Internet Laboratory for Predicting Harmful Effects Triggered by Drugs and Chemicals. Concept and Call for Co-operation

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Summary
It is our objective to establish a virtual laboratory on the Internet to allow for an in silico estimation of harmful effects triggered by drugs, chemicals and their metabolites. Presently, our database includes validated models for five biological targets – the Aryl hydrocarbon, the serotonin 5HT-2A, the cannabinoid, the GABA (gamma-amino butter acid), and the steroid receptors. It shall be continuously extended to include surrogates for any bioregulator known or presumed to mediate harmful effects. Free access to this virtual laboratory shall allow any interested party to estimate the harmful potential of a given substance prior to its synthesis. This is achieved by generating the three-dimensional structure of the compound and its possible metabolites in the computer, followed by calculating their binding affinity towards each receptor surrogate in the database. Only compounds and metabolites passing through this surrogate battery without displaying a significant affinity towards any member may be cleared for synthesis and preclinical studies. This way, potentially harmful compounds can be withdrawn from the evaluation pipeline before in vivo test are conducted, hence contributing to the reduction of animal testing in chemical and pharmaceutical research and development.

Zusammenfassung: Internet-Labor zur Voraussage schädlicher Wirkungen von Arzneistoffen und Chemikalien. Konzept und Kooperationsaufruf
Unser Ziel ist die Erstellung eines virtuellen Labors im Internet zur computer-gestützten Abschätzung von unerwünschten Wirkungen, die von Arzneistoffen, Chemikalien und deren Metaboliten ausgelöst werden. Zur Zeit umfasst unsere Datenbank validierte Modelle für fünf biologische Zielproteine – den Aryl hydrocarbon, 5HT-2A, Cannabinoid, GABA-A und Steroidrezeptor. Sie soll kontinuierlich erweitert werden und Modelle aller Rezeptoren umfassen, die nachweislich oder vermutetermassen unerwünschte Wirkungen vermitteln. Ein freier Zugriff auf dieses virtuelle Labor würde es allen interessierten Kreisen ermöglichen, das schädliche Potential einer beliebigen Substanz vor deren Synthese abzuschätzen. Dies wird durch die Computergenerierung der dreidimensionalen Struktur und ihrer möglichen Metaboliten, gefolgt von der Berechnung der Bindungsaffinität gegenüber allen Rezeptormodellen der Datenbank erreicht. Nur diejenigen Substanzen, welche die Surrogat-Batterie ohne signifikante Affinität gegenüber irgendeinem Modell durchlaufen, sollten für Synthese und präklinische Studien freigegeben werden. Daher können potentiell schädliche Substanzen aus der Entwicklung genommen werden, bevor in vivo Versuche angesagt sind und so beitragen, die Anzahl Tierversuche in der pharmazeutischen und chemischen Forschung und Entwicklung zu vermindern.

Keywords: Internet laboratory, virtual experiments, prediction of harmful effects of drugs and chemicals, multi-dimensional QSAR, reducing animal tests

1 Introduction

Toxicity testing as required by international regulations for drug development and chemical safety is still associated with stressful animal tests. While many *in vitro* approaches have been validated for various aspects of pharmacological and toxicological phenomena, these technologies require a substance being physically available (i.e. synthesized) before testing and may not produce all metabolic products of interest. Computational (*in silico*) approaches have the advantage of being applicable to any hypothetical substance as their three-dimensional structure and those of their metabolites can readily be generated in the computer. Moreover, nowadays computer power permits to scan larger batches of compounds in short time spans.

Toxic agents, particularly those that exert their actions with a great deal of specificity, sometimes act via receptors to which they bind with high affinity. This phenomenon is referred to as receptor-mediated toxicity. Examples of soluble intracellular receptors, which are important in mediating toxic responses, include the glucocorticoid receptor which can act as a model for other receptors but is also involved in mediating toxicity associated effects such as apoptosis of lymphocytes as well as neuronal degeneration as a response to stress, the peroxisome proliferator activated receptor which is associated with hepatocarcinogenesis in rodents, and the Aryl hydrocarbon receptor ("dioxin receptor") which is involved in a whole range of toxic effects (Gustafsson,

1995). Harmful effects of drugs and chemicals can often be associated with their binding to other than their primary target – macromolecules involved in biosynthesis, signal transduction, transport, storage, and metabolism (cf., for example, Rymer and Good, 2001; Hestermann et al., 2000; Fischer, 2000; Lukasink and Pitkunen, 2000; Rihova, 1998; Ammon, 1991).

Quasi-atomistic receptor modeling, a 4D-QSAR technique more recently developed at our laboratory, allows to quantitatively predict the binding affinity of small molecules towards an unknown or a hypothetical receptor. The approach combines receptor modeling and QSAR techniques based on genetic algorithms. In addition to the fourth dimension – the possibility to represent each molecule by an ensemble of conformations, orientations, protonation states, or metabolites – it allows for the simulation of induced-fit and solvations phenomena, key aspects for a realistic simulation of drug-receptor interactions. Our concept has been validated for various pharmacological targets (cf., Vedani et al., 2000a,b; Vedani et al., 1998; Schulze-Alexandru et al., 1999; Streich et al., 2000) as well as for receptor-mediated toxicological phenomena (Vedani and Dobler, 2000; Vedani et al., 1999a,b).

2 Toxicity-mediating bioregulators

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds represent serious environmental health hazards, whose effects include tumor promotion, dermal toxicity, immunotoxicity, developmental and reproductive toxicity as well as induction and inhibition of various enzyme activities. TCDD also induces differentiation changes affecting, for example, the human epidermis – manifesting itself as chloracne. There is strong evidence that the toxicity is mediated by the Aryl hydrocarbon (Ah) receptor, a regulatory element involved in the mammalian metabolism of xenobiotics. In target cells, TCDD initially binds to the Ah receptor, which accumulates in the nucleus as an "Ah receptor-aryl hydrocarbon nuclear translocator" heterodimeric complex. The nuclear Ah-receptor complex acts as a ligand-induced transcription factor which binds to transacting genomic dioxin/xenobiotics responsive elements located in the 5'-regulatory region upstream from the initiation site. This interaction results in transactivation of gene transcription (Safe and Krishnan, 1995). Treatment of animals or cells with TCDD and related Ah-receptor agonists can result in decreased enzyme activities and decreased gene expression (cf., for example, Putzrath, 1997; Okey et al., 1994; Swanson and Bradfield, 1993; Rappe 1993; Whitlock, 1993, 1990; Landers and Bunce, 1991; Safe 1990).

The glucocorticoid receptor (GR) regulates target gene expression in response to corticosteroid hormones. Both *in vivo* and *in vitro* protein-protein interaction assays revealed a ligand-dependent interaction between the GR and RIP140, a receptor-interacting protein. In a yeast-transactivation assay, RIP140 and SRC-1, a member of the steroid receptor co-activator family of proteins, both enhanced the transactivation activity of a GR protein (GRA-1) in which the potent N-terminal τ1 transactivation domain has been deleted. In contrast, in COS-7 cells increasing amounts of RIP140 significantly inhibited GR$\delta\tau$1 function. In cotransfection studies in COS-7 cells, RIP140 also inhibited receptor activity in presence of both SRC-1 and the coactivator protein CBP together. In yeast cells a stimulation of receptor activity was observed, while in mammalian cells RIP 140 repressed GR function. These data suggest that RIP140 is a target protein for the GR and RIP140 can modulate the transactivation activity of the receptor (Windahl et al., 1999, Gustafsson, 1995).

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily of ligand-activated transcription factors. The PPARs are believed to play a physiological role in the regulation of lipid metabolism. They can be activated by high concentrations of fatty acids and have been shown to regulate the expression levels of fatty-acid binding proteins or enzymes involved in fatty-acid oxidation (Wilsson et al., 1996; Desvergne et al., 1995). Since the discovery of rodent peroxisome proliferation, a wide variety of structurally diverse and industrial important compounds have been shown to have similar effects; these compounds include hypolipidaemic drugs (e.g. clofibrate), leukotriene antagonists, herbicide and plasticisers (Reddy and Lalwani, 1982). This rodent hepatocarcinogenesis is important for human risk assessment, since humans are exposed to many peroxisome proliferators (PPs) both environmentally and otherwise. There is experimental evidence that PPAR toxicity is subject to receptor-mediated phenomena (Tugwood, 1995).

Further receptor-mediated toxicological phenomena have been observed with the NMDA receptor (Harada et al., 1999; Oosterink et al., 1998a,b), the AMPA receptor (Ohno et al., 1998), the glutamate receptor (Matute et al., 1997; Kwak and Nakamura, 1995), and the cannabinoid receptor (Maneuf et al., 1997).

3 Methods

The 4D–QSAR concept developed at our laboratory (software *Quasar*) allows the construction of a three-dimensional binding-site model about any molecular framework of interest, e.g. a pharmacophore. The essential information about the hypothetical receptor site is provided by means of a van-der-Waals surface populated with atomistic properties. The shape of the surface represents information about the steric nature of the receptor site; the associated properties represent other information of interest, such as hydrophobicity, electrostatic potential and hydrogen-bonding propensity (Hahn, 1995). In contrast to other techniques, our approach allows for multiple ligand representation, induced fit, H-bond flip-flop, and dynamic cavity shaping. As the technology and its validation have been published in detail (Vedani et al., 2000a,b; Vedani and Dobler, Vedani et al., 1998), the technical details shall not be reiterated at this point. Instead, we present the results for the most complex system simulated so far: the Ah (dioxin) receptor, binding dibenzodioxins, dibenzofurans, biphenyls, and polyaromatic hydrocarbons. Our simulation included a total of 121 compounds and yielded a cross-validated r^2 of 0.851 and a predictive r^2 of 0.792 with no compound in both training and test set being predicted false-positive or false-negative (Figure 1) (Vedani and Dobler 2000; Vedani et al.,1999b).

The proposed virtual laboratory on the Internet to predict harmful effects triggered by drugs or chemicals and their

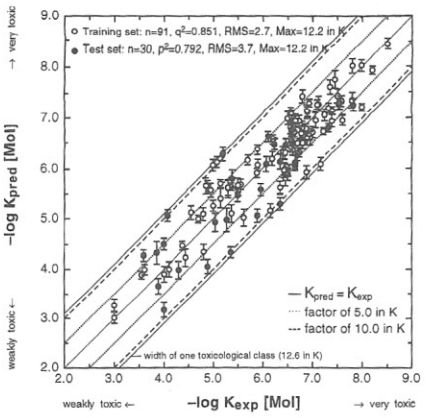

Figure 1: Graphical comparison of experimental and predicted binding affinities for the 121 dibenzodioxins, dibenzofurans, biphenyls and polyaromatic hydrocarbons with respect to the Ah receptor. The 91 substances of the training set are marked by open circles; the 30 compounds with filled circles. The width of one (of the five) toxicological classes is determined by the fifth root of the affinity range (3.2×10^5).

metabolites shall be configured as a local mirror of the database including all software, freely accessible to any interested party (academic, industrial and government). Security is guaranteed as for compound evaluation the pertinent data is downloaded to a local host and feedback is disabled by the algorithm. Extension of the surrogate database – model generation requires the use of confidential compound data – may be performed on a stand-alone computer at our laboratory or at the depositor's site.

3.1 Testing a compound for harmful effects (Scheme 1a)

Testing a new compound for harmful effects involves the following steps: 1. Deposition of its three-dimensional structure on our server (or a local mirror) via an Internet protocol. 2. Identification and structure generation of potential metabolites using appropriate concepts (see, for example, Cornish-Bowden and Eisenthal, 2000). 3. An ensemble of energetically favorable conformations is identified for the parent compound and all of its metabolites using a conformational search protocol. 4. A Monte-Carlo search procedure then identifies position and orientation for parent compound and metabolites with respect to every receptor surrogate. 5. Finally, the harmful potential of a substance is estimated by calculating its binding affinity and that of all metabolites towards each receptor surrogate in the database (Figure 2). Only compounds along with all of their metabolites passing through this surrogate battery

Scheme 1a

Compound deposition → Generation of 3–D structure → Generation of metabolites → Generation of conformers → **Activity scan** (all receptor surrogates)

binding weaker than a threshold affinity (e.g. $K > 1.0 \times 10^{-5}$ M) towards any model may be cleared for synthesis and further preclinical studies.

3.2 Extending the database by a new model (Scheme 1b)

Presently, our database includes validated models for the Ah receptor (receptor-mediated toxicity), the 5HT–2A receptor (hallucinogenic activity), the cannabinoid receptor (psychotropic effects), the GABA–A (receptor-mediated toxicity), and the steroid receptor (various undesired effects). The database shall be continuously extended to include surrogates for any bioregulator known or presumed to mediate toxicity or being associated with other harmful effects. The following steps are necessary for adding a new entry to the database: 1. Selection of training and test set for which experimental binding data is available. 2. Generation of all 3-D structures. 3. Conformational search. 4. Generation and validation of the receptor surrogate (*Quasar*). 5. Model deposition with the data base and testing (external compound set; scramble tests).

Additional advantages of the concept include:

1. The potential harmfulness of a compound can be accessed well before its synthesis, i.e. during the very first phase of development. Should it test positive for any of the included surrogates, the compound may not be cleared for further studies, in particular *in vivo* toxicity tests.
2. This virtual test is fast: the estimated computing time in *Quasar* is less than 30 seconds per surrogate – i.e. for a database with 100 entries this would add up to a total time of 50 minutes (on a high-end

Scheme 1b

Training set selection → 3–D structure generation → Conformational search → Model generation and validation → **Model deposition**

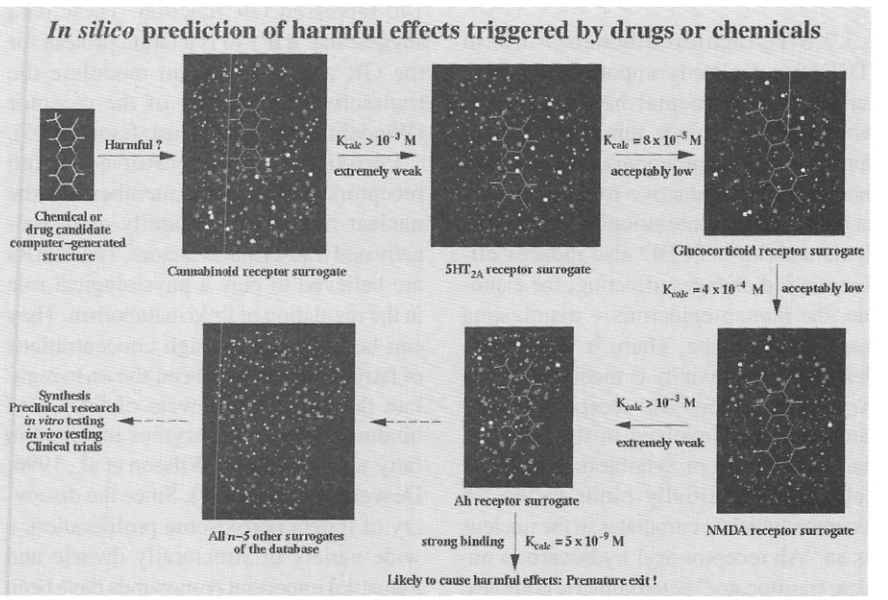

Figure 2: Flowchart of the proposed surrogate battery shown with the example of 2-trifluoromethyl-3,7,8-trichlorodibenzo-*p*-dioxin. The corresponding figure can be downloaded in color from http://www.biograf.ch/ GIFS/Sideeffects.gif

PC, Macintosh or Unix server). When using distributed computing, an overnight task may handle as much as 500–1000 compounds at a university or corporate laboratory.

3. This surrogate test battery will be available at effective costs for other if support services are requested from our laboratory.

4. The content of the database is constantly being augmented and improved; any new experimentally tested compound will be added to the existing training set, thus improving the range of validity as well as its accountability.

5. A widely used database of this kind would reduce the number of otherwise doubly–conducted (toxicity) tests at research laboratories focussed on identical or closely related biomedical targets. This should be facilitated by its absolute security (cf. 6.).

6. Most important, there is a 100% data security as the sensitive compound data used to validate the model is *not* deposited with the database and it cannot be backward regenerated: the dimensionality of the property space (typically, n=10,000–25,000) would seem to be absolutely permissive for such an undertaking.

7. The Biographics Laboratory is prepared to assist any party in both the setup process (structure generation and optimisation, conformational search) as well as during model generation.

8. Any mirror of this database can easily be installed on sites outside our laboratory (pharmaceutical industry, academia, regulatory bodies). A 100-receptor model database including all pertinent software (Quasar, setup protocols) is expected to use less than 1.0 GB of disk space – i.e. it could even be installed on laptop computers.

4 3R-Relevance

The proposed Internet laboratory contributes to two aspects of the 3R philosophy: First, it allows for an early recognition of potentially harmful substances, thus, replacing stressful animal tests in preclinical research and development as compounds with a significant activity towards any of the database surrogates are not cleared for further studies – including pharmacological and toxicological testing. Second, a widely used database of this kind would reduce the number of otherwise doubly-conducted (toxicity) tests at research laboratories focussed on identical or closely related biomedical targets.

The main advantage of the proposed virtual laboratory is that it can be applied to hypothetical substances, i.e. well before their synthesis. If a harmful potential is recognized *in silico*, it may not be cleared for synthesis and any preclinical tests, thus preventing *in vivo* toxicity tests that would otherwise simply confirm the computational finding. Such tests may then be used instead for compounds not identifiable as harmful *in silico* – leading to a reduction of *in vivo* tests.

It is the aim of the proposed virtual laboratory to allow for a free access to this database via an Internet browser/navigator. This would seem of utmost importance as a single laboratory – due to limited access to sensitive compound data – cannot validate the whole database. Equally vital is the contribution of academic and industrial laboratories to database augmentation. This can be performed locally (the sensitive data is only used for model construction but not for testing and validation) and should, therefore, increase the attractiveness of the project.

5 Call for co-operation

Although the basic concept (4D–QSAR, software Quasar) – currently used by 7 large pharmaceutical companies and 19 academic laboratories – has been validated on a variety of systems (cf. above), its use for testing compounds of possibly very different structural classes and its concatenation to form a receptor-model battery represents treading new grounds on various aspects. Therefore, its implementation and validation may turn out to be a more difficult task than presently assumed. As the expertise of our laboratory is clearly in the computational, chemical, and pharmaceutical area, it would be of great advantage to co-operate with distinguished research groups in the fields of toxicology and preclinical testing.

A co-operation may include both knowledge and data transfer. We would greatly appreciate any comments – both, criticism and suggestions – to improve or modify the presented concept. Of course, any contribution of substance data (towards a bioregulator known or presumed to mediate adverse effects) from third parties will be vital to the expansion and general usefulness of the database.

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