inhibitors reverse this error by blocking the enzymes that remove the acetyl groups; as a result, DNA can unwrap itself and make the required gene products. In their study, Qian *et al.* [1] reported that treatment with the HDAC inhibitor NVP-LAQ824 alone affected tumor and endothelial cells and was associated with increased histone acetylation, p21 upregulation and growth inhibition. Treatment with this agent also inhibited the expression of a number of angiogenesis-related genes, such as *angiopoietin-2*, *Tie-2* and *survivin*, in endothelial cells, and downregulated hypoxia-inducible factor 1-α and VEGF expression in tumor cells.

When the HDAC inhibitor was combined with the angiogenesis inhibitor PTK878/ZK222584, the number of endothelial cells in culture dishes was reduced by 51%, compared with only 21% reduction when used individually. 'VEGF inhibitors are know to have most effect on endothelial cells, the bricks and mortar of blood vessels,' explains Pili. 'However, HDAC inhibitors target both endothelial and epithelial cells, which line organs, and are the origin of many cancers.'

When these drugs were applied in combination to mice that had prostate and breast cancer, prostate tumor development was reduced by 85%; this was compared with 35% and 75% when the mice were treated with VEGF and HDAC inhibitors, respectively. The combination treatment was also more effective against breast tumor development, with an ~80% inhibition in tumor growth being observed; this was compared with 54% and 60% tumor growth reduction when the inhibitors were used on their own. Importantly these effects were obtained without the adverse side effect of toxicity.

The results reported in this study [1] suggest that such combination therapy might represent a novel therapeutic approach to the fight against cancer and are so promising that preliminary testing of similar drug combinations in humans is now planned.

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Ontology-based knowledge management of troglitazone-induced hepatotoxicity

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Drug safety and development has serious limitations in the use of all available knowledge for efficiency and risk prediction because the underlying knowledge is complex and diverse. Here, a system that enables the prediction and discovery of meaningful associations of possible adverse drug reactions (ADRs) with drug treatments by *in silico* pre-screening is introduced. The presented ontology-based knowledge management method enables efficient analysis and visualization of complex knowledge and data, which are not penetrable without auxiliary means. Such analysis enables the early identification of patients with a potentially elevated risk for the development of serious ADRs when exposed to an offending drug. Moreover, the evaluation enables the recognition of crucial substructures in drugs, which could be defused or deactivated in an appropriate secondary drug design. The example discussed here is troglitazone-induced hepatotoxicity, which is displayed variously by patients with different genotypes.

Drug response variability

The person-to-person variability of drug response is a major problem in drug development and clinical practice [1]. It can lead to both ADRs or therapeutic failure in individual patients or in sub-populations of patients. A meta-analysis of ~40 prospective studies in hospitals in the USA indicates that 6–7% of inpatients might suffer from serious ADRs and 0.32% of patients have fatal ADRs [2]. Overall, this results in ~100,000 deaths per year caused by serious ADRs, making them the fourth

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to sixth leading cause of death [2]. Moreover, serious and sometimes fatal ADRs such as drug-induced idiosyncratic hepatotoxicity cause post-marketing withdrawal of drugs [3], and pose a major problem for regulatory authorities, pharmaceutical companies, and affected patients alike. For example, of ~200 drugs licensed by the Medicines Control Agency of the UK (http://www.mca.gov.uk) between 1997 and 2002, 12 drugs were withdrawn from the market because of serious safety concerns, among them products such as troglitazone, pemoline, sertindole, cisapride, cerivastatin, and the herbal product kava-kava [4].

Individualized drug safety aims at the prospective identification of individual patients who carry genetic predispositions for the development of serious or fatal ADRs under drug exposure. Chemical structures of parent compounds, genetic variations, and environmental and other confounding factors influence the response of an individual to drugs; in other words, how likely it is that someone recovers from disease (efficacy of a therapy) and how likely ADRs occur [5–7]. The analysis and the predictive understanding of the molecular factors that eventually predispose an individual patient to the development of a serious ADR rely on various underlying data, such as chemical structures and metabolic pathways, gene and protein sequences, pharmaco- and toxicokinetic as well as pharmaco- and toxicogenetic data, clinical and single patient data, including geno- and haplotypes, which all need to be integrated for comprehensive drug safety evaluation.

The available, yet very dispersed data provides the basis for the development and integration of algorithms to derive useful predictive safety information for given drugs or individual patients. The data also provide the basis for visualization in easily understandable concepts, which are the starting point to extract crucial predictive safety information. However, due to the complexity and diversity of the underlying data, knowledge management tools and methods for mining and linking genomic, drug, metabolic, toxicological, clinical and individual patient data are still in their infancy and not broadly applied; thus, the bottleneck is not availability of data but the tools to analyze them.

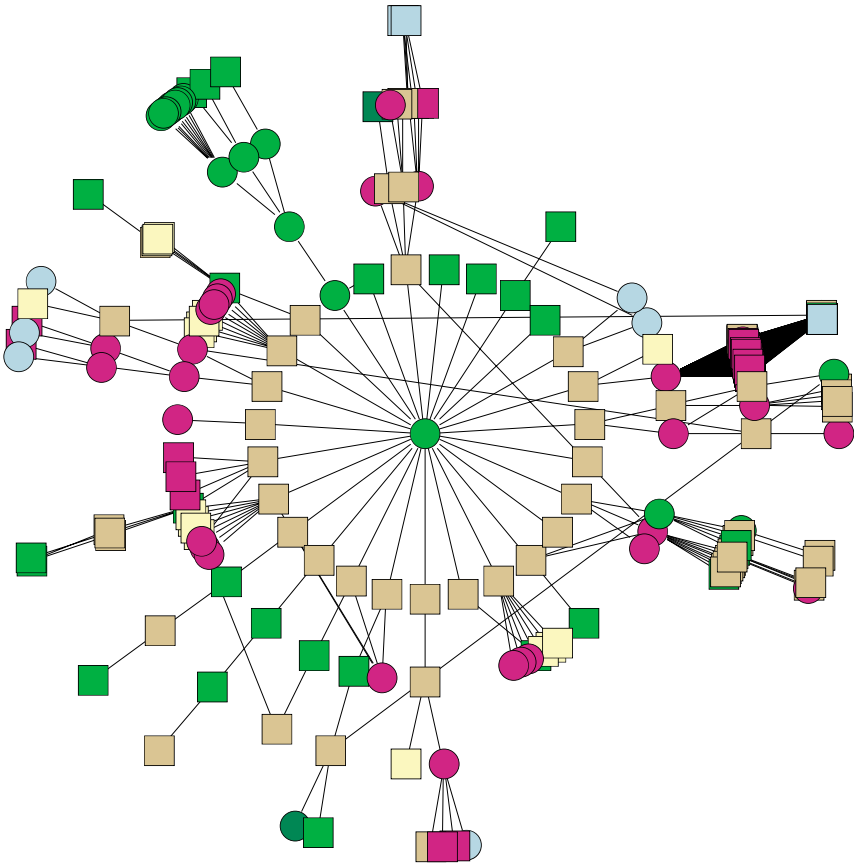

Figure 1. Ontological overview of troglitazone-related data. The central node is troglitazone. The first node circle represents the directly related neighbours of troglitazone, the second node circle the directly related neighbours of the nodes of the first circle. Different node types are shown in different colour: sand, processes; green, chemical compounds; magenta, proteins; yellow, outcomes; cyan, genes.

The Adverse Drug Reactions Information Scheme

The recently introduced Adverse Drug Reactions Information Scheme (ADRIS) [8] addresses, on a conceptional level, the ontological organization as well as the logical and semantic relation of the molecular factors that are relevant to the etiology of ADRs. A holistic view on the whole knowledge space that relates to drug safety and ADRs is provided. This knowledge space is mined, molecular factors potentially predictive for ADRs on a single patients' basis are abstracted, and this abstracted knowledge is visualized in easily understandable concepts by ADRIS, when integrated into a suitable front end. Thus, ADRIS constitutes an ontological prerequisite for the creation of knowledge base and knowledge discovery systems such as SafeBase™, which comprises interactive tools for editing, arranging and visualization of knowledge and data by connection-, neighbour- and pathfinding, sequence editing and chemical similarity searches.

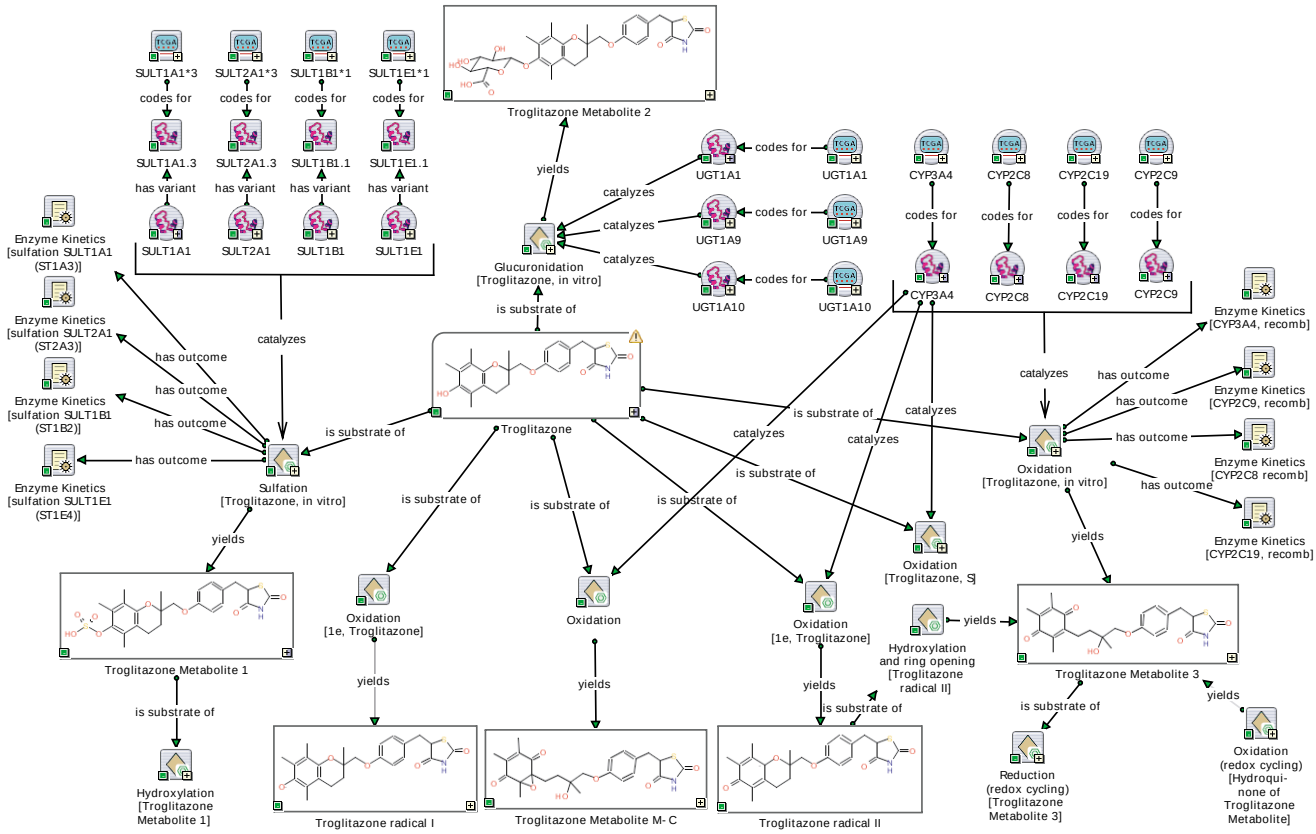

Figure 2. Metabolism and kinetics of troglitazone. Some major metabolic pathways of troglitazone and its metabolites are shown. Kinetic data are present in the attached pharmacokinetic node types. The exclamation mark (warning) at the right upper edge of troglitazone means (in this case) that the drug is withdrawn from the market.

SafeBase™ aims at the abstraction and visualization of genetic and drug related risk profiles of individual patients – theragenomic concepts.

Knowledge management systems that are based on an ontology such as ADRIS help to design better and safer drugs by at least two mechanisms: (1) the prediction of the potential for future ADRs in early phases of drug development and (2) the prediction for the exclusion of selected drugs, exposure to which should be avoided by a patient due to the given patients predisposition for the development of ADRs in later phases. This can be achieved by matching ADRs with genetic characteristics, which have impact on metabolic pathways and toxicological endpoints.

The vast amount of accumulated knowledge in the public domain on troglitazone, an oral type 2 antidiabetic drug of the thiazolidinediones class of compounds that increases the insulin sensitivity of target tissues, was mined. Troglitazone was an approved drug on the market for the treatment of type 2 or non-insulin dependent diabetes mellitus from 1997 to 2000 [9,10]. Its main ADR is direct hepatocyte toxicity; thus, in clinical trials >1.9% of patients had elevated serum transaminases [10]. Severe (ADR), with several fatalities associated idiosyncratic hepatotoxicity eventually led to the market withdrawal of troglitazone in 2000.

ADRIS-based evaluation of troglitazone action

Ontology-based knowledge management systems

A prerequisite for the efficient use of available knowledge in drug safety is an ontology-based knowledge management system for evaluation of ADRs. The exponential rise in the amount of available sequence and related data has led to the development of several databases with toxicogenomic knowledge [11,12]. These data are not yet organized within a comprehensive ontology.

Protein interaction and post-translational modification data are managed within a designed ontology in the Biomolecular Interaction Network Database (BIND) [13]. However, such data are only a subset of the data necessary for the understanding of ADRs.

Under ADRIS, data related to ADRs from various public sources [14], from the literature and from information obtained through cooperations were collected and analyzed by a combination of several algorithms in a

structured ontology. This enabled the detection of knowledge relations, which were barely evident from the publicly available, yet dispersed individual pieces of knowledge itself. These relations opened perspectives that so far seemed to be unconnected to troglitazone actions.

In a first step, a troglitazone centered deep search on the knowledge and data in SafeBase™ was performed and a general overview of the troglitazone-related knowledge was generated (Figure 1). The obtained graph contains 376 nodes of 6 different types and 501 relations. Four layers of related datasets became evident. The ADRIS-based ontology revealed several hot spots of information – regions with many nodes of the same or different types – but also revealed apparent gaps of knowledge in the second through fourth level.

Effects of metabolism

Starting from the overview, more detailed analysis and visualizations of the resulting clips were performed. Already the primary steps (i.e. sulfation, glucuronidation, oxidation, radical formation) of the metabolic pathways of troglitazone revealed diverse and complex ways of molecular interactions (Figure 2; for clarity, no secondary metabolic steps are shown).

The predominant metabolic fate of troglitazone is sulfation in the liver. Phenol sulfotransferase (ST1A3, SULT1A1) has been described as the main enzyme for sulfation of troglitazone in human liver [15]. It became evident that inhibition of sulfotransferase should be studied (Figure 2) because inhibition of sulfotransferases probably results in higher concentrations of troglitazone in the body, which might increase the potential of ADRs. Variability of sulfotransferases, alleles and expression levels, should be determined for patient cohorts suffering from idiosyncratic hepatoxicity.

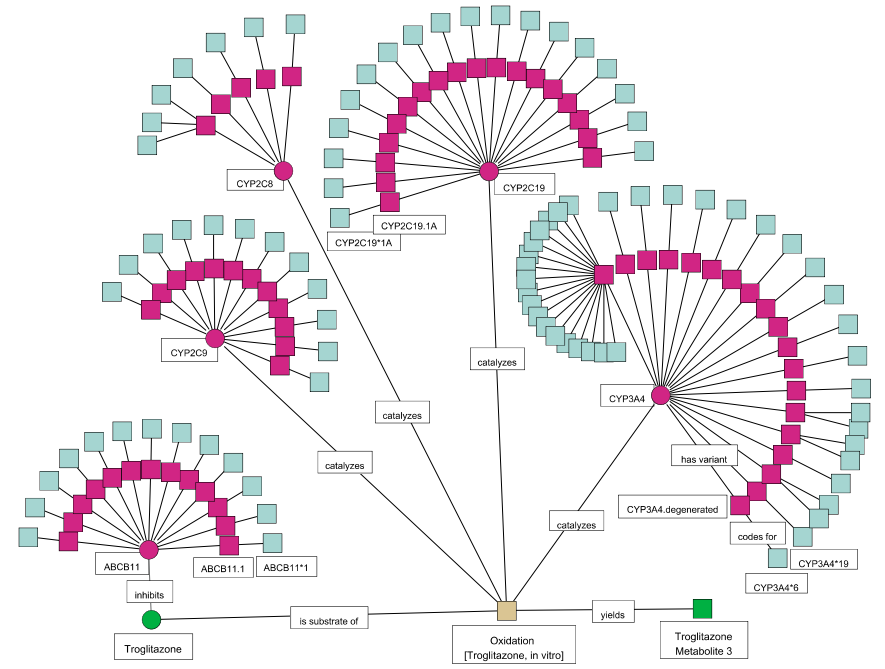

Figure 3. Allelic world of troglitazone. The protein and allelic variants of the CYP enzymes that catalyse the oxidation of troglitazone to troglitazone metabolite 3 and of the canalicular bile salt export pump (ABCB11 or Bsep), which is inhibited by troglitazone and some of its metabolites are shown as simple view. Different node types are shown in different colour: sand, processes; green, chemical compounds; magenta, proteins; cyan, allelic variants. For clarity, only a few alleles and protein variants are labelled.

Inhibition of sulfation results in a higher concentration of troglitazone and its oxidized metabolites. Thus, the combination of inhibitors of SULT1A1 together with troglitazone is expected to enhance troglitazone mediated ADRs. Strong inhibitors of SULT1A1 are present in certain diets [16] and medications like mefenamic acid [17]. We conclude that diets should have been considered during troglitazone treatments. The dietary ingredients cianidanol (catechin) and butylated hydroxytoluene (BHT) were shown to increase α-tocopherol (vitamin E) in rats [18]. Like vitamin E, troglitazone contains a chroman ring (see below), therefore, one might speculate that such dietary ingredients, which are omnipresent (e.g. in tea and chocolate) are able to increase troglitazone concentration by inhibition of SULT1A1 leading to the typical ADRs.

Effects of polymorphisms

The mechanisms of pathogenesis mediated by troglitazone are still not completely understood. Detailed information on ADR mechanisms connected with troglitazone metabolites was included in ADRIS whenever available. Allelic variants of the genes coding for the cytochrome P450 isoenzymes CYP3A4, CYP2C8, CYP2C9 and CYP2C19 involved in the oxidation of troglitazone to troglitazone metabolite 3 [19,20] and allelic variants of the gene coding for the canalicular bile salt export pump ABCB11 (Bsep) involved in troglitazone-induced intrahepatic cholestasis [21] were identified (Figure 3).

The incidence of the polymorphisms of CYP2C19 and CYP2D6 genes was correlated to the occurrence of troglitazone-induced hepatotoxicity [22]. For these variants individual patient-based data were available and

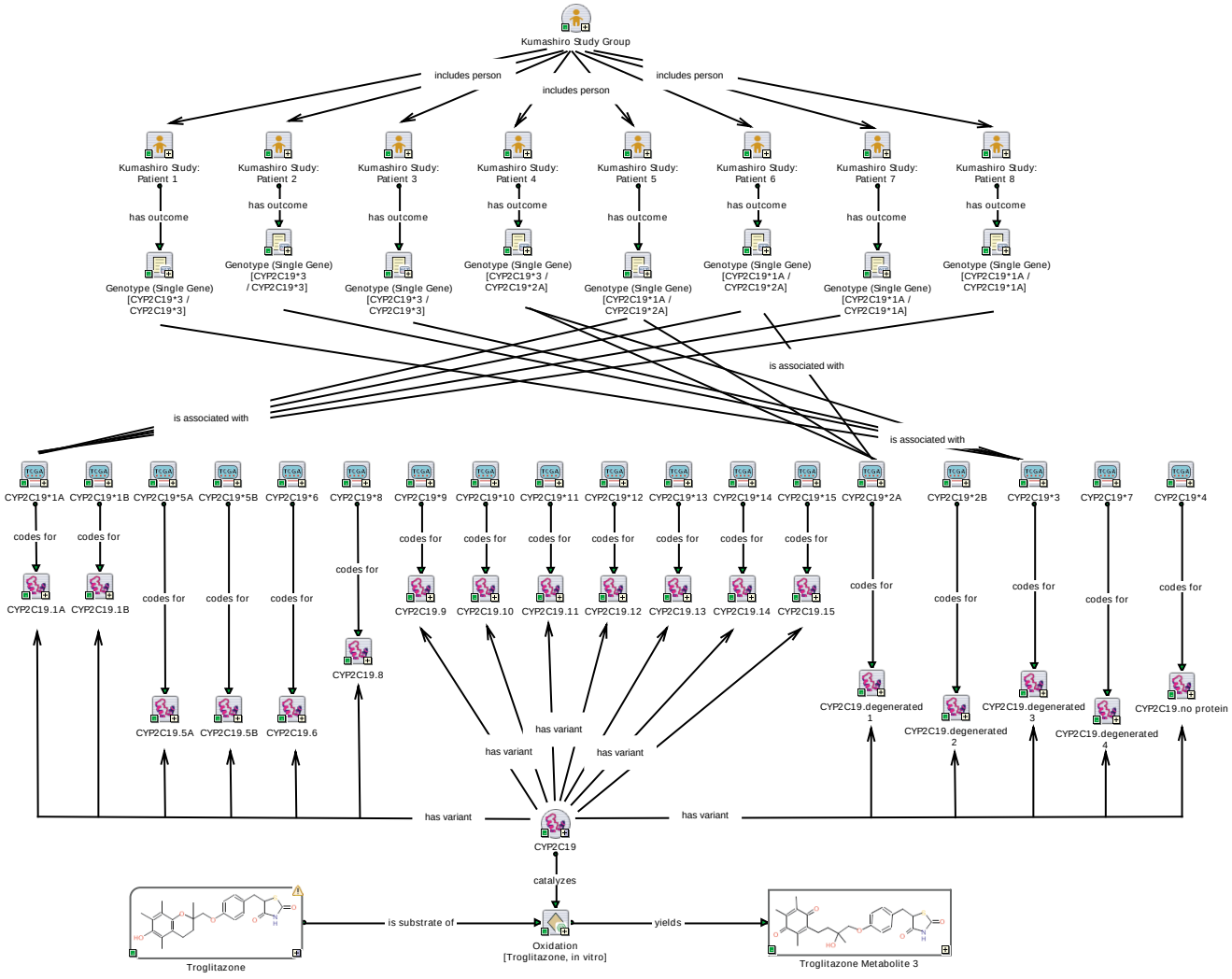

Figure 4. Relations of patient genotypes to troglitazone metabolism. The data set is from a Japanese study [22]. The CYP2D6 and CYP2C19 genotypes of individual patients are visualized and correlated with an oxidation process of troglitazone.

the genotypes of individuals were linked to defined biochemical pathways (Figure 4). The main result of this study was that the occurrence of certain CYP2C19 SNPs correlated with troglitazone-induced hepatotoxicity in the Japanese population, whereas the studied CYP2D6 SNPs did not show a significant influence. An evident gap of knowledge of this study is the missing information on polymorphisms of CYP3A4, CYP2C8 and CYP2C9, which also play a major role in troglitazone oxidation (Figure 2).

Particular information could be extracted by neighbour searching. One of the genotypes under study displayed also an effect in the demethylation of fluoxetine catalyzed by the CYP variant CYP2D6.2, as revealed in an ADRIS concept. Because ADRIS combines information from various fields, linkages were disclosed, which would have been difficult to detect by separate pieces of information.

Chemical similarity searches

Similarities of a compound with other compounds that are stored in ADRIS are revealed by comparisons between digital compound fingerprints. Substructure similarity compares whether a given structure is included in another structure. Parent compounds, intermediates and metabolites with similar properties can be identified.

A chemical similarity search with troglitazone as query was performed. Troglitazone substructures are a cryptic quinone, glitazone, thiazolidinediones and a chroman ring. A search with thiazolidinedion, the substructure of troglitazone, which is suspect of being responsible for some of the ADRs, detected among others KRP-297 (MK-767, L-410198). This compound is under development for the potential treatment of diabetes [23].

Other important troglitazone interactions

The reported ADRs of troglitazone consist mainly of various types of liver toxicity. Troglitazone affects sugar and low-density lipoprotein metabolism, phosphorylations and other intracellular signal transduction mechanisms by activation, induction or inhibition of several enzymes. The detailed evaluation of metabolic and intracellular signal transduction pathways in regard to troglitazone induced toxicity, treatment of type 2 diabetes and the suggested usage of thiazolidinediones in the treatment of various cancers is ongoing.

Thiazolidinediones are activators of the peroxisome proliferators-activated receptor (PPAR) γ, which is a member of the nuclear receptor family of ligand-activated transcription factors [24]. PPAR γ ligands might generally serve as promising drugs for the treatment of breast cancer. In this regard, it has been postulated that troglitazone can inhibit the growth of breast cancer cells [25,26], most likely by induction of apoptosis, but a phase II study showed that PPAR γ activation by troglitazone had no significant effect on patients with treatment-refractory metastatic breast cancer [27].

Conclusion and outlook

A model was built to investigate the scientific reasons underlying the withdrawal of troglitazone. Such kinds of investigations could help to identify potentially unsafe drugs before marketing. Moreover, obtaining the genotype of some patient candidate genes before drug treatment can reduce the risk of ADRs. In this perspective, it is even considerable that drugs withdrawn from the market could be re-introduced for patients with defined polymorphisms.

The concepts generated with ADRIS help to identify metabolic pathways that should undergo further study. Where appropriate, concepts might include not only data generated from human *in vivo* studies but also from *in vitro* systems and animals. The interpretation depends on the quality of the data. For example, the epoxide metabolite of troglitazone M-C is toxic in cell culture [28]. A possible conclusion is that pathways like the sulfation of troglitazone, which lead to a decrease in the epoxide, could diminish the occurrence of troglitazone-mediated ADRs. On the other hand, troglitazone itself and the sulfated product inhibit the canalicular bile salt export pump ABCB11 in rats, [21] thereby helping to induce cholestasis. It has been hypothesized that troglitazone and troglitazone sulfate are responsible for toxicity, whereas oxidation by CYP3A4 and glucuronidation are detoxification pathways [29]. The concepts show the direction of future research by helping to identify the critical steps in drug metabolism. Critical structures and compounds are identified with the help of chemical similarity searches.

Prospective safety profiles are helpful in the optimization of leads, in the preclinical and clinical phases and in post marketing. In the near future, tools to identify new molecules or pharmacophores as drug targets will be integrated into ADRIS. The safety profiles are sharpened for serious risk factors, expected frequency of affected alleles, targeting for indications and patient populations. The increased value for leads or early drug candidates will guide to more successful in house development and a higher value at any stage of licensing.

The strategy for the 'right medicine for the right patient at the right dose' is initiated and will evoke ethical issues [30,31]. The problem of generating false positives or false negatives can be handled by careful data editing. Diagnostic data could be exploited by medical insurances and therefore, patient data must be kept and handled anonymously. Regulatory organizations are concerned to limit the occurrences of ADRs and regulate pharmacogenomic data submissions (http://www.fda.gov/cber/gdlns/pharmdtasub.htm).

In principle, an analysis as shown here for troglitazone is possible for all chemical compounds. Taken together, such early identification of serious risk factors will help pharmaceutical companies to develop safer drugs.

Note added in proof

The recent market withdrawal of the COX-2 inhibitor rofecoxib (Vioxx®) underlines once more the necessity of a more comprehensive intelligence on and visualization of all safety relevant information and data related to a single compound and its induced ADRs.

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Cite and oversight

The research publication is the currency of our scientific lives, and the value of our careers is measured with what we publish. It is not enough to document what we have done, it is also important to know whether we have made a meaning ful difference, hence the desire to measure our worth and value. Enter citation analysis and impact factors.

I have been interested in this issue for along time, but the recent stimulation came from reading the recent article in *Drug Discovery Today* by Raymond C. Rowe entitled *Publish or perish* [1]. This response to Rowe's article represents one individual's opinion on a controversial issue. It is not a meta-analysis or a review, therefore, there will not be a surfeit of references; the citations will be idiosyncratic to suit my purpose. I am following in the footsteps of many, only honest enough to admit it. If at the end of it all, I have provoked a response, I will rest content.

The research publication that plays such an inordinate part of our professional lives was largely the creation of a single individual, Henry Oldenberg. His tale has often been told [2–4], therefore, a simple summary will suffice here. Born in Bremen between 1617 and 1620, he settled in England after 1653, becoming friendly with those who were trying to propagate a new way of

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