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Investigative and Mechanistic Studies

Unexplained observations from Safety or DMPK studies?

Unknown relevance to man?

- **Due to inter-species differences, toxicity or ADME issues observed in animals may not necessarily be relevant to Man**
- **By determining mechanisms in animals, one can make predictions about the likely effects in humans**
- **By using human *in vitro* systems these predictions can be tested to determine the relevance to man**
- **A rationale that is substantiated with experimental evidence can then be used to make accurate assessments about the risk to human health**

CXR BIOSCIENCES HAS A PROVEN TRACK RECORD IN DETERMINING TOXICOLOGICAL AND DRUG METABOLISM MECHANISMS, DESIGNING CUSTOMISED ASSAYS, AND PROVIDING DATA TO SUPPORT SCIENCE BASED HAZARD AND RISK/BENEFIT ASSESSMENT

Introduction

A key issue in the development and registration of chemicals and drug candidates is establishing the relevance to Man of experimental observations made in non-human species. Toxicity observed in animals can lead to adverse classification and labelling that may impact negatively on sales and can often be a reason for stopping product development completely. Inappropriate bioavailability or drug metabolism can have similarly negative implications for development. However, experience, and our growing knowledge of species differences, has shown that such observations in rodents do not necessarily translate into the same effects in humans. By understanding the mechanism(s) of the biological response in animals, CXR Biosciences can help determine the relevance to man, and provide data to support a realistic hazard assessment in humans and allow informed decisions on further development.

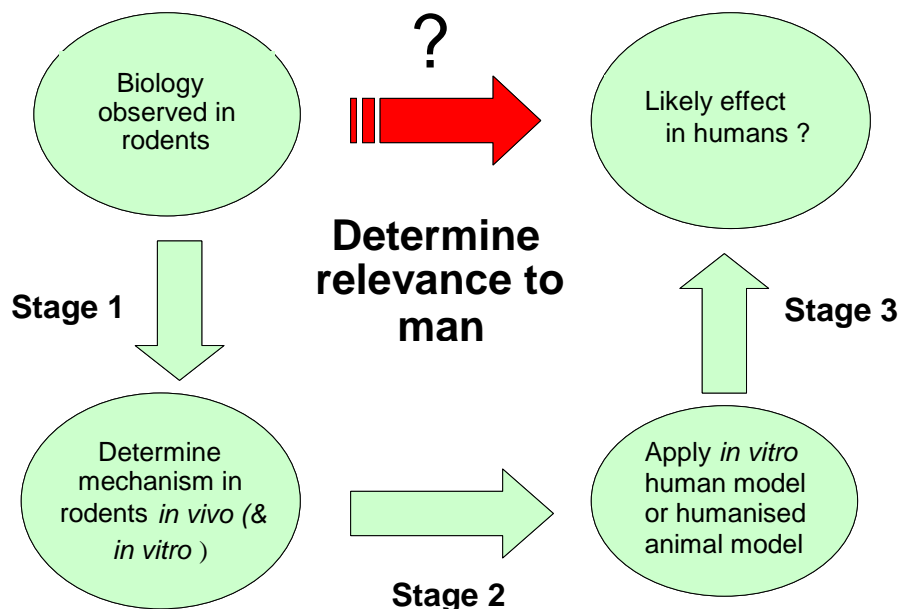
An in depth understanding and expert interpretation of the results of preclinical studies are pivotal to qualifying the development risks of a substance. The unique combination of toxicology, ADME, molecular biology and bioinformatics expertise at CXR Biosciences facilitates rapid and sophisticated studies to explain toxicological or other observations. CXR Biosciences also has staff experienced in working with the regulatory authorities to assist in their understanding of relevance to humans.

CXR has a proven track record in elucidating mechanisms of action, investigative toxicology, and product rescue and advocacy in the pharmaceutical and chemical industry. We currently assist a number of large and small companies in the Pharmaceutical, Agrochemical and Chemical Industries and provide specific support to Cefic sector groups including European Council for Plasticisers and Intermediates (ECPI), Plastics Europe Fluoropolymers Committee (formerly APME), and Euro Chlor.

CXR Biosciences' Problem-Solving Expertise

CXR Biosciences has developed an approach to problem solving that provides cost effective solutions to address the issues faced by industry. Through expert interpretation of existing data, and by applying state of the art technologies to investigate the underlying mechanisms in animals, CXR can determine whether the observed effect is relevant to man. The Company has extensive experience with a wide range of conventional, molecular biology and genomics related technologies, including a number of proprietary models. Key to the success of these projects is the design of a customised study program that meets the unique needs of the problem, and a high level of flexibility and interaction with clients.

The diagram below illustrates our problem solving approach:



Stage 1. Hypothesis formulation and testing

Existing data is reviewed and the results interpreted in light of the available information, and bioinformatic analysis if relevant. Based on this review, either (a) a

putative mechanism will be proposed and a series of studies designed to test the hypothesis or (b) studies will be suggested to aid in the formulation of a hypothesis.

Stage 2: Develop an *in vitro* rodent model and/or humanised mouse model

Having elucidated an *in vivo* mechanism in rodents, a relevant rodent *in vitro* model of the cellular mechanism will be applied or developed. This may comprise a primary cell line or immortalised cell line and / or the development of a non-cell based assay. The aim will be to provide a simple assay that can confirm our hypothesis, and can also be used as a primary screen to evaluate the potential of other candidate compounds to induce the same toxic response.

CXR's proprietary tools can also be applied where relevant, such as humanised mouse models in which certain murine genes involved in pathways of relevance are replaced by their human counterparts. This is particularly relevant where species differences between rodent and human homologues are known to exist, for example with the transcription factors PXR and CAR. CXR has already developed a range of humanised mouse models for key genes involved in drug metabolism and disposition.

Stage 3: Develop an *in vitro* human model

The final stage is to develop an *in vitro* human cell model that is equivalent to the *in vitro* rodent model. The ultimate aim of the programme will be to develop an *in vitro* screen using human cells that can be used to screen for potential human hazard.

In some cases, humanised mouse models are a relevant model for performing *in vivo* experiments as an additional avenue for establishing relevance to man.

Tools for Problem Solving

Expertise

Capitalising on the extensive academic and industrial experience of its founders Dr Cliff Elcombe and Prof. Roland Wolf, the company combines scientific excellence with effective project management to deliver cost effective solutions. A key part of the success of CXR Biosciences in problem solving is its multi-disciplinary team, combining extensive expertise in a range of conventional techniques and cutting edge technologies. The judicious use of appropriate tools and techniques maximises the chance of successfully elucidating toxic mechanisms.

Toxicology, ADME and molecular biology

In addition to the standard toxicology and ADME studies that are performed routinely at CXR Biosciences, an experienced team of molecular biologists can develop new assays or models where required. This approach can, for example, include the cloning of rodent and human receptors to compare species differences or the development of an assay to demonstrate the mechanism of toxicity of a chemical.

Transcriptional profiling and bioinformatics

Transcriptional profiling involves the measurement of changes in global gene expression that occur as a result of a toxic insult. This is particularly useful when the mechanism(s) of toxicity are unknown. Using gene-chip microarrays and sophisticated bioinformatics and pathway analysis software, CXR Biosciences can generate biologically relevant hypotheses for mechanisms of action or toxicity. Applied in this focused and thoughtful manner transcriptional profiling provides a powerful approach towards elucidating and understanding biological mechanisms.

CXR Biosciences has is accredited as an 'Agilent Service Provider'. This is an independent measure of the high standard of CXR Biosciences expertise and quality in transcriptional profiling. The Company also has an ongoing collaboration with Ingenuity Systems in the area of toxicological pathway analysis.

Novel proprietary models

Rapid *in vitro* and *in vivo* toxicity screens

CXR Biosciences has developed revolutionary systems for the rapid assessment of potential mechanisms of toxicity. Bioinformatic searching has been used to identify a series of genes that are activated in response to toxic agents. The promoters of these genes have been linked to proprietary biomarker molecules, to provide rapid *in vitro* and *in vivo* methods for the assessment of candidate compounds. CXR Biosciences can also develop customised systems that measure changes in the transcriptional regulation of any gene of interest, which can be applied to resolve specific problems.

Transgenic mouse models

In vivo studies are a key component of any problem solving programme but species differences are a significant confounding factor to understanding the relevance to man. As drug metabolism and drug transport are key areas that affect the demonstration of safety and efficacy, CXR and its partner TaconicArtemis have jointly developed, and are continuing to develop, a panel of mouse models that have been humanised for key genes including:

- The nuclear receptors PXR, CAR and AHR;
- The major Cytochrome P450 enzymes;
- Drug transporters.

In many cases, mice with multiple humanisations or these genes knocked out are also available.

These new models provide a powerful addition to the toolset available to CXR to resolve problems in drug development or safety.

An example of a problem solving project performed at CXR is described in the following pages.

CXR Biosciences Role in Safety Evaluation and Human Risk Assessment of Substance C: Mechanisms of Non-Genotoxic Carcinogenesis

Substance C has low mammalian toxicity and is non-genotoxic in conventional assays for mutagenicity and clastogenicity. However, when administered to Fischer 344 rats in a two year bioassay, Substance C induced liver tumours and thyroid follicular cell neoplasms in male and female rats, and renal adenocarcinomas in male rats only. Two 90-day rat experiments, with Substance C administered in the diet for one study and by gavage in corn oil for the other study, revealed similar pathological trends to those seen in the two-year study. There was marked hepatomegaly accompanied by hepatocellular hypertrophy in both sexes, chronic nephritis in male rats and thyroid follicular cell hypertrophy and hyperplasia in both sexes.

Significant human exposure to Substance C occurs, and the discovery of its carcinogenicity led to concerns over potential human health problems. Without an understanding of the mechanisms for the induction of these tumours, the regulatory authorities will assume that these tumours are relevant to humans, classify the substance as a carcinogen and conduct any risk assessment accordingly. This necessitated the initiation of research aimed at understanding the mechanism of carcinogenicity in rats, in order to evaluate the potential hazard of exposure of humans to Substance C.

In light of the non-genotoxicity of Substance C and the spectrum of tumours induced in rats and mice, it was hypothesised that the tumours were induced *via* three separate mechanisms. A series of experiments were designed and performed to test these hypotheses.

1. Liver Tumours – *The Peroxisome Proliferation Phenomenon*

Characterisation of the hepatomegaly induced in rats demonstrated Substance C as a peroxisome proliferator. Chemicals of this class induce liver tumours in rodents *via* a non-genotoxic mechanism. These chemicals interact with the peroxisome proliferator activated receptor (PPAR α) and initiate transcription. This interaction leads to the characteristic pleiotrophic response involving hepatomegaly that is characterised by proliferation of peroxisomes, inhibition of apoptosis, stimulation of replicative DNA synthesis and cell proliferation. This sustained hepatomegaly ultimately leads to the development of hepatocellular carcinoma.

Activation of the PPAR α receptor is a prerequisite for the induction of liver tumours *via* this mechanism. The lack of hepatocarcinogenicity of peroxisome proliferators in PPAR α null mice substantiates this contention. In the human liver, PPAR α is expressed at levels of 1-10% of that found in rat and mouse liver. This level of expression appears to be too low to support the receptor-mediated mechanism of hepatocarcinogenesis observed in rodents. Hence, non-genotoxic peroxisome proliferators are generally not considered to pose a hepatocarcinogenic hazard to humans.

Substance C clearly fitted into this class of rodent selective hepatocellular carcinogen and would be unlikely to pose a carcinogenic hazard to humans.

2. Thyroid Follicular Cell Tumours – Perturbation of Thyroid Hormone Homeostasis

The regulation of the hypothalamic-pituitary-thyroid-liver axis is similar in mice, rats and humans. Decreased plasma thyroxine concentrations lead to the suppression of the negative feedback control of TSH (thyroid stimulating hormone) release from the pituitary gland leading to thyroid hypertrophy and hyperplasia (i.e. goitre). While goitre does not usually progress to thyroid cancer in humans, it may be implied that sustained thyroid growth may predispose an individual to carcinogenesis.

In rats, Substance C caused a decrease in plasma thyroxine (T4) levels and therefore an increase in TSH which, in turn, leads to the development of thyroid tumours. The decrease in the levels of T4 is due to the induction of glucuronosyl transferase, the enzyme responsible for conjugating thyroxine prior to its elimination as T4-glucuronide in the bile.

The induction of glucuronosyl transferase by Substance C was reproduced in rat primary hepatocyte cultures. However, using primary cultures of human hepatocytes, we were able to demonstrate that Substance C did not induce human glucuronosyl transferase. Hence, Substance C will not perturb plasma concentrations of thyroxine in humans and the consequential thyroid gland growth will not occur. Hence, this chemical does not pose a thyroid cancer hazard to humans.

3. Kidney Tumours - α 2u globulin nephropathy

28 day and 90 day studies showed that Substance C induced the accumulation of α 2u globulin in male rat kidneys. The α 2u globulin-mediated mechanism of renal tubular carcinogenesis is dependent ultimately upon the synthesis of α 2u globulin in the liver. This is specific to the male rat. Female rats do not express the protein and do not develop nephropathy or renal tumours. Similarly, other studies with Substance C in mice (non-expressers of α 2u globulin) did not demonstrate nephropathy or kidney tumour induction.

Therefore, as humans do not express α 2u globulin, it is reasonable to conclude that Substance C will not elicit kidney tumours in humans.

Summary

Such marked species differences in response underline the need for caution when using rodent toxicity data to assess potential hazard and risk to humans. When extrapolating from rodents to humans it is important to have an understanding of carcinogenic mechanism, especially in the case of potential non-genotoxic carcinogens. The demonstration that the carcinogenic effects of Substance C arise *via* rodent-specific mechanisms which do not occur in humans made it possible to deduce that Substance C does not represent a carcinogenic risk to humans.