High Throughput Screening for Natural Products to Find Biologically Active Compounds: Natural Products versus Combinatorial Chemistry

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Drug development began with the finding of biologically active compounds which are obtained by chemical synthesis or from natural sources. The advent of Combinatorial Chemistry is recognized as a strategy which has a potential to change the methodology of research and development(R&D) of new drugs. Drug deveopment has been carried out with diverse strategies. In the past several decades a variety of new methodology have been introduced in R&D. Random screening of accumulated synthetic samples which had been synthesized for development of other drugs led to the discovery of new drugs. The typical examples are anti-asthma drug trimethoquinol and calcium antagonist diltiazem.(herbesser). In particular the latter drug has been used as a calcium antagonist worldwide, however it was first synthesized to find new tranquilizer and this is the reason why diltiazem has benzodiazepam skeleton. The random screening contributed in the finding of new drugs were carried out with whole animal test and it is a standard methodology in R&D of new drugs. Aspirin is the first synthetic non-steroidal antiinflammatory drug(NSAID) and has been used for more than one hundred years. It is the first example of drug developed from natural product. Salicin is the main constituent of willow bark which had been used in Europe for a long time to treat arthritis and aspirin was developed from salicin. Most of NSAID used clinically were developed from the structure of aspirin, however it took 70 years to clarify why aspirin exhibits its antiinflammatory, analgesic and antipyretic activities. The target of aspirin is cyclooxygenase(COX)which is the first enzyme involved in arachidonate cascade leading to the procuction of prostaglandins(PG) and thromboxan(TX). Side effect of aspirin causing ulcer in stomach is rather serious problem, since aspirin is so popular drug easily obtained in drug store(OTP). This problem is now going to be solved by a new finding on COX, which have two different types, one is constitutionally expressed COX 1 in almost all organs and the other is inducible COX 2. COX 2 is the responsible enzyme in inflammation etc and now the search of COX 2 specific inhibitors is the target of R&D of next generation NSAID.

Apart from random screening other methodologies have been extensively investigated in R&D. Captopril is an example of glorious success with rational design, in which snake venom played the role of leads in designing peptice mimics inhibiting angiotensin converting enzyme. This was the successful epoch making event in R&D of drug and followed by the second generation of ACE drugs. Success of ACE inhibitors open a new area in R&D. That is computer aided drug design(CAD) technology. In the first stage of CAD application in drug design is limited use the comparison of the structures of known therapeutic drugs and designed the compounds which showed maximum matching to essential parts of structures in exhibiting desired action. The ultimate

targets of medicinal drugs in exhibiting their actions are enzyme or receptor proteins. When the three dimensional structures were available including active sites computer assisted drug design(CAD) should be the powerful tool in R&D. At this moment CAD is recognized as the tool of R&d in 21st century and no as far as I know any success has been reported in journals or patents. It is generally recognized that CAD is premature to bring new drugs in the market and needs a time to mature enough contributing the birth of new drugs. However pharmaceutical and venture companies have the laboratories of CAD where the CAD systems are mostly employed refining the structures of hopeful candidates of new drugs.

On the contrary random screening of natural products brought numerous success in microbial metabolites since the finding of penicillin. In earlier time the search of microbial metabolites were limited to antibacterial activities, then the screening systems were expanded to those for antitumour, enzyme inhibition and protein binding including receptors. From microbial origins potent anticancer drugs such as daunorubicin, doxorubicin, acrabubicin and mitomycin C, actinomycin D and bleomycin. Antitumour screening extensively carried out world wide on plant constituents brought several drugs of plant origin in cancer therapy. Vinca alkaloids, podphylotoxin derivative(etoposide) and camptothecin derivatives (irinotecan etc) have been used clinically and recently a bright new ace anticancer drug taxol brought so called 'taxol effect' to the study of natural procucts in the U.S.A. New drug discovery studies were launched and groups were organized to find promising natural procucts from natural sources, microorganisms such as Actinomycetes and fungi, marine organisms including marine microorganisms such as microalgae and bacteria. The feature of New Drug Discovery Study in the organization of groups. Each group consists of a couple of university laboratories and at one ompany. In most cases ompany takes a part of bio-activity evaluation and the universities concentrate to isolation and strucural elucidation with close collaboration with company member. When promising natural products are found during the collaborative studies the member company is able to hold a right for drug development on those compounds.

The strategies of testing bio-active compounds developed very fast and in vitro bioassays are now prerequisite testing methods in R&D. In the screening of antitumour compounds National Cancer Institute(NCI) in U.S.A. changed their strategy in more than 10 years ago and set up in vitro panel screening with established human cancer cell lines for the first screening. The screening repeated twice and then the results are submited for the Biological Evaluation Committee if the active compounds are worth to be tested in vivo Holow Fiber Assay with nude mouse. An example of plant constituent will be shown in the lecture.

The development of in vitro bioassay strategy bought ironical situation in R&D. Because now a day in vitro bioassays are carried out with automated robot systems which work for 24 hrs and this resulted in the shortage of samples for testing. As it

is in a proverb 'Necessity is the mother of invention', the lack of samples for automated screening systems working 24 hrs let Combinatorial Chemistry brought up on the stage of new drug development. Combinatorial Chemistry consists of the construction of library which contains up to 100,000 compounds and high throughput bioassay. Before I joined the present laboratory I have been thinking to introduce high throughput bioassays in the screening of natural products, since we engaged in the screening and identification of natural products in the University of Tokyo more than two decades ago we introduced in vitro bioassay systems to detect the inhibitors of enzyme reactions such as phosphodiesterase, COX and arachidonate 5-peroxidase, the compound to inhibit degranulation of mast cellls, calcium antagonists, serotonin antagonists and PAF antagonists on medicinal plant extracts of more than 200 collected in Japansese, Hong Kong, Taiwan and mainland China markets. In Toyama Medical and Pharmaceutical University we developed the screening methods with high sensitivity as well as high efficiency. Screening, isolation and identification of natural products should proceed in parallel, which is my concept on natural product chemistry and we expanded our screening targets to not only medicinal plant extracts but also to microbial culture broth's which have been supplied from Prof. Haruo Seto of the University of Tokyo. The gaps between binding to proteins or inhibition of enzymes and real action of human beings are so large and how to minimize the gapes is the problems which we have to make effort and to solve. The possible methodology to overcome the problems is to use established cultured cells possessing functions to exhibit desired responses. In ideal the cells preferable derived from human being or genetically transformed cells transformed with the genes of human origin. Following the line of this concept we introduced ELISA methods to detect interleukin release from cultured cells. Financial situation in the university is far behind ideal screening system, however the efforts of the members in our laboratories we made screening of more than 2000 samples and obtained a significant number of samples which showed activity. We are also interested in establishing new bioassay using cultured cells to detect the compounds binding to receptors which are present naturally in the cultured cells or geneticallly introduced into cells. Highly sensitive binding bioassays are usrally carried out with probe labelled with radioisotopes, however it is sometimes very difficult to carry out for a large number of samples though beta-counter with 96 well plate are available. New method to change this situation came out recently. Luminescent probes witn metal chlate of Europium(Eu) can be the substitute of radioisotopes without loositn their high sensitivity. Eu chelate can be substitute ELISA bioassay. The disadvantage of this method is in the measurement of micro-second fluorescence and definitely require the machine specially designed for this technology. This principle however applied in the measurement of binding to protein such as receptors with two different dyes, one of which absorb certain wave length light transmitted to the second dye that corresponds excitation wave length and emit a different fluorescent. This proximity assay method

has a potential to detect the binding of protein-protein, antigen-antibody and antigen-antigen-second antibody. The proximity assay method can be applied to CPR in which two primer are labelled with two different dyes and time course of amplification of DNA can be measured by proximity assay with emission of fluorescence.

At this moment we are in the beginning of our work and I show some examples of our studies on bio-active natural products which have been carried out in Toyama Medical& Pharmaceutical University.

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