined by the choice of what is to be measured. Various items of apparatus therefore have to be available for fitting into a purpose-designed system connected to the computer.

The ability to differentiate between drug effects and spontaneous physiological variations is particularly dependent upon obtaining reliable baseline control data. The nature of the experimental design is such that it is necessary to establish physiological profiles of the experimental subjects so as to obtain such data. It has been shown previously that control baselines are unsatisfactory when unprofiled experimental subjects are selected at random, and standard 'normal' ranges are used as criteria of the limits of 'normality'. (Joubert, Rivera-Calimlim & Lasagna, 1975).

A unique system of data management and mathematical techniques was developed. The experimental design is such that it is not possible to attach statistical significance to the results obtained, therefore the objective is to obtain early indication of consistent changes from control values, even if the changes are relatively small. The sensitivity of the system is therefore of critical importance, and adequate sensitivity was demonstrated in the validation experiments described, in which the inotropic effect of isoprenaline was differentiated from its chronotropic effect at low infusion rates.

Published work indicates that the lower intravenous infusion rates of isoprenaline used in experiments with humans are usually of the order of $10.0 \text{ ng kg}^{-1} \text{ min}^{-1}$. With doses of this order separation of inotropic and chronotropic responses was not demonstrated in selected patients (Jewitt, Mitchell, Birkhead & Dollery, 1974) or in normal subjects (Easley & Rodbard, 1977). However, Crouchman (1973) showed a separation of chronotropic and inotropic effects when infusing a lower dose into patients with aortic valve prostheses, and so also did Jackson (1974), in a normal volunteer, using the same method of measuring inotropic activity as in the system described above.

Another observation of interest was that reduction in the height of the T-wave of the ECG was related to the inotropic effect of isoprenaline. Simoons & Hugenholtz (1975) showed that the effect of exercise in reducing the magnitude of the T-wave was related to changes in heart rate. However, since cardiac inotropic action was not measured, a relationship between the T-wave changes and cardiac contractility was not investigated in that study.

It may be argued that the chances of discovering novel drug actions under the conditions of these experiments, and at this early stage of drug development, are so remote that the value of such screening is questionable. But the screening system described is only the first part of a process of searching for novel drug action, which goes on throughout the development of a NCE. By itself it may be insufficient, but any possible novel approach to discovering new drug actions is worth exploring, because the success rate is so low when compared with the number of NCEs investigated. Furthermore, if NCEs are tested only for predetermined types of action, other actions which they may have will never be discovered because they are not looked for, or will only be found by clinical serendipity if and when compounds come into therapeutic use for a different purpose.

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