Likewise, NS-B was found comparably toxic in male and female primary hepatocytes being rapidly metabolized in the two cases. Same metabolites were identified in both genders. Overall, results in minipigs and humans exclude major sex differences in pharmacokinetics and toxicity in non-rodents. One can not exclude a role of sex-related CYPs in the sex-dependant toxicity and kinetics in rats.

Melius is a CENIT project granted by CDTI, Spain.

doi:10.1016/j.toxlet.2010.03.883

P302-054

Nypta (NP031112) does not possess mutagenic potential. An activity of the Melius project

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The main objective of the preclinical safety program is to determine the potential side effects of the drug candidates being developed. However, other related chemical entities, such as metabolites or impurities, may also be important regarding safety aspects.

Nypta (NP031112) is a new compound for the treatment of neurodegenerative diseases. Currently, it is under Phase IIb clinical development for Progressive Supranuclear Palsy, Furthermore, after the recent completion of a Phase IIa trial on Alzheimer's Disease, a follow up Phase IIb study is planned to start shortly.

To fulfil the regulatory guidelines (parent drug, related metabolites and impurities), it was mandatory to provide relevant information about their potential mutagenic risk.

Accordingly, and following the ICH guidelines about mutagenesis, in vitro tests were performed with NP031112, its main metabolite and its main impurity.

Screening results obtained in the umu test showed no effect in this screening model, and gave confidence to initiate the regulatory development.

GLP-compliant Ames tests showed that none of the tested compounds was genotoxic in this model. In addition, GLP-compliant mouse lymphoma cell assays and in vitro micronucleus were used as eukaryotic cell models for additional testing. NP031112, its main metabolite and its main impurity resulted in non-genotoxic responses. However, since NP031112 was cytotoxic to cells in culture, it was decided to confirm the lack of genotoxicity under in vivo conditions conducting a micronucleus assay (also in compliance with the guideline ICH S2).

After confirming the good exposure of the product in the mouse, no evidence of induction of micronuclei in the polychromatic erythrocytes was observed.

In conclusion, neither NP031112 nor its main metabolite nor its main impurity possess mutagenic potential and the lack of genotoxic risk of Nypta is confirmed.

Melius is a CENIT project granted by CDTI, Spain.

doi:10.1016/j.toxlet.2010.03.884

P302-055

The Melius project: A Spanish industrial project to improve the translational prediction of the non-clinical safety studies to man

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The society is waiting for new effective and safe treatments to fight against the existing diseases. Despite the effort made both by researchers and the Regulatory Authorities, unexpected safety events occur in clinical development or, still worse, when the new drug reaches the market.

It is assumed by the scientific community that the X-Omics technologies today available might help in finding safer and more effective drugs. However, these technologies require very important investments and may not be available to small to medium size companies.

With the support of CDTI, the Melius Consortium was established in 2007, with a budget of 20 million €, which 50% was financed by the CENIT-Ingenio 2010 programme. The objective of the project was the application of X-Omics technologies (Cellomics, Genomics, Proteomics, Metabolomics) to compounds from the discovery phase to non-clinical and clinical development, integrating the new technologies with more classical safety assessment approaches, including regulatory animal studies. The Melius Consortium consists of 4 drug companies (Pharmamar, Faes, Rovi and Noscira) that bring their own compounds under R&D and 4 technological companies (PGK, PTK, OWL, NBT) that bring their technologies. Research activities started in 2007 and will end in early 2011.

In addition to the members of the Consortium, several Spanish public research institutions are involved in the project to cover both fundamental and technological aspects not available in the know-how of the members of the Consortium.

Up to now, several general conclusions have been obtained from the project. First of all, the project demonstrates that competitors can collaborate with an ethical objective. We have also learned how to use these new technologies to make decisions, their strengths and limitations. Finally and very important, how to integrate them in the classical R&D process.

Melius is a CENIT project granted by CDTI (Spain).

doi:10.1016/j.toxlet.2010.03.885

P302-056

Toxic effects associated to the use of the delivery enhancer caprylocaproyl macrogol-8 glyceride (labrasol) in early toxicological studies in rats. An activity of the Melius project

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The emulsifier caprylocaproyl macrogol-8 glyceride (labrasol) is frequently used in the course of the early toxicological characterization of new candidate drugs to enhance the intestinal absorption of poorly soluble compounds.

40% and 50% labrasol in water (V/V) were used as emulsifier for a new candidate drugs. In one case, 40% labrasol was used to administer an anticholinesterasic compound. Adult female rats received a single oral administration of vehicle (10 ml/kg, $n = 12$) or compound ($n = 20$) and the presence of body tremors was evaluated as a marker of efficacy, associated to the presence of anticholinesterasic activity.

All animals, irrespective of the treatment applied, showed unspecific clinical signs (mainly piloerection, chromodacryorrhea, hunched back, hypersalivation, nasal secretion, decreased motor activity, dyspnea, respiratory noises and abdominal distension). Significant lost of body weight was observed at 24 h after treatment, although food intake was not affected. Six vehicle-treated and 2 compound-treated animals died or were euthanized, due to the severity of their clinical signs, within 48 h after treatment. At necropsy, these animals showed signs of pneumonia with a foamy content in the airways. In addition, 86% of the compound-treated animals showed body tremors ($P < 0.05$ vs 0% incidence in vehicle-treated group). At termination (14 days after treatment), animals showed presence of gas and/or distension of the gastrointestinal tract, hyperemia of the gastric mucosa and emphysematous lungs. These observations indicate that tremors are the only clinical sign directly attributable to the compound, while all other signs are likely attributable to the vehicle. Spontaneous mortality was associated to the vehicle; likely due to its chemical composition, leading to foam formation upon administration, which causes both respiratory and abdominal signs. Labrasol, and similar emulsifying compounds, should be avoided in toxicological studies because of their physicochemical properties, leading to confounding side effects and associated high rates of mortality.

doi:10.1016/j.toxlet.2010.03.886

P302-057

Sex-related acute toxicological effects of NS-B in rats. An activity of the Melius project

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NS-B is a compound in development for the treatment of neurodegenerative diseases. NS-B decreases plaque load in treated transgenic mice and possess anticholinesterasic potential. Early studies reported increased toxicity in female animals (including deaths). Moreover, there were evidences showing strain-related differential toxicity between Sprague–Dawley and Wistar Hannover rats. The influence of strain (Sprague–Dawley vs. Wistar Hannover), age (6 vs. 10 weeks) and sex (female vs. male) was evaluated. NP0361 or vehicle (water, 15 ml/kg) was administered orally ($n = 6$ per group) and clinical signs, body weight and food and water intake were monitored for 14 days.

No spontaneous mortality was observed associated to the NP0361 treatment. Clinical sings (mainly piloerection, chromodacryorrhea, hypersalivation, nasal secretion, postration and alterations of motor activity) were occasionally observed within the 8-h period post-administration; irrespective of the treatment, strain, sex or age considered. Only the presence of tremors was attributable to the test item (50% incidence in NS-B-treated animals; $P < 0.001$ vs. incidence in vehicle-treated animals: 6%). When considering the sex, only NS-B-treated females, irrespective of the strain or the age considered, showed tremors (100% incidence; $P < 0.001$ vs. males, 0% incidence). Tremors affected forelimbs, head and/or anterior part of the body; and were observed between 1 and 8 h post-dosing, only in one case persisted up to 24 h post-dosing. Regardless the group considered, no effects on body weight gain or food or water intake were observed. These observations indicate that potential acute toxicological effects of an anticholinesterasic compound are a reflection of an exacerbated pharmacology manifested specifically in female rats. Since the presence of sex-related differences in metabolism is frequent in rats, the metabolism of NS-B in rodents and non-rodents must be explored.

Melius is a CENIT project granted by CDTI, Spain.

doi:10.1016/j.toxlet.2010.03.887

P302-058

Characterization of exacerbated anticholinesterasic-related pharmacological effects in female rats treated with NP0361. An activity of the Melius project

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Previous toxicological observations suggested a sex-related (restricted to females) exacerbated pharmacology, manifested as body tremors, following treatment with NS-B.

The purpose of this study was to characterize the effects of NS-B on body tremors in female rats and to establish a toxicokinetic–toxicodynamic correlation.

Female rats (Wistar Hannover, 10-week old) received a single oral dose of NS-B (low or high; $n = 5$ each) or vehicle (15 ml/kg; $n = 5$) and were monitored for signs of toxicity for the 48-h period following treatments. The incidence and intensity of tremors (using a visual analogue scale, VAS, and a numerical rating scale, NRS) were assessed. Satellite groups ($n = 4$–6) were used to obtain plasma samples for toxicokinetic analysis. No spontaneous mortality was observed. Body tremors, affecting the forelimbs, head and/or the anterior part of the body, were present in all animals receiving high dose of NS-B and in 4 out of 5 animals in the low dose group ($P < 0.05$ vs vehicle). Time of appearance of tremors was dose-dependent, starting 30–65 min and 20–45 min after the low and high doses, respectively. Similarly, tremor scoring (VAS and NRS) was also dose-dependent. For the high dose, peak average scores were 33 ± 4 (VAS), at 155–160 min post-dosing, and 2 ± 1 (NRS), at 6 h post-dosing. At low dose, peak average scores were 8 ± 6 (VAS), at 145 min post dosing, and 1 ± 1 (NRS), at 5 h post-dosing. Tremors ceased by 6 h post-dosing. Total plasma levels of NS-B were also dose-dependent (C_{max}: 783 ± 383 and 2208 ± 1102 μg/l; T_{max}: 5.7 ± 2.3 and 4 ± 1.3 min; and AUC 0-t 5872 ± 2752 and 15742 ± 6376 ng h/ml) for low and high dose, respectively. These observations confirm an exacerbated pharmacological anticholinesterasic activity in female rats. Toxicokinetic data correlated with the toxicodynamic effects (incidence and intensity of tremors). Thus suggesting that tremors are associated to the test substance.

doi:10.1016/j.toxlet.2010.03.888