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SCREENING CENTRE FOR PHARMACEUTICALS

DP/ROK/86/003

REPUBLIC OF KOREA

Technical Report: Design of a general screen for pharmacological activity*

Prepared for the Government of the Republic of Korea
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

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Explanatory notes

The value of the local currency in July, 1987 was
800 won = 1 U.S. dollar

Abbreviations used:

ROK Republic of Korea
KRICT Korean Research Institute of Chemical Technology
CD Candidate drug (as synthesized by by a chemist for submission to a screening test for pharmacological activity)
min minute(s)
cns central nervous system
cvs cardiovascular system
h hour
LD_{50} median lethal dose, in this case determined approximately
sc subcutaneously
iv intravenously

Abstract

Screening centre for pharmaceuticals DP/ROK/86/003.

The object of the activity, which occurred in July 1987, was to advise and assist in setting up a pharmacological screening laboratory. Most of the visit was concerned with identifying the needs of the institute through discussions, and then writing a proposal for a general screen designed to detect pharmacological activity in new candidate drugs. The absence of any basic research and library facilities were identified as weaknesses in the present organisation, and recommendations were made to rectify these omissions. Advice is needed in the area of ligand binding, and a recommendation was made to overcome this problem. In general, the balance between the chemical and pharmacological effort is inappropriate: the latter facility needs considerable expansion.

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INTRODUCTION

I arrived at KRICT on Monday, 6th July, 1987, after being briefed in Vienna (3rd July) and Seoul (6th July). The activity of my visit to KRICT ended on 23rd July, so that I could be de-briefed in Seoul on the following day and in Vienna during the next week.

The objective of my visit to KRICT was to advise and assist in setting up a pharmacological screening laboratory. Although clear in general terms, I was unable to determine the exact scope of the iniative and my precise role, before my discussions with those at KRICT. One of my first tasks, therefore, was to clarify the objective, then assess the stage already reached in the development, and subsequently give advice and assistance that was appropriate.

At the time of my visit (July, 1987) the pharmacological screening unit was housed in two small laboratories, which were occupied by two Ph.D-level scientists, three UNIDP advisors and at least two technicians. Additionally, the range of equipment already purchased for installation in the new building is housed in this confined space. As the demonstration of experimental techniques was impossible, I was asked to propose a strategy for testing CDs. Chapter 1 gives details of this plan, which includes a list of equipment necessary for its operation, an estimate of the number and level of staff required, suggests a possible rate of evaluation of CD, and provides a basis for calculating the unit cost of evaluation.

Thus my period of duty at KRICT can be viewed as only an initial step in establishing the facility required. This much was attained, but the scientists at KRICT will have to decide if this is the right approach for them to take (there are alternatives). The successful implementation of my proposal will depend upon recruitment to KRICT of appropriate

staff, acquisition of relevant equipment, and reaction to this and subsequent advice, and to the programme of training.

RECOMMENDATIONS

It is very early in the establishment of pharmacology at KRICT, and no doubt many of the problems will be solved as the new building becomes operative and new staff are recruited. However, my recommendations, all to the directors of KRICT, are distinct from the logistical problems covered above.

1. Some basic pharmacological research should be instigated at KRICT as this would complement the screening approach to drug discovery.

2. An adequate pharmacological library should be provided at KRICT, so that the pharmacologists can keep in touch with developments elsewhere.

3. Advice is needed with respect to the design, conduct and analysis of ligand binding experiments. A Scientist such as Dr J. M. Sneddon, The William Harvey Research Institute, St. Bartholomew's Hospital Medical School, Charterhouse Square, London EC1M 6BQ, England, should be invited to act as a consultant in this respect. Dr Sneddon was the Sandoz Prize winner in 1973 (awarded to the most outstanding British pharmacologist under the age of 35) and has a distinguished research record. He is an expert in the interaction of naturally occurring and foreign compounds with biological tissues, and has made extensive use of radioactive techniques to monitor events in tissue culture. Apart from advice on ligand binding, Dr Sneddon would also be of help in the inflammation and cardiovascular programmes.

4. At this early stage for KRICT, some control should be established over the storage and labelling of drugs and CDs. There should be a central store for all drugs and CDs (permitting any retrospective chemical analysis, if the biological results require it, as they sometimes do). Samples should be labelled with the supplier's name, batch number and stability. A pharmacist could be given this responsibility.

In addition to these specific proposals, I think it is worth placing on record that a very considerable expansion in the biological capabilities of KRICT must take place. This will require the expenditure of <u>considerable</u> funds on equipment, and the recruitment of <u>many</u> staff. A really good synthetic chemist should be able to provide enough CD for about 5-10 pharmacologists. At KRICT there are many chemists and few pharmacologists. This disproportionate emphasis on chemistry will lead to failure of the screening unit unless a more appropriate balance of effort can be reached.

CHAPTER 1

PROPOSED GENERAL SCREEN OF CD FOR PHARMACOLOGICAL ACTIVITY

Context

After my various briefings (see Introduction), and particularly following discussions with Dr Roh at KRICT, it became clear that the Government wished to develop pharmaceutical research in ROK to an extent that would enable them to compete with the existing multinational companies in the process of drug innovation. KRICT has been established towards this end: with financial support from the Government and access to expertise via the UNIDO-UN DP agencies, the objective should be achieved.

KRICT will attempt to develop drugs itself from its already established skill in chemistry and the new (to Korea) disipline of innovative pharmacology. In addition to this role, it is envisaged that KRICT will act on a contract basis as an enabling facility for small-scale pharmaceutical companies in ROK, and furthermore set standards of excellence in performance that will be a 'pacemaker' for the emerging Korean industry.

The general modus opperandi adopted by KRICT is that of chemical synthesis of CDs with their evaluation for activity via pharmacological screening tests. For reasons already presented (see Introduction), I have confined my attention to the design of a general screen for useful activity, leaving the proposals for specific screens to my fellow advisors (see Figure 1). This chapter describes my proposal in general terms.

Objectives

The objective was to design a general screen that could be implimented at KRICT and would be capable of detecting useful pharmacological activity. To be of value the screening process must be rapid, and operatable by the existing and future staff of KRICT. Protocols for the component experiments have not been written at this stage because before this is done it is essential that:

a). the new building is functicning
b). the right equipment is ordered, delivered, and installed in working order
c). additional staff are appointed.

If required, I, or some other general pharmacologist, can demonstrate the simple techniques on a subsequent visit. Only at this stage can operating standards be set, protocols be evolved, and the calibration of tests be accomplished.

Assumptions

It is never possible to detect all useful pharmacological activity of a CD. The general screen has been designed so that no CD would be rejected on the basis of:

a). the result of a single experiment
b). results from a single species of animal.

The general screen has been made open-ended, so that additional tests can be added as more knowledge becomes available or technical capabilities develop (particularly as new labelled probes become available that permit new receptors to be studied in ligand binding experiments).

In addition to the general screen for all CDs, there will be specific screens for types of activity considered important by the directors of KRICT. No doubt CDs will be synthetised with a particular use in mind and for which they will be

tested, but these CDs should also be submitted to the general screen.

It is essential that there is a background of basic research and supportive reading of the primary literature associated with the general and specific screens, so that new information discovered elsewhere can be accommodated in the programme of tests, and the standards of performance and level of understanding do not remain static (see recommendations).

Description of the general screen

Figure 1 shows the structure of the general screen. Furthermore, it relates the general screen to those specific screens already identified as of interest to the scientists at KRICT. The individual tests of the general screen are described below.

Although administration of drugs to whole animals in the general screen is via the iv or sc routes in the descriptions that follow, this can be varied. Oral activity is a prerequisite for most drug usage in man, so should be demonstrated at some stage. However, activity can be missed if the initial screen relies on oral administration of CDs and there is poor absorption from this route. Because an active drug with poor bioavailability may represent the starting point in the discovery of a worthwhile new drug, the general screen relies on parenteral administration of CDs.

Test 1: Behavioural profile in mice (to be conducted in the cns laboratory).

Mice are injected iv with a CD and the overt effects observed according to a check-list of signs. This check-list could be transferred to a microcomputer, so that the operator responds to a sequence of computer prompts: this would ensure that the test is always completed. The test is based on that introduced by Irwin (1962, Science, 136, 123).

The Irwin test serves two functions:

a). by indicating the approximate LD_{50} value of the CD, there is the basis for the selection of an appropriate dose in the subsequent tests (e.g. 0.5 times the LD_{50} value, etc.

b). there is an indication of what tests should be employed subsequently, i.e. referral to the specific screens.

Test 2: Cardiovascular profile in the anaesthetised cat (to be conducted in the cvs laboratory).

Cats are anaesthetised and the trachea (for respiration), femoral vein (for iv administration of drugs) and artery (for arterial blood pressure and heart rate measurement) are cannulated. One ascending cervical sympathetic nerve is located, then cut preganglionically (acute decentralisation) and electrodes applied distal to the lesion and proximal to the superior cervical ganglion: the ipsilateral nictitating membrane is prepared so that tension in this muscle can be measured.

Control drugs are first administered iv, followed by control electrical stimulation of the nerve, then the CD is infused (iv in a fraction of the mouse LD_{50} value: see Test 1) and the control procedures repeated. Any direct effect of the CD, and any change in the reaction of the cat to the control measures is recorded. Essential control

drugs would include: angiotensin II, noradrenaline, tyramine and acetylcholine. The choice of any additional drugs would depend upon the particular activity sought in this experiment. Each CD would be applied to two such cats, and no one cat would receive more than one CD.

The purpose of this experiment is to:

a). select those CD with overt actions on the systems monitored.

b). select the CDs that interact with the control drugs.

c). show by the absence of an effect that some other detected action of a CD is not secondary to vascular changes.

Test 3: Prolongation of barbiturate-induced sleeping time in mice (to be conducted in the cns laboratory).

Mice are placed in a temperature-controlled room or cabinate (20°C). After a period of acclimatisation, they are dosed (sc) with a CD (at a fraction of the LD_{50}: see Test 1) or the vehicle for the CD (control). Thirty min later, the barbiturate (pentobarbitone or hexobarbitone) is administered iv, and the duration of sleep (signified by the lack of a righting reflex) determined. Ideally, control mice should sleep for 10 min. A prolongation of sleeping time (end the experiment when the mice have slept for 60 min!) indicates either:

a). a subtle depression of the cns (as is seen with some anti-histamine drugs)

or b). changed metabolism of the barbiturate.

Test 4: CD-induced changes in the physiological chemistry of rats

Rats are allowed water ad libitum but not food for 12 h before this test is performed. Each rat is placed in a metabolism cage so that urine uncontaminated with faeces can be collected. One group of rats receives vehicle (sc), whilst the other group is given 0.5 times the LD_{50} dose of the CD (also sc: see Test 1). Blood samples are taken from each rat immediately before dosing, and at 1 h and 4 h afterwards (pooled if necessary). Urine is collected over the 4 h period. Plasma and cells are separated by centrifugation, then all three biological materials are examined in an auto-analyser.

The substances measured depend upon the interests of KRICT, but should include:
urine: glucose, protein, blood, electrolytes, urea, uric acid.
blood cells: acetylcholinesterase, ATPase, electrolytes.
plasma: glucose, fatty acids, butyrylcholinesterase, electrolytes, thrombin levels, etc.
Any direct effect (e.g. diuretic or hypoglycaemic action) or indirect effect via hormones should be detectable by this test.

Test 5: CD profile on guinea-pig ileum in vitro

Segments of guinea-pig ileum are prepared for:
a). stimulation by drugs (acetylcholine, histamine, nicotine and barium ions)
b). electrical stimulation via co-axial electrodes (0.1 Hz, 1 ms, 80 v).

These tests detect the action of drugs on various pharmacological receptors, nerves, the receptor-effector coupling process, or contractility. These tests should be considered primarily as detectors of the limitations (side-effects) of other, more useful actions.

These tests should be performed twice, and should be situated in the in vitro or cardiovascular laboratory.

Test 6: Other tissues in vitro

Additional tests on isolated tissues (as required) should include some examination of CDs on skeletal muscle(e.g. the rat diaphragm stimulated via the phrenic nerve) and cardiac muscle (if guinea-pig atria were chosen, histamine causes tachycardia by an action on histamine H_2 receptors).

Ligand binding experiments

Although I do not possess the expertise to comment on these techniques in detail, it is clear that everybody searching for new drugs today employs this experimental capability. In many ways, the underlying principle of this approach to drug evaluation is the converse of that described above, because in tests 1-6 no presumption is made about the mechanism of action of the CD. However, with ligand binding studies, a key mechanism (here the pharmacological receptor) is first identified then CD are made in the hope of eventually interfering with the control process subserved by this receptor. If such a bodily control process is disturbed in a disease, the clinician will then have the ability to effect a change with the receptor-specific drug.

Performance of the experiment

First a preparation of membranes is obtained by homogenisation and centrifugation of a relevant tissue, usually rat brain. The membranes are then solubilized by a surfactant. A ^3H-ligand for a specific receptor is incubated with an aliquot of the membrane preparation, and after equlibrium has been established, the membranes are separated from the buffer by ultrafiltration, followed by washing. The radioactivity

associated with the membranes is counted by conventional means (A). This procedure is repeated in the presence of an excess of a second, unlabelled, drug that also has affinity for the receptor (B). The rationale of the experiment is that these two drugs (^{3}H-ligand and 'cold' drug) only compete for the receptor, their non-specific binding to other sites on the membranes being distinct. Thus by subtraction of (B) from (A), the specific binding is measured.

Subsequent determinations are made of the ^{3}H-ligand to the membranes in the presence of various concentrations of CD. The concentration of CD that reduces the specific binding by 50% is measured as the affinity of the CD for receptor under consideration.

Value of ligand binding experiments

The technique is simple, rapid, uses little CD, and is suitable for automation. However, there are many potential problems in the interpretation of the results; for example:

a). Is the binding subject to optical preference?
b). Does one molecule of drug interact with one molecule of receptor?
c). Is only one type of receptor involved in the binding?
d). Is there any co-operativity (where binding to one particular receptor changes the probability of binding to adjacent ones)?

It should also be remembered that with this technique there is no way to distinguish between stimulant and antagonist drugs, as only affinity and capacity are measured. In general, the agreement between values for the fundamental constant (equlibrium-dissociation constant, equivalent to 50% occupation of the receptors) between binding studies and isolated organ experiments is not always good. It is because the analysis is complicated that I suggest that expert advice is sought (see recommendations).

Operation of the general screen

Figure 1 indicates that all CD should be submitted to the general screen (this includes the ligand binding tests). Clearly the maximal rate at which CDs can be evaluated depends upon three factors:

a). the time to complete the slowest experiment (the rate-limiting step), which is the cvs profile in the cat.

b). the number of test units operated for each test (e.g. having two cat cvs profile set ups would double the rate of testing).

c). the proportion of tests that needs to be repeated.

Staff requirements for the general screen

TEST NUMBER	1	2	3	4	5
sets of equipment	1	2	1	8	2
tests/CD	1	2	1	1	2
staff: Ph.D	0	1	0	0	0
juniors	1	1	1	1	1
Rate of testing CD/week	16	8	16	8	8
Animals for each CD species	50 mice	2 cats	10 mice	8 rats	2 guinea-pigs

These estimates are made on the assumption that 4 full days are worked each week, the remaining time being allowed for basic research, reading and administration.

Thus if tests 3 and 4 were performed by the same person, 1 senior member of staff (Ph.D) and 4 juniors (first degree or technical qualification)should be able to test 8 CD/week. Additionally, 1 Ph.D and 1 junior would be required for the ligand binding studies. This makes the total team: 2 Ph.Ds, and 5 juniors.

It would be possible to scale up this operation (e.g. by two-fold, or three-fold, etc) to increase the rate of testing, with some economy of scale.

About 50 standard drugs would need to be examined in this general screen. If this calibration was done on a 'blind' basis, it would be possible to determine the actual rate of testing and the efficiency of the screen in detecting activity.

Equipment needed to operate the general screen

Test 1: No equipment, unless the prompts were made from a microcomputer, then one would be needed.

Test 2: Two units of equipment are needed to operate the basic plan (see P 17), so multiply the list below by a factor of two. I would suggest that you use polygraphs and associated equipment from Grass Instruments or equivalent and have indicated the type numbers from their catalogue.

Cat operating table
4-channel polygraph (model 79)
Four driver amplifiers (7DA)
Three bridge circuits (7P1 dc preamplifiers)
heart rate meter (7P44)
blood pressure transducer (Statham P23)
force-displacement transducer (FT 03)
iv infusion pump
ventilation pump
electronic stimulator (suggest Harvard Inst 50-7442 or equivalent)
electrode
tracheal cannula
rectal temperature with digital display
various clamps, X-blocks, etc

Test 3: Temperature controlled cabinate
eight clocks

Test 4: Eight metabolism cages
use of auto-analyser

Test 5: Four isolated organ baths (suggest Harvard 50-2146 or equivalent)
Two electronic stimulators (see Test 2 above)
isotonic transducer
Three isometric force-displacement transducers (FT 03)
Three Grass polygraphs (79), together with driver amplifiers (7DA) and preamplifiers (7P1): see Test 2.

One flatbed recorder
coaxial electrode
phrenic nerve electrode (if used)
Four rack and pinnion sets (small)
Four gas cylinders and heads for 95% O_2 5%CO_2
deioniser, still, and distilled water containers

Utilisation of the results of the activity

The procedure for the general screen has been discussed with the scientists at KRICT, but I do not know if they intend to implement the plan. The utilisation of the plan would depend upon appropriate recruitment of staff to KRICT, and the provision of equipment.

Conclusion

No conclusion can be drawn at this stage. Subsequent visits by myself, or somebody of equivalent experience and background, will be needed to effect the proposal.

CHAPTER 2

REPORT OF SEMINARS PRESENTED AT KRICT

Currently, there are few staff qualified in experimental pharmacology at KRICT. Consequently, the seminars I presented were of a very general nature, and were all related to the main activity (see Chapter 1). The seminars were as follows.

1: Stategy of the search for new drugs, delivered on Thursday 9th July in Seoul. About 70 attended from various pharmaceutical organisations, including KRICT.

I reviewed the process of drug development and indicated the small but essential part occupied by detection of new pharmacological activity. The origin of screening tests was next described, and how drug treatment could be related to our current view of disease. Types of screeningtest were classified according to the level of biological organisation associated with the measurements. Finally, the importance of performing screening tests in conjunction with basic research and detailed reading of the scientific literature was emphasised.

2: Advantages and disadvantages of the use of screening tests to detect useful pharmacological activity, delivered on Thursday, 16th July, at KRICT.

This seminar was basically similar to the one above, but concentrated particularly on the problems of missed activity, false-positive results and other weaknesses of screening tests.

3: Receptor theory and introduction to ligand binding, presented at KRICT on Monday, 20th July. The advantages of receptor theory in the search for new drugs was reviewed. The section on ligand binding experiments contained much of the argument covered in Chapter 1.

4: Use of isolated organ experiments in drug discovery, presented at KRICT on Tuesday, 21st July. There was little experience of isolated organ experiments in the scientists at KRICT, and there was no apparatus for any such experimentation, so I took the opportunity to illustrate the strengths and weaknesses of this approach to pharmacology, and compare it with in vivo experimentation. On the following day, I demonstrated the location of some of the tissues I had considered on cadaver animals.

Utilisation of the results of the activity

The seminars were used more to introduce me to the scientists at KRICT and elsewhere than to provide detailed information. In this context , they were successful.

Figure 1: Operation of the proposed general screen.