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Target organ profiles in toxicity studies supporting human dosing: Does severity progress with longer duration of exposure?

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ABSTRACT

We have previously reported the profile of target organs (defined as organs showing histopathological changes) in rodent and non-rodent toxicity studies conducted prior to first-time-in-man (FTiM) for 77 AstraZeneca candidate drugs (CDs). Here, we test the assumption that toxicity is exacerbated by dosing duration by comparing the incidence and severity of target organ toxicities in these ≤6 week FTiM studies with those observed in subsequent subchronic/chronic (≥3 month) studies. Looking at the effect of dosing duration on severity (pathological score) and incidence (percentage of animals within the group) for the 39 CDs that met the criteria for inclusion (comparable doses between FTiM and subchronic/chronic studies), new toxicities appeared for 31 target organs but existing ones resolved for 29 target organs. Increased severity was more frequent for rodent (16 target organs) than for non-rodent (4 target organs). Most notable changes were a large increase in severity/incidence in liver and in non-rodent lung in contrast to a large decrease in severity and incidence for kidneys/ureter and for the non-rodent thymus. Overall this analysis shows that, even with continued exposure, target organ toxicities of CDs are as likely to show partial or complete recovery as they are to progress in severity.

1. Introduction

The potential for new drugs and other chemicals to cause toxicity is assessed using a range of in silico, in vitro and in vivo tests that are designed to assess, limit and manage risk to humans, wildife and to the environment. In performing risk assessments for new entities or for new or extended use of existing entities, the data sets available can be highly diverse depending on previous and intended use. Regulations are also complex on the need for new animal tests with emphasis correctly placed on preferential utilisation of existing data wherever possible.

For new small molecule drugs, nonclinical safety packages are conducted stepwise in accordance with regulatory guidance; in general studies of up to one month duration in rodents and non-rodents can support First-Time-in-Man (FTiM) (Phase I) (ICH M3(R2), 2009) but longer duration animal studies are required to support longer duration clinical exposure; 6 month rodent and 9 month non-rodent studies would generally support dosing for longer than 6 months in clinical trials and are also required for registration (ICH M3(R2), 2009).

For industrial chemicals, the duration of toxicity studies required is driven by production volume as well as by import tonnage (ECHA, 2014). A short term repeated dose toxicity study (one month) is required when production exceeds 10 tonnes/year. A subchonic toxicity study (3 months) is required if production exceeds 100 tonnes/year but might be avoided if the 90 day no adverse effect level (NOAEL-90) can be extrapolated from the NOAEL-28 and only if the substance already has a hazard label for repeated dose toxicity.

A longer term repeated dose toxicity study (>12 months) may be required for >1000 tonnes/year, especially if there is concern around frequency and duration of potential human exposure. Further toxicity testing in animals can be omitted altogether if combined weight of evidence from previous tests and from alternative methods such as qualitative or quantitative structure–activity relationships ((Q)SARs) is sufficient for the purpose of classification and labelling and risk assessment. For many

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substances only short-term animal studies might be available for the evaluation of long-term human exposure. Therefore extrapolation factors (EF) are generally used to extrapolate NOAELs from existing short-term studies to NOAELs for long term exposure.

For agrochemicals, plant protection products and their active ingredients are assessed stepwise in repeated dose one month studies (OECD Guideline 407, 2008), repeated dose subchronic (3 month) studies in rodents (OECD Guideline 408, 1998) and non-rodents (OECD Guideline 409, 1998) and chronic studies in rodents (OECD Guideline 452, 2009 , OECD Guideline 453, 2009; EU, 2013a,b). This can potentially be avoided but only for natural products and only if there is sufficient data in the literature to support labelling and risk assessment. Overall, the testing strategy, risk assessment and labelling approaches for drugs, industrial chemicals and agrochemicals are predicated on the assumption that severity of toxicity increases with duration of exposure (Batke et al., 2011).

In general, in the risk assessment of chemicals, exposure is estimated over a similar period to that over which the toxicty is manifest (WHO, 2009). This means that for longer term exposures, shorter-term fluctations will be averaged out, on the assumption that it is cumulative exposure over the entire period of concern that determines toxicity.

The pre-clinical toxicological profiles of candidate drugs (CDs) provide a rich dataset with which to explore commonly-held assumptions in the risk assessment of chemicals. We have previously reported an analysis of target organ profiles in FtiM-supporting toxicity studies for a set of 77 AstraZeneca CDs across a range of therapy areas (Cardiovascular/Gastrointestinal: CVGI; CNS/Pain: CNSP; Respiratory and Inflammation: RITA; Oncology/Infection: OI) (Horner et al., 2013). Target organ toxicity was primarily defined as compound-related histopathological changes. Here we report on how target organ toxicity progresses with prolonged exposure by comparing toxicity profiles in the one month FTiM studies with toxicity profiles in the subchronic//chronic (≥3 month) studies for the same CDs.

2. Materials and methods

2.1. Included CDs and studies

A previously-reported database (Horner et al., 2014) of general toxicology studies on 42 AstraZeneca candidate drugs (CDs) was updated to include a further 11 CDs, totalling 53 CDs where both FTiM-supporting studies and subchronic/chronic studies in rodents and/or non-rodents had been conducted. This was based on an earlier database of 77 CDs (Horner et al., 2013) where FTiM studies but not necessarily subchronic/chonic studies had been conducted, hence the differences in number of CDs. FTiM studies were typically one month duration (range 2–6 weeks) and subchronic/chronic studies were typically 6 months duration (range 3–12 months) (Table 1). All studies were conducted to GLP and in accordance with ICH guidelines.

For the 53 CDs where there both FTiM and subchronic/chronic studies were conducted, dose levels were analysed to determine comparibility between doses tested in the FTiM studies and subsequent doses in the subchronic/chronic studies. There was equivalence (±10%) between the high doses tested for 36 CDs, 25 in rodents and 24 in non-rodents. For 2 CDs in rodents and 2 CDs in non-rodents (encompassing 3 additional CDs = 39 CDs total) there was equivalence between the mid dose in the FTiM study and the high dose in the subchronic/chronic studies. For the remainder, the chronic high dose in the rodent studies was ≥67% of the FTiM dose for around half of them and for 3 of these it was >100%. Similarly, in the non-rodent the top dose in chronic studies for 7 CDs was ≥67% of the FTiM dose and was >100% for 2 CDs. Hence, exlcusion of these CDs from the analysis would not have introduced any bias. The majority of studies were dosed via oral routes with a small number dosed by inhalation or by subcutaneous administration. For all studies irrespective of route the dose was administered as mg/kg bodyweight.

A summary of included studies is shown in Table 1.

The rodent and non-rodent species used in the majority of studies were rats (Wistar-derived) and beagle dogs, respectively (Table 1), with mice or Sprague Dawley rats and cynomolgus monkeys, respectively used in the remaining studies. As in our previous analyses (Horner et al., 2013, 2014) the parameters typically measured in these studies included clinical observations, bodyweight, food/water consumption, ophthalmoscopy, haematology (including coagulation in non-rodents), clinical chemistry, urinalysis, ECG/blood pressure measurements (in non-rodents) and terminal investigations (organ weights, macroscopic abnormalities and histopathology). The tissues/organs typically collected for histopathological evaluation from these studies are detailed in these analyses.

2.2. Analysis and comparison of target organ toxicity profiles

For the 39 CDs meeting the criteria for inclusion in the analysis (comparable doses between FTiM and subchronic/chronic studies), the presence of target organ toxicities in the FTiM and subchronic/chronic studies was analysed and compared for each CD in the rodent and non-rodent to determine if the target organs noted in the FTiM studies persisted in the subchronic/chronic studies and whether there were new target organs in the subchronic/chronic studies. Three sub-analyses were also conducted to compare target organ toxicities in the FTiM with the subchronic (3 month) studies (25 CDs), FTiM with the chronic (6–12 month) studies (22 CDs) and the subchronic (3 month) studies with the chronic (6–12 month) studies (5 CDs). Target organ toxicity was primarily defined as compound-related histopathological changes: other changes such as altered organ weights or clinical pathology findings, in the absence of associated histopathological changes, were considered not to be evidence of target organ toxicity for the purpose of this analysis. Note that the totals for the presence of target organ toxicity exceeded the number of CDs since there were multiple target organs for several of the CDs. In this first analysis and the 3 sub-analyses of presence or absence, single or multiple findings within a tissue, or the severity of the findings, were not discriminated.

However, in two further analyses, severity and incidence were introduced as additional criteria. These analyses focused on the target organs (adrenal glands, kidneys & ureters, liver (including bile ducts), spleen and thymus) that were the most frequent target organs in previously reported FTiM studies (Horner et al., 2013) plus additional target organs (lung, lymph nodes, male reproductive organs and thyroids & parathyroids) showing high incidence in this updated data set. Focussing just on these 9 target organs reduced the dataset to 31 CDs since there were no findings in these 9 target organs for the other 8 CDs.

The first of these further two analyses looked at the change in severity of target organ pathologies between the FTiM and the subchronic/chronic studies. A mean severity score was defined based on the pathology severity/grading (0 – normal, 1 – minimal; 2 – mild or slight; 3 – moderate; 4 – marked; 5 – severe or massive) for each target organ for each animal in the group. For the purposes of this analysis, individual lesions within each target organ were assessed separately, and the findings for the “most severe” used in the analysis. Changes in severity were then classified for each CD as: +: New target organ in subchronic/chronic study; ⇧: