J. Biochem. 2011;149(5):499–505 doi:10.1093/jb/mvr032

JB THE JOURNAL OF BIOCHEMISTRY

JB Review

Drug discovery and development focusing on existing medicines: drug re-profiling strategy

Received January 28, 2011; accepted February 24, 2011; published online March 24, 2011

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As a new strategy for drug discovery and development, I focus on drug re-profiling as a way to identify new treatments for diseases. In this strategy, the actions of existing medicines, whose safety and pharmacokinetic effects in humans have already been confirmed clinically and approved for use, are examined comprehensively at the molecular level and the results used for the development of new medicines. This strategy is based on the fact that we still do not understand the underlying mechanisms of action of many existing medicines, and as such the cellular responses that give rise to their main effects and side effects are yet to be elucidated. To this extent, identification of the mechanisms underlying the side effects of medicines offers a means for us to develop safer drugs. The results can also be used for developing existing drugs for use as medicines for the treatment of other diseases. Promoting this research strategy could provide breakthroughs in drug discovery and development.

Keywords: drug re-profiling/drug discovery and development/existing medicines/comprehensive analysis.

Abbreviations: Aβ, amyloid-β peptide; AD, Alzheimer's disease; CHOP, C/EBP homologous protein; COX, cyclooxygenase; DDS, drug delivery system; ER, endoplasmic reticulum; GGA, geranylgeranylacetone; HSF1, heat shock factor 1; HSP, heat shock protein; IBD, inflammatory bowel disease; NSAIDs, non-steroidal anti-inflammatory drugs; PGE_2, prostaglandin E_2; TJ, tight junction.

Key words to describe major industries that are likely to sustain developed countries, including Japan, in the 21st century are 'high added value' and 'knowledge-intensive'. Considering the high level of personnel costs in these countries, goods of high added value (marketable though expensive) and knowledge-intensive goods (unable to be produced in developing countries) are required. Medicines are ideal as such goods, but the pharmaceutical industry responsible for producing them must reinvent itself and continually develop in order to meet economic growth objectives.

To achieve this outcome, huge amounts of money have been invested to promote drug discovery and development. Moreover, in order to raise the efficiency of drug discovery and development, major pharmaceutical companies have repeatedly merged with each other, and novel techniques for drug discovery, such as genomic drug discovery, high-throughput screening, and combinatorial chemistry have been established. While it was thus thought that the beginning of the 21st century would be heralded by an avalanche of new medicines coming onto the market, the number of drugs reaching the marketplace has decreased year by year (Fig. 1). This is because unexpected side effects and poor pharmacokinetics of potential drugs are being revealed at various stages of clinical trials, thus rendering the drugs not fit for use on humans. I consider that this is due to the fact that a large proportion of developable drugs (high safety and good pharmacokinetics) have actually already been discovered. Thus, I would like to focus attention on a new strategy for drug discovery and development, which focuses on the use of existing medicines; in other words, to employ a drug re-profiling strategy.

Background to the drug re-profiling strategy

In the drug re-profiling strategy, the actions of drugs already in clinical use, whose safety and pharmacokinetics in humans have already been confirmed, are examined comprehensively at the molecular level, using current and/or ground-breaking technologies, and the results used for the development of new medicines (Fig. 2). This refers not only to medicines currently in the market place, but also to medicines that have been withdrawn from the market or medicines whose clinical trials failed due to ineffectiveness (not because of safety issues).

In addition to an apparent deadlock in current drug discovery and development strategies, another aspect of the drug re-profiling strategy is the fact that among existing medicines, there are many of them for whom it is unclear how their underlying mechanisms of action give rise to their main effects and side effects. Many drugs that have been on the market for a long time (in most cases, good drugs) can be included in this group. This is because a significant proportion of them are derived from natural products that are traditionally thought to be effective for the treatment of particular

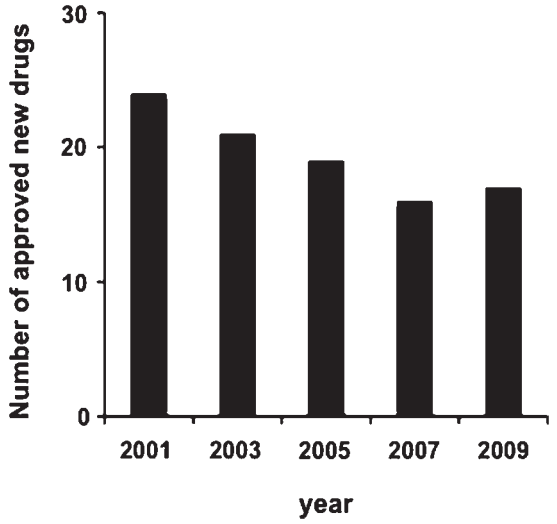

Fig. 1 Decrease in the number of new drugs approved by FDA.

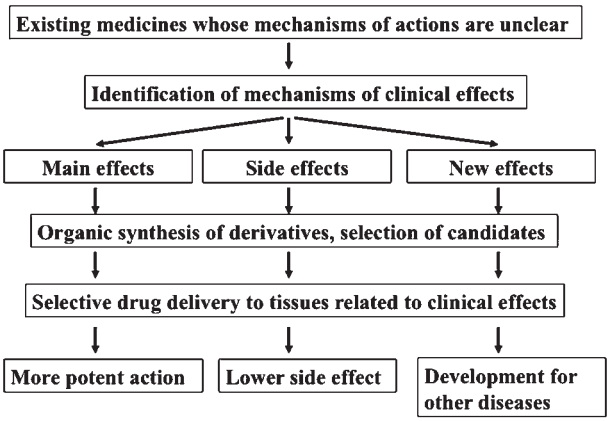

Fig. 2 Drug re-profiling strategy.

conditions. Nevertheless, the mechanisms underlying how these drugs achieve their clinical effect have not been examined. Furthermore, when such traditional medicines were developed, it was difficult, if not impossible to investigate the molecular mechanisms of action that give rise to their main effects and side effects, due to a lack of analytical technology.

On the other hand, epidemiological studies have revealed a number of novel clinical effects of existing medicines (for example, the anti-tumour and anti-Alzheimer's disease (AD) effects of non-steroidal anti-inflammatory drugs (NSAIDs), as described below); however, the mechanisms governing these novel clinical effects are unclear at present.

With this background in mind, in drug re-profiling the actions of clinically employed drugs are subjected to a comprehensive examination at the molecular level to identify the mechanisms underlying their main actions and side effects, with the aim to develop more potent and safer medicines, or to identify novel clinical effects and their underlying molecular mechanisms. This may enable the development of existing drugs as treatments for other diseases (Fig. 2). The advantage of this strategy is that there is a decreased risk for unexpected side effects and poor pharmacokinetics in humans because their safety and pharmacokinetics have already been well characterized. By employing this strategy, we could also improve the efficiency of drug development by reducing the enormous amount of time, money and energy that goes into getting a product to market. For example, pre-clinical tests (such as evaluation for safety, metabolism, absorption and excretion in animals) and phase I clinical trials in humans can be omitted. Drug re-profiling has consequently been linked to the concept of 'eco-medicine'.

This research strategy can also be considered as a new type of basic chemical biology. When the mechanisms of action of existing medicines are poorly understood, this means that they act in an unknown biological manner; in other words, the identification of such mechanisms may lead us to new biological outcomes. For example, as described below, analysis of the anti-tumour activity of NSAIDs led us to identify that tight junction (TJ)-associated proteins regulate the metastasis of tumours (*1*, *2*).

Recently, a number of successful results of indication expansion have been reported. For example, sildenafil and minoxidil were originally developed as medicines for the treatment of cardiovascular diseases. However, in the clinical setting, other pharmacological activities were identified and these drugs were re-developed for the treatment of erectile dysfunction and alopecia, respectively (*3*, *4*). In Japan, ramosetron was originally developed as an antiemetic drug and thereafter, taking into account its principal side effect, constipation, this drug was re-developed as a treatment for diarrhea-predominant irritable bowel syndrome (*5*).

In these indication expansions, the strategy was found by chance, giving rise to the possibility of there being many un-identified pharmacological activities of existing medicines. Thus, in drug re-profiling, the pharmacological activities of existing medicines are identified scientifically and comprehensively using innovative technologies and the results are used for drug development, including indication expansion.

Methods underlying the drug re-profiling strategy

Steps in the drug re-profiling strategy can be described as follows:

(i) Targeting and selection of existing drugs to be subjected to analysis.
(ii) Comprehensive analysis of the actions of these drugs and to identify the manner in which they exert their clinical action (main effect, side effects and novel effects) with the aim to select compounds that might warrant further analysis.
(iii) Organic synthesis of derivatives of the selected drug to obtain more-effective homologues. A principle advantage of the drug re-profiling strategy is that existing medicines can be subjected to drug development in order to reduce costs associated with development and risk of

failure. However, when a drug with desirable characteristics cannot be found in existing medicines, the slight modification of existing medicines should be considered (see our study for NSAIDs with reduced gastric side effects).

(iv) Drug delivery system (DDS) studies to deliver drugs to tissues related to the drug's main effects or novel effects, or to avoid delivery in cases where side effects occur.

We applied this strategy to existing medicines to identify new possibilities for drug development. Furthermore, since some beneficial effects of existing medicines may not be identified by this strategy only, we recently prepared a library of existing medicines and applied various screening methods to these compounds in order to comprehensively search for existing medicines with clinically beneficial effects.

The following discussion provides more detailed methods for each step, which are currently being performed in our work.

Targeting and selection of existing medicines

Candidate existing medicines to be subjected to drug re-profiling are selected on the basis of data from epidemiological studies and previous clinical trials (including examples of failure), as well as from analyses of existing drugs whose mechanism of action is unclear.

Comprehensive analysis of the mechanisms of action of targeted medicines

Analysis of genes whose expression is induced by the target drug. Using DNAchip and proteinchip techniques, genes and proteins induced by the target drug are identified in various cells types (such as cells from different tissues, and cells expressing proteins related to specific diseases). As for genes possibly related to some diseases, the drug and the gene are analysed in an *in vitro* system (for example, using siRNA) and also in animal models. Through these studies, we select existing medicines that are possibly linked to new drug development strategies. On the other hand, the mechanism of action of targeted drugs is analysed to identify new biological outcomes.

Analysis of proteins bound to the target drug. To identify proteins bound to the target drug, total human proteins are separated by 2D gel electrophoresis and detected with the labeled drug. Analysis of the identified proteins is performed as described earlier.

Other analyses. Other comprehensive analyses using innovative techniques are also performed. For example, alterations in the concentrations of various signal transduction-related molecules (such as cAMP) after the treatment of cells with the drug are monitored, or systematic screening of receptors that bind the drug is performed.

Analysis of a library of existing medicines. A library of existing medicines is subjected to the various screening systems. When novel, clinically beneficial actions are identified, further drug development and analysis of underlying molecular mechanisms are carried out as described above.

Organic synthesis of derivatives and analysis of their actions

Derivatives of existing medicines selected are synthesized. In such cases, clear strategies for the synthesis are needed. For example, in the case of the synthesis of NSAIDs with lower membrane permeabilizing activity, we computer-simulated the interaction between the target NSAID and the membrane and used the results to synthesize derivatives of the target NSAID (*6*). The activities of the newly synthesized drugs are estimated *in vitro* and *in vivo* for the subsequent selection of promising compounds as candidates for new medicines.

DDS-mediated modification of drugs

DDS is a technique that permits selective drug delivery to specific tissues, and as such is essential in the quest for drug re-profiling. For example, when the underlying mechanisms related to the side effects of existing medicines are revealed, DDS can be used to avoid delivery of the drug to tissues related to the side effect. Conversely, when the novel clinical effects of drugs are revealed, DDS can be used to selectively deliver the drugs to the relevant tissues related to this effect.

Embedding of the medicine into nanoparticles and modification of the surface of nanoparticles (for example, loading antibodies that recognize tissue-specific proteins) is a useful DDS technique. Using more traditional techniques, it was impossible to embed hydrophilic drugs; however, we recently found a way around this by introducing a phosphate side chain into the drug and its insolubilization with zinc ion. By this method, we were able to embed PGE_1 (a stimulator of vascularization) into nanoparticles and deliver this drug to the site of vascular disorders (*7–10*). Further progression of this technique may lead us to be able to deliver the target drug to the preferable position.

Examples of drug re-profiling

NSAIDs

NSAIDs are one of the most frequently used classes of medicines in the world and account for ~5% of all prescribed medications (*11*). NSAIDs are inhibitors of cyclooxygenase (COX), a protein essential for the synthesis of prostaglandins (PGs), which have a strong capacity to induce inflammation. However, NSAID administration is associated with gastro-intestinal complications, such as gastric ulcers and bleeding. In the United States, about 16,500 people per year die as a result of NSAID-associated gastrointestinal complications (*12*). Inhibition of COX by NSAIDs was previously thought to be fully responsible for their gastrointestinal side effects; however, recent reports suggest that some additional, unknown mechanisms might contribute to this side effect. On the other hand, a range of epidemiological studies have revealed that prolonged NSAID use reduces the risk of cancer and AD (*13–17*). However, the molecular mechanisms

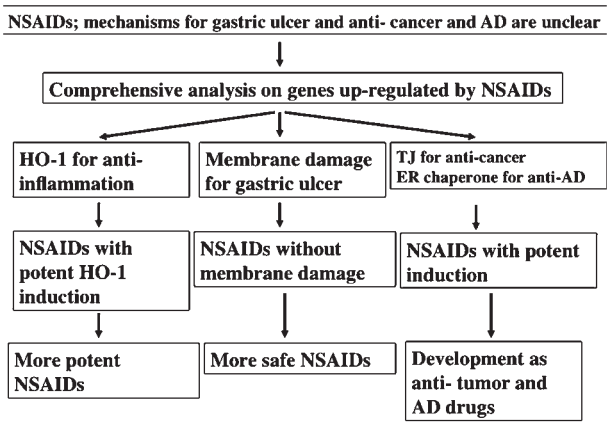

Fig. 3 Drug re-profiling study for NSAIDs.

governing these newly identified effects of NSAIDs are unclear at present.

In order to identify the molecular mechanism underlying the gastric side effect of NSAIDS, as well as their anti-inflammatory, anti-tumour and anti-AD effects, we comprehensively examined the actions of NSAIDs at the molecular level using a number of state-of-the-art techniques (for example, by using DNAchip analysis to search for genes whose expression is up-regulated by NSAIDs). We made several observations that can be outlined as follows: the induction of HO-1 (an anti-inflammatory protein) is involved in the anti-inflammatory action of NSAIDs (*18*); NSAID-dependent membrane permeabilization and the resulting induction of the endoplasmic reticulum (ER) stress response [induction of C/EBP homologous protein (CHOP)] and apoptosis are involved in the gastric side effects of NSAIDs (*19–23*); the COX-inhibition and induction of expression of TJ-associated proteins is involved in NSAIDs' anti-tumour effect (*1*, *2*, *24*); not only the COX-inhibition and resulting inhibition of EP2 and EP4 receptors but also the ER stress response (induction of ER chaperones) are involved in NSAIDs' anti-AD effect (*25–28*) (Fig. 3).

As for the gastric side effects of NSAIDs, we found that loxoprofen has the lowest membrane permeabilizing activity among existing NSAIDs (*29*). Loxoprofen has been used clinically for a long time as a standard NSAID in Japan, and clinical studies have suggested that it is safer than other NSAIDs, such as indomethacin. We therefore synthesized a series of loxoprofen derivatives and found that fluoro-loxoprofen does not have membrane permeabilizing activity yet still exerts an anti-inflammatory effect and causes fewer gastric ulcers in mice than loxoprofen (*6*). These results suggest that the drug re-profiling strategy used here is a useful means to identify the molecular mechanisms governing the side effects of existing medicines and to develop new drugs with reduced side effects.

Further to the above, we selected NSAIDs with potent activity for inducing the expression of TJ-associated proteins and ER chaperones, and we are developing these NSAIDs with a view to using them as anti-tumour and anti-AD drugs, respectively. From these achievements, we realized that the drug re-profiling strategy could help us to develop existing drugs for use in the treatment of other diseases.

As a consequence of our findings, we suggested that claudins transmembrane proteins consisting of TJs positively or negatively affect the migration and invasion activity of cancer cells, depending on the claudin species, and that this action plays an important role in conferring the chemopreventive effect of NSAIDs through the inhibition of metastasis (*1*, *2*). Furthermore, based on the finding that EP2 and EP4 receptors are involved in the anti-AD effect of NSAIDs, in other words, promoting the progression of AD, we examined the mechanism underlying this involvement. By using EP_2- or EP_4-receptor-null mice, we found that activation of the EP_2 receptor stimulates the production of amyloid-β peptide (Aβ) through the activation of adenylate cyclase, as well as causing an increase in the cellular level of cAMP and activation of protein kinase A (*28*). On the other hand, activation of the EP_4 receptor causes its co-internalization with PS-1 (γ-secretase) into endosomes, which in turn activates γ-secretase, resulting in the upregulation of Aβ production (*28*). These results led us to develop antagonists for these receptors as anti-AD drugs. In fact, we recently found that oral administration of an antagonist specific for the EP_4 receptor improves cognitive functions in AD model mice (Hoshino *et al.*, unpublished data). Thus, the drug re-profiling strategy has also enabled us to identify new biological outcomes and new targets of existing medicines.

Geranylgeranylacetone

Geranylgeranylacetone (GGA) was developed 27 years ago and has been used clinically since 1983 as a standard anti-ulcer drug in Japan. However, the molecular mechanism underlying this anti-ulcer action of GGA was, until recently, unclear. Rokutan and his co-workers comprehensively examined the action of GGA at the molecular level and found that it is a non-toxic heat shock protein (HSP)-inducer (*30*). Since HSPs protect cells from various stressors (*31*, *32*), we hypothesized that GGA achieves its anti-ulcer effect by making gastric mucosal cells resistant to various gastric irritants by induction of HSPs. We successfully proved this hypothesis of the contribution of the HSP-inducing activity of GGA to its anti-ulcer activity by showing that GGA does not exhibit anti-ulcer activity in heat shock factor 1 (HSF1)-null mice, where the induction of HSPs is suppressed (*33*, *34*). These results suggest that the drug re-profiling strategy may contribute to identification of the molecular mechanisms underlying the clinical effects of existing medicines and that transgenic mice are useful tools to understand such molecular mechanisms (Fig. 4).

It was recently revealed that HSP70 has an anti-inflammatory activity by means of its inhibition of nuclear factor kappa B and a resulting suppression of pro-inflammatory cytokine and chemokine expression (*35–38*). Therefore, we consider that inducers of

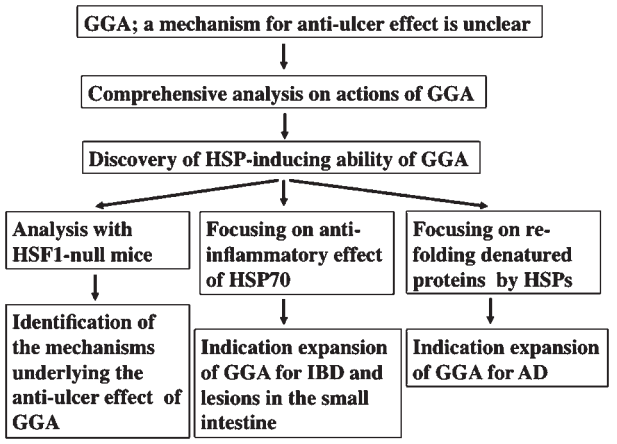

Fig. 4 Drug re-profiling study for GGA.

HSP70, such as GGA, could be effective for treating diseases that involve stressor-induced cell death and inflammation. To commence this work, we focused on inflammatory bowel disease (IBD), which has become a significant health problem with an actual prevalence of 200–500 per 100,000 people in Western countries, with a doubling rate of just over 10 years (*39*). Recent studies suggest that IBD involves chronic inflammatory disorders in the intestine due to 'a vicious cycle'. Infiltration of leukocytes into intestinal tissues causes intestinal mucosal damage induced by reactive oxygen species that are released from the activated leukocytes, with this damage further stimulating the infiltration of leukocytes (*40*). Based on the cytoprotective and anti-inflammatory effects of HSP70, we speculated that expression of HSP70 would be effective for treating IBD. Using animal models for colitis, we found that transgenic mice expressing HSP70 are more resistant to colitis than the wild-type mice. Furthermore, we revealed that expression of HSP70 achieves this protective effect against colitis through its cytoprotective and anti-inflammatory activity (*33*). Ohkawara *et al*. (*42*) addressed this issue by employing GGA in their studies, and reported that oral administration of GGA suppressed IBD-related colitis. Furthermore, they showed that GGA up-regulated the expression of HSP70 and HSP40 but not other HSPs in the colon (*41*, *42*). These results support the idea that HSP70 is protective against IBD-related colitis and suggest that non-toxic inducers of HSP70 are therapeutically beneficial for IBD (Fig. 4).

More attention has generally been paid to NSAID-induced gastric lesions rather than lesions of the small intestine, because the latter are usually asymptomatic and their diagnosis is difficult to make. However, recent improvements in diagnostic techniques such as capsule endoscopy and double-balloon endoscopy have revealed that NSAID-induced lesions of the small intestine occur very frequently and that the small intestine is even more susceptible than gastric tissue to the detrimental effects of NSAIDs (*43*, *44*). Nevertheless, clinical protocols for the treatment of NSAID-induced lesions of the small intestine have not been established. For example, acid control drugs are not as effective for treating NSAID-induced lesions of the small intestine compared with their effect on gastric lesions (*45*, *46*). Recent studies suggest that NSAID-induced lesions of the small intestine involve the direct cytotoxicity (topical effect) of the NSAID, and inflammatory responses. Thus, it is reasonable to speculate that HSP70 protects against NSAID-induced lesions of the small intestine. Using transgenic mice expressing HSP70 and wild-type mice, we compared the development of lesions in the small intestine after administration of indomethacin. Indomethacin-induced such lesions in a dose-dependent manner in wild-type mice and this production was significantly reduced in transgenic mice expressing HSP70. We also found that expression of HSP70 achieves this protective effect through its cytoprotective and anti-inflammatory activity. Furthermore, pre-administration of GGA suppressed the indomethacin-induced lesions in a dose-dependent manner, and the GGA-induced expression of HSP70 suppressed the extent of indomethacin-induced lesions by inhibiting indomethacin-induced mucosal cell apoptosis and reducing the inflammatory response (*47*). These results strongly suggest that oral administration of GGA could be therapeutically beneficial against NSAID-induced lesions of the small intestine in humans owing to its HSP-inducing activity (Fig. 4).

Based on the activity of HSPs for re-folding denatured proteins, a group from Nagoya University speculated that GGA would be effective for treating spinal and bulbar muscular atrophy (whose major cause is protein denaturation) and succeeded in verifying this (*48*). On the other hand, we recently reported that expression of HSP70 is an effective treatment against AD in a mouse model. AD model mice showed less of an apparent cognitive deficit when they were crossed with transgenic mice expressing HSP70. Transgenic mice expressing HSP70 also displayed lower levels of Aβ, Aβ plaque deposition and neuronal and synaptic loss than control mice. These results suggest that expression of HSP70 in mice suppresses not only the pathological but also the functional phenotypes of AD (*25*). These studies on GGA suggest that the drug re-profiling strategy is useful for the indication expansion of existing medicines (Fig. 4).

Conclusions and perspectives

In order to successfully carry out a drug re-profiling strategy, specialists in various fields of drug development, epidemiology, clinical medicine, molecular biology, genomic analysis, organic chemistry, DDS and material chemistry must pool their resources and know-how. The bringing together of such experts is currently underway in Japan, and success in the re-profiling of different drugs will surely affect the drug development strategy of pharmaceutical companies in the future. In other words, our final goals are to contribute to the development of the pharmaceutical industry and to promote efficient drug development through drug re-profiling. Many pharmaceutical companies have had disappointing economic growth because they expected a rush of new drugs to come

onto the market with the development of novel techniques such as genomic drug discovery. I want to contribute to the revival of the pharmaceutical industry by promoting a paradigm shift in their drug development strategy that is based on drug re-profiling.

If drug re-profiling is to be performed in an efficient manner then a network of researchers from universities and industry is required. In universities, numerous researchers have developed original screening systems for medicines, giving rise to the real possibility to establish a research network in which existing medicines are made available by pharmaceutical companies and subjected to such screening procedures to obtain clues for new uses of currently available drugs. Since such screening systems are closely related to the basic research carried out by the researchers in question, the identification of drugs by these screening systems also greatly contributes to the progress of their basic research. Given the potential that a university–industry network would have, it is highly recommendable that such a system be established.

Conflict of interest
None declared.

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