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Animals and alternative models for CNS drug discovery

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ABSTRACT

Drug discovery can be described as the process of identifying chemical entities that have the potential to become therapeutic agents. A key goal of drug discovery campaigns is the recognition of new molecular entities that may be of value in the treatment of diseases that qualify as presenting unmet medical needs. These diseases do not have definitively useful therapies, and are actually or potentially life-threatening. Marketed drugs at this point in time represent a relatively small number of drug target types. The development of drugs for CNS indications by the pharmaceutical industry generally follows a path from biochemical or cell-based assays to testing in animal models to human clinical trials. In particular, only 8% of CNS drug candidates that enter clinical trials win FDA approval, among the lowest success rates of any therapeutic area. The preclinical studies for drug screening involve the use of animals which is very time consuming and expensive and at times leads to suffering of the used organism. This has forced the researchers to find ways to not only decrease the time involved in drug screening procedures but also decrease the number of animals used and also increase the humane care of animals. To fulfil this goal a number of new in vitro techniques have been devised which are called 'Alternatives' or 'Substitutes' for use of animals in research involving drugs. These 'Alternatives' are defined as the adjuncts which help to decrease the use as well as the number of animals in biomedical research.

Keywords: Drug discovery, CNS drug discovery, Drug development, Preclinical screening.

INTRODUCTION

Drug discovery can be described as the process of identifying chemical entities that have the potential to become therapeutic agents. A key goal of drug discovery campaigns is the recognition of new molecular entities that may be of value in the treatment of diseases that qualify as presenting unmet medical needs. These diseases do not have definitively useful therapies, and are actually or potentially life-threatening. Marketed drugs at this point in time represent a relatively small number of drug target types. Drugs targeted against G-protein coupled receptors, nuclear receptors, and ion channels represent slightly less than 50% of the marketed drugs. drugs directed against enzymes represent the largest

portion of marketed drugs. Expansion into new types of drug targets may be necessary to fill certain therapeutic voids, but a matter of great intellectual challenge is how to choose a target likely to be of value, especially when venturing into less well-explored types of drug targets.

Drug development comprises all the activities involved in transforming a compound from drug candidate (the end-product of the discovery phase) to a product approved for marketing by the appropriate regulatory authorities. Efficiency in drug development is critical for commercial success, for two main reasons:

1. Development accounts for about two-thirds of the total R&D costs. The cost per project is very much greater in the development phase, and increases sharply as the project moves into the later phases of clinical development. Keeping these costs under control is a major concern for management. Failure of a compound late in development represents a lot of money wasted.
2. Speed in development is an important factor in determining sales revenue, as time spent in development detracts from the period of patent protection once the drug goes to market.

PRECLINICAL SCREENING MODELS FOR CNS DRUGS (INVIVOAND INVITRO)

The development of drugs for CNS indications by the pharmaceutical industry generally follows a path from biochemical or cell-based assays to testing in animal models to human clinical trials. In particular, only 8% of CNS drug candidates that enter clinical trials win FDA approval, among the lowest success rates of any therapeutic area. The majority of these drug candidates fail in late-stage clinical trials, with attrition most commonly caused by inefficacy or undesirable side effects. This low success rate is due in part to the limited predictive power of animal testing for human CNS pharmacology .Additionally, drug candidates with peripheral targets can be lost from the development pipeline if they produce unwanted CNS side effects .Thus, there is a clear need to develop improved physiological and behavioural tests in animals that accurately predict human responses to CNS-active drugs .In rodents, functional tests that assess CNS drug action can be roughly divided into 4 categories based on behavioural complexity:

1. simple stimulus-response paradigms, such as the startle reflex, prepulse inhibition, paw withdrawal from a hot plate, or vocalization after isolation;
2. Stereotyped motor tasks, such as balancing on a rotarod.
3. Complex innate behaviors, such as circadian entrainment or open-field activity.
4. Learned responses, such as maze running.

Tests in the first 2 categories can be performed rapidly with simple equipment and can measure a variety of changes in CNS function, such as sedation, analgesia, and ataxia. By contrast, tests in the third and fourth categories require more extensive monitoring or preliminary training trials, constraints that substantially increase the time and expense of animal testing.

CNS drugs can be of stimulants and depressant.

Central nervous system stimulants used for attention deficit disorder, narcolepsy or excessive sleepiness include the amphetamines, methylphenidate, atomoxetine, modafinil, armodafinil. Stimulants that are no longer used for medical conditions.[2] The CNS stimulants can be classified as analeptic stimulants, psychomotor stimulants, or methylxanthines. Doxapram activates peripheral chemoreceptors and central respiratory centres in a dose-dependent manner. Psychomotor stimulants (e.g. cocaine and amfetamines) increase sympathetic nervous system activity.

Central Nervous System (CNS) depressants are medicines that include sedatives, tranquilizers, and hypnotics. These drugs can slow brain activity, making them useful for treating anxiety, panic, acute stress reactions, and sleep disorders. CNS depressants cause drowsiness; sedatives are often prescribed to treat sleep disorders like insomnia and hypnotics can induce sleep, whereas tranquilizers are prescribed to treat anxiety or to relieve muscle spasms. Some examples of CNS depressants grouped by their respective drug class are:

- Benzodiazepines: diazepam, clonazepa , alprazolam
- Non-Benzodiazepine Sedative Hypnotics: zolpidem, eszopiclone
- Barbiturates mephobarbital pentobarbital sodium

INVIVO METHODS FOR CNS STIMULANTS DRUGS ELEVATED PLUS MAZE APPARATUS

Elevated Plus Maze Apparatus

The EPM has been widely used as a tool in the investigation of the psychological and neurochemical bases of anxiety, for screening anxiety- modulating drugs. The EPM is in the form of a plus- sign, with two open elevated arms facing each other and separated by a central square and two arms of the same dimensions, but enclosed by walls. The maze is raised off the ground so that the open arms combine elements of unfamiliarity, openness, and elevation. The EPM is based on the natural aversion of rodents to open spaces, and uses conflict between exploration and this aversion. Mice taken from their home cages will generally show a pattern of behavior characterized by open-arm avoidance, with a consistent preference for the closed arms. The rank order preference profile is closed → centre → open, indicative of a penchant for relatively secured sections of the maze. This tendency is suppressed by anxiolytics and potentiated by anxiogenic agents. The measures of anxiety are the number of open-arm entries and the number of closed-arm entries expressed as a percentage of the total number of arm entries and the amount of time spent on the open arms. Although automated plusmaze apparatus (eg, photobeam-based, video tracking systems) is now used in a few laboratories, most research groups still observe the behavior of their animals during testing.

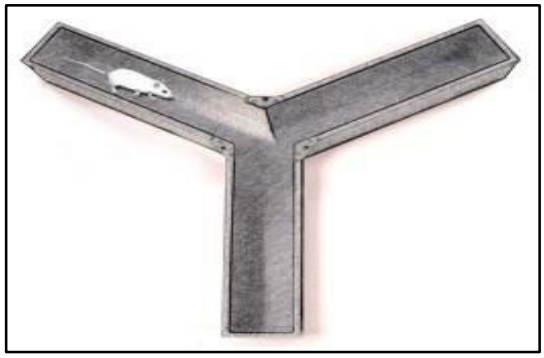
FORCED SWIM TESTAPPARATUS

Forced Swim Test Apparatus

Albino rats of either sex (150 - 200 g) were selected. Rats were placed individually in a transparent glassbcylinder (12cm in diameter, height 25 cm), which was filled with water to a height of 15 cm. Two swim sessions were conducted. An initial 15-min pre-test followed 24 hr later by a 6 min test. In the pre test session, the mice which have in a glass cylinder for 15 min. In the second session, each mouse received a respective dose of sample 1 hour prior to test, and placed in the cylinders again for 6 min. The following behaviours were recorded during the last 4 min.

- Immobility: floating in water without swimming.
- Climbing active movements of forelimbs on the container wall.
- Swimming active movements of extremities and circling in the container.

RUNAWAY TEST OR Y MAZE TEST



Run Way Test or Y Maze Test

This test is used to study the effect of a drug on spontaneous activity and motor coordination. Swiss albino rats of either sex were selected. The mice were placed individually in a symmetrical Y–shaped runway (33 cm x 38 cm x 13 cm) for 3 min and the number of the maze with all 4 ft (an‘entry’) were counted.

INVITRO METHODS

In vitro screening systems and high-throughput screening are critical aspects of the modern drug discovery process. Prior to the advent of these technologies, screening candidate compounds for biological activity was a major bottleneck in the identification of novel therapeutics. By the turn of the

twenty-first century, however, In vitro screening methods had become capable of generating data on hundreds to thousands of compounds per day, greatly enhancing the acquisition of biological data. Detecting the presence of radiolabeled material, as we are monitoring changes in fluorescence or absorbance, can be used in conjunction with microtiter plates, advanced robotics, and sophisticated software to determine the biological activity of a candidate compound. "Label-free" systems have also been developed as a means of identifying potential useful therapeutic agents without the need to perturb the biological systems of interest.

INVITRO MODELS FOR CNS DEPRESSANT DRUGS

INHIBITION OF [3H]-NOREPINEPHRINE UPTAKE IN RAT BRAIN SYNAPTOSOMES

Tissue preparation: Male Wistar rats are decapitated and the brains rapidly removed. The hypothalamic region is prepared, weighed, and homogenized in 9 volumes of ice-cold 0.32 M sucrose solution using a Potter-Elvejhem homogenizer. The homogenate is centrifuged at 1000 g at 0–4 °C for 10 min. The supernatant is decanted and used for the uptake experiments. Assay: 200 µl of tissue suspension are incubated with 800 µl 62.5 nM 3H- norepinephrine in Krebs-Henseleit bicarbonate buffer and 20 µl of the appropriate drug concentration at 37 °C under a 95% O2/5% CO2 atmosphere for 5 min. For each assay, 3 tubes are incubated with 20 µl of vehicle at 0 °C in an ice bath. After incubation all tubes are immediately centrifuged at 4000 g for 10 min. The supernatant fluid is aspirated and the pellets dissolved adding 1 ml of solubilizer (Triton X-100 + 50% ethanol, 1 : 4). The tubes are vigorously shaken, decanted into scintillation vials, and counted in 10 ml of liquid scintillation cocktail. Active uptake is the difference between cpm at 37 °C and 0 °C.

INHIBITION OF [3H]-DOPAMINE UPTAKE IN RAT STRIATAL SYNAPTOSOMES

Tissue preparation: Male Wistar rats are decapitated and the brains rapidly removed. Corpora striata are prepared, weighed and homogenized in 9 volumes of ice cold 0.32 M sucrose solution using a PotterElvehjem homogenizer. The homogenate is centrifuged at 1000 g at 0–4 °C for 10 min. The supernatant is decanted and used for the experiments.

Assay: 100 µl of tissue suspension are mixed with 900 µl 55.5 nM 3H-dopamine solution in KrebsHenseleit bicarbonate buffer and 20 µl of drug solution in appropriate concentration . The tubes are incubated at 37 °C under a 95% O2/5% CO2 atmosphere for 5 min. For each assay, 3 tubes are incubated with 20 µl of vehicle at 0 °C in an ice bath. After incubation all tubes are immediately centrifuged at 4000 g for 10 min. The supernatant fluid is aspirated and the pellets dissolved by adding 1 ml of solubilizer (Triton X-100 + 50% ethanol,1 : 4). The tubes are vigorously shaken, decanted into scintillation vials, and counted in 10 ml liquid scintillation counting cocktail. Active uptake is the difference between cpm at 37 °C and 0 °C.

INHIBITION OF [3H]-SEROTONIN UPTAKE IN SYNATOSOMES

Tissue preparation: Male Wistar rats are decapitated and their brains rapidly removed. Either the whole brain minus cerebellum or the hypothalamus is weighed and homogenized in 9 volumes of ice-cold 0.32 M sucrose solution using a Potter- Elvehjem homogenizer. The homogenate is centrifuged at 1 000 g at 0–4 °C for 10 min. The supernatant is decanted and used for further uptake experiments.

Assay: Two hundred µl of tissue suspension are mixed with 800 µl 62.5 nM 3 H-5-HT solution in Krebs Henseleit bicarbonate buffer and 20 µl of drug solution in the appropriate concentration (or the vehicle as control). The tubes are incubated at 37 °C under 95% O2/5% CO2 atmosphere for 5 min. For each assay, 3 tubes are incubated with 20 µl of the vehicle at 0 °C in an ice bath. After incubation all tubes are immediately centrifuged at 4000 g for 10 min. The supernatant is aspirated and the pellets are dissolved by adding 1 ml of solubilizer (Triton X100 + 50% ethanol, 1 + 4). The tubes are vigorously shaken, decanted into scintillation vials, and counted in 10 ml of liquid scintillation counting cocktail. Active uptake is the difference between cpm at 37 °C and 0 °C.

DRUG EFFECTS ON LEARNING AND MEMORY

INVITRO INHIBITION OF ACETYLCHOLINE-ESTERASE ACTIVITY IN RAT STRIATUM

Tissue preparation: Male Wistar rats are decapitated, brains rapidly removed, corpora striata dissected free, weighed and homogenised in 19 volumes (approximately 7 mg protein/ml) of 0.05 M NaH2PO4, pH 7.2 using a Potter- Elvehjem homogenizer. A 25 µl aliquot of this suspension is added to 1 ml of the vehicle or various concentrations of the test drug and incubated for 10 min at 37°C.

Assay: Enzyme activity is measured with the Beckman DU-50 spectrophotometer. This method can be used for IC50 determinations and for measuring kinetic constants.

Reagents are added to the blank and sample cuvettes as follows:

- Blank: 0.8 ml PO4 buffer/DTNB,0.8 ml buffer/Substrate
- Control: 0.8 ml PO4 buffer/DTNB/Enzyme,0.8 ml PO4 buffer/Substrate
- Drug: 0.8 ml PO4 buffer/DTNB/Drug/Enzyme,0.8 ml PO4 buffer/Substrate Blank values are determined for each run to control for non-enzymatic hydrolysis of substrate and these values are automatically subtracted by the kindata program available on kinetics soft-pac module.

INVITRO INHIBITION OF BUTYRYLCHOLINE-ESTERASE ACTIVITY IN HUMAN SERUM

Enzyme Preparation: A vial of lyophilized human serum is reconstituted in 3 ml of distilled water. A 25 ml aliquot of this suspension is added to 1 ml of the vehicle or various concentrations of the test drug and pre-incubated for 10 min at 37 °C.

Assay: Enzyme activity is measured with the Beckman DU-50 spectrophotometer. This method can be used for IC50 determinations and for measuring kinetic constants. Reagents are added to the blank and sample cuvettes as follows:

- Blank: 0.8 ml PO4 buffer/DTNB.0.8 ml buffer/Substrate

- Control: 0.8 ml PO4 buffer/DTNB/Enzyme,0.8 ml PO4 buffer/Substrate
- Drug: 0.8 ml PO4 buffer/DTNB/Drug/Enzyme,0.8 ml PO4 buffer/Substrate Blank values are determined for each run to control for non-enzymatic hydrolysis of substrate and these values are automatically subtracted by the kindata program available on kinetics soft-pac module. This program also calculates the rate of absorbance change for each cuvette.

INVITRO METHODS FOR CENTRALANALGESIC ACTIVITY

3H-NALOXONE BINDINGASSAY

Tissue preparation: Male Wistar rats are decapitated and their brains rapidly removed. Whole brains minus cerebella are weighed and homogenized in 50 volumes of ice-cold 0.05 M Tris buffer with a Tekmar tissue homogenizer. The homogenate is centrifuged at 40 000 g for 15 min, the supernatant is decanted and the pellet resuspended in fresh buffer and recentrifuged at 40 000 g. The final pellet is resuspended in the original volume of fresh 0.05 M Tris buffer. This yields a tissue concentration in the assay of 10 mg/ml.

Assay: The tubes are incubated for 30 min at 37 °C. The assay is stopped by vacuum filtration through Whatman GF/B filters which are then washed 3 times with icecold 0.05 M Tris buffer, pH 7,7. The filters are then counted in 10 ml of Liquiscint liquid scintillation cocktail. Stereospecific binding is defined as the differencebetween binding in the presence of 0.1 µM dextrorphan and 0.1 µM levorphanol. Specific binding is roughly 1% of the total added ligand and 50% of the total bound in the absence of Na+ and 2% of the total added ligand and 65% of the total bound ligand in the presence of Na+ (100 mM). The increase in binding is due to an increase in specific binding.

Evaluation: Data are converted into % stereospecific 3H-naloxonebinding displaced by the test drug. IC50 values are determined from computer-derived log-probit analysis.

3H-DIHYDROMORPHINE BINDING TO µ OPIATE RECEPTORS IN RAT BRAIN

Tissue preparation: Male Wistar rats are sacrificed by decapitation. Whole brains minus cerebella are removed, weighed and homogenized in 30 volumes of ice-cold M Tris buffer, pH 7.7. The homogenate is centrifuged at 48 000 g for 15 min, the supernatant is decanted and the pellet resuspended in the same volume of buffer. This homogenate is then incubated for 30 min at 37 °C to remove the endogenous opiate peptides and centrifuged again as before. The final pellet is resuspended in 50 volumes of 0.05 M Tris buffer, pH 7.7.

Assay: Tubes are incubated for 30 min at 25 °C. The assay is stopped by vacuum filtration through Whatman GF/B filters which are washed twice with 5 ml of 0.05 M Tris buffer. The filters are then placed into scintillation vials with 10 ml Liquiscint scintillation cocktail and counted.

Evaluation: Specific binding is defined as the difference between total binding and binding in the presence of 0.1 mM levallorphan. IC50 values are calculated from the percent specific binding at each drug concentration.

MAIN COMMON INVITRO MODELS CELLCULTURE

All receptors were individually expressed in Chinese hamster ovary cells stably transfected with human receptor cDNA developed in our laboratory. Receptor expression levels were 1.2, 1.6, 1.8, and 3.7 pmol per milligram of protein for the NOP, µ, κ, and δ opioid receptors, respectively. The cells were grown in Dulbecco's modified Eagle medium with 10% fetal bovine serum in the presence of 0.4 mg/ml G418 (Geneticin) and 0.1% penicillin/streptomycin in 100-mm plastic culture dishes. For binding assays, the cells were scraped off the plate at confluence.

CELLLINES

Cell lines are in vitro model systems that are widely used in different fields of medical research, especially basic cancer research and drug discovery. Their usefulness is primarily linked to their ability to provide an indefinite source of biological material for experimental purposes. Among cell line model systems, immortalized cell lines such as Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines (LCLs),NCI-60 panel have been used most often to test the effect of germline genetic variation on drug efficacy and toxicity.

RECEPTOR BINDING

Binding to cell membranes was conducted in a 96-well format as described previously. Cells were removed from the plates by scraping with a rubber policeman, homogenized in 50 mM Tris pH 7.5, using a Polytron homogenizer then centrifuged once and washed by an additional centrifugation at 27,000g for 15 min. The pellet was resuspended in Tris, and the suspension was incubated with Nmethyl-2-phenyl-N-[(5R,7S,8S)-7-(pyrrolidin-1-yl)-1-oxaspiro[4.5]dec-8-yl]acetamide (U69593) (41.7 Ci/mmol, 1.9 nM), or [3H]N/OFQ (120 Ci/mmol, 0.2 nM) for binding to, µ, δ, κ, and NOP receptors, respectively. Nonspecific binding was determined with 1 µM unlabeled DAMGO, DPDPE, ethylketocyclazocine, and N/OFQ, respectively. Total volume of incubation was 1.0 ml, and samples were incubated for 60 min at 25°C. The amount of protein in the binding assay was 15 µg. The reaction was terminated by filtration using a Tomtec 96 harvester through glass fiber filters. Bound radioactivity was counted on a β-plate liquid scintillation counter and expressed in cpm. IC50 values were determined using at least six concentrations of each peptide analog and calculated using Prism.

[35S]GTPγS BINDING

Cells were scraped from tissue culture dishes into 20 mM HEPES and 1 mM EDTA, then centrifuged at 500g for 10 min. Cells were resuspended in this buffer and homogenized using a Polytron homogenizer. The homogenate was centrifuged at 27,000g for 15 min, and the pellet was resuspended in buffer A containing 20 mM HEPES, 10 mM MgCl2, and 100 mM NaCl, pH 7.4. The suspension was recentrifuged at 27,000g and suspended once more in buffer A. For the binding assay, membranes (8–15 µg protein) were incubated with [35S]GTPγS (50 pM), GDP (10 µM), and the appropriate compound in a total volume of 1.0 ml for 60 min at 25°C. Samples were filtered over glass fiber filters and

counted as described for the binding assays. Statistical analysis was conducted using the program Prism.

ALTERNATIVE TO ANIMAL EXPERIMENTS

The preclinical studies for drug screening involve the use of animals which is very time consuming and expensive and at times leads to suffering of the used organism. This has forced the researchers to find ways to not only decrease the time involved in drug screening procedures but also decrease the number of animals used and also increase the humane care of animals. To fulfil this goal a number of new in vitro techniques have been devised which are called 'Alternatives' or 'Substitutes' for use of animals in research involving drugs. These 'Alternatives' are defined as the adjuncts which help to decrease the use as well as the number of animals in biomedical research.

"Alternatives" or "Substitutes" is defined as anything from absolute to partial replacement of live animals in biomedical research and testing. The use of animals in biomedical research are adjuncts, aids, shortcuts, or supplements which help an investigator to decide whether an experiment on an animal is likely to produce a useful result. Russell and Burch have given their definition of "alternatives" as "the three Rs - Replacement, Reduction, and Refinement". The 4th R was added in 1995.

Replacing: A test method that substitutes traditional animal models with non-animal systems such as computer models or biochemical or cell-based systems, or replaces one animal species with a less developed one (for example, replacing a mouse with a worm).

Reducing: A test method that decreases the number of animals required for testing to a minimum while still achieving testing objectives.

Refining: A test method that eliminates pain or distress in animals, or enhances animal well- being, such as by providing better housing or enrichment. Test methods that incorporate the 3Rs are referred to as new alternative methods.

Responsibility: The 4th R of Research implies addition of 'responsibility' to the original three R's of Russell and Burch. It has grown into a new era of performance-based outcomes, which reflects integrity, honesty, and scientific correctness in appropriate and reasonable use of laboratory animals. This ensures that animal life is required and necessary for biomedical advancement.

Alternative methods

These alternatives can be physico-chemical techniques, microbiological systems, tissue/organ culture preparation, computer or mathematical analysis (in silico testing), epidemiological surveys, and plant analysis (e.g. toxicity assays in plants). Research methods superior to using animals to learn about human disease or predict the safety of new drugs are stem cells, microdosing, DNA chips, microfluidics chips, human tissue, new imaging technologies, and post-marketing drug surveillance.

Various methods have been suggested to avoid the animal use in experimentation. These methods provide an alternative means for the drug and chemical testing, up to some levels. Advantages associated with these methods are, time efficiency, requires less man power, and cost effectiveness.

PHYSICO-CHEMICAL TECHNIQUES

These help to identify human responses to chemicals and biological substances e.g. Gas chromatography which separates complex substances and solutions into their basic elements which are further identified and measured through the use of mass spectrometry. This is frequently done in vitamin and drug research.

COMPUTER MODELS

Specialized computer models and software programs help to design new medicines. Computer generated simulations are used to predict the various possible biological and toxic effects of a chemical or potential drug candidate without animal dissection. Software known as Computer Aided Drug Design (CADD) is used to predict the receptor binding site for a potential drug molecule. CADD works to identify probable binding site and hence avoids testing of unwanted chemicals having no biological activity. Another popular tool is the Structure Activity Relationship (SARs) computer programs. It predicts biological activity of a drug candidate based on the presence of chemical moieties attached to the parent compound. Quantitative Structure Activity Relationship (QSAR) is the mathematical description of the relationship between physicochemical properties of a drug molecule and its biological activity. The activities like carcinogenicity and mutagenicity of a potential drug candidate are well predicted.

CELLS AND TISSUE CULTURES

Use of in vitro cell and tissue cultures which involves growth of cells outside the body in laboratory environment can be an important alternative for animal experiments. In vitro culture of animal/human cells includes their isolation from each other and growing as a monolayer over the surface of culture plates/flasks. Used for preliminary screening of potential drug molecules/chemicals to check their toxicity and efficacy.

MICRODOSING

It is a new method of obtaining human metabolism data which enables potential new drugs to be tested safely in humans at an earlier stage. Microdosing relies on the ultrasensitivity of accelerator mass spectrometry (AMS) which is a very sensitive device. Microdosing could screen out drugs destined to fail earlier, faster and cheaper. Micro dosing takes only 4-6 months andIts accuracy at predicting human metabolism is excellent.

DNA CHIPS

These enable the study of pharmacogenetics which helps in personalized drug treatment. DNA chips are glass slides studded with an array of genes or fragments of DNA. A sample of DNA tagged with fluorescent dyes is exposed to a new drug, and then washed over the chip. When the genes on the chip match the DNA in the sample, they stick together and the colours reveal which genes have been activated or suppressed by the experimental drug. This technique helps to design drugs for a particular individual.

MICROFLUIDICS CHIPS

These are just 2 cm wide and contain a series of tiny chambers each containing a sample of tissue from different

parts of the body. The compartments are linked by micro channels through which a blood substitute flows. The test drug is added to the blood substitute and circulates around the device. Sensors in the chip feed back information for computer analysis. This can mimic what goes on in the body on a micro scale.

ORGAN ON CHIP

It revolves around the culture of one or more cell systems in a small chamber or bioreactor with a laminar flow, providing nutrients and appropriate levels of shear stress. An organ-on-chip system constitutes the following elements:

- microfluidic chip, a credit card size device containing microchannels for medium flow and microchambers for cell culturing;
- compartment to culture cells (commercial cell line, primary cells, stem cell–derived cells or organoid) or tissue segments/slices;
- microfluidic flow through one or multiple microchannels/tubing providing culture medium and a test compound of interest and may additionally constitute:
- optionally: scaffold or 3D gel on which cells are grown to simulate physiological structures or create barrier models.
- Optionally: biosensors to measure endpoints such as shear stress, metabolism, barrier integrity and/or viability, or bio-actuators that stimulate relevant physical stimuli.

MICROORGANISMS

Yeast, Saccharomyces cerevisiae is the most popular and important model organism due to its rapid growth, ease of replica plating and mutant isolation, dispersed cells, well defined genetic system and highly versatile DNA transformation system. Yeasts can be grown in solid or liquid culture and isolated as colonies derived from a single cell on solid media. The generation time is very short i.e. about 90 min, hence it is very easy to grow a large population and analyze it.

Other organisms are:

Escherichia coli -Model for molecular and genetic studies.

Bacillus subtilis-Model for cellular differentiation.

STEM CELL RESEARCH

Stem cells may provide a complementary alternative to animals as in vitro models of disease and for toxicological testing. Disease genes are inserted into embryonic stem cells, which are then induced to differentiate into human disease tissues that can be used to screen for drugs. Embryonic stem cells can grow and differentiate in a Petri dish into the variety of cells that build a human organ.

NEW IMAGING TECHNOLOGIES

Magnetoencephalography (MEG), magnetic resonance imaging (MRI), functional MRI (fMRI), magnetic resonance spectroscopy (MRS), positron emission tomography (PET), single-photon emission computed tomography (SPECT), event-related optical signals (EROS) and transcranial magnetic stimulation (TMS) are the techniques offering a view of the human body –in particular, the brain – that cannot be gained by studying animals.

PLANT ANALYSIS

Plant substitution has had limited success in animal research. Some effects of exposure to certain substances have been demonstrated and the effects related to humans. A recent study on the effect of pharmaceuticals and their residues as environmental contaminants was performed on Brassica juncea, and demonstrated drug induced defense responses and activation of detoxification mechanisms as a result of oxidative stress.

CONCLUSION

Modern drug discovery strategies include both methods in tandem or in an iterative way .This review primarily provides a succinct overview and comparison of experimental and in silico screening techniques , selected case studies where both methods were used in cocert to investigate their performance and complementary nature and a statement on the developments in experimental and in silico approaches which is a biological experiments conducted on a computer via computer simulation in near future.

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