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LES PROGESTATIFS, LES POISSONS ET LES MILIEUX AQUATIQUES : APPORTS DU PROJET ANR « PROOFS »

Contributeurs

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During these twenty last years, the question of the effects of endocrine disruptors (ED) on physiological functions such as reproduction has been the subject of numerous works because of the risks incurred for organisms and aquatic environments. In this regard, many works have been carried out on the risks associated with oestrogenic compounds and, in particular, the oestrogens used in contraceptive pills [1; 2]. However, the presence of other steroid pharmaceuticals in aquatic environments is today proven without that we can grasp the consequences. Among these steroids, synthetic progestins represent a set of substances largely used in human and veterinary medicine. Some progestins have been quantified in effluents of treatment plants and surface waters, at low concentrations (ng/L-µg/L) but capable of affecting the reproduction of fish exposed in the laboratory. However, there is a lack of information on the mechanisms, effects and exposure of organisms to progestins that it is necessary to fill in the perspective of evaluating the dangers and risks that these substances raise for aquatic species and the environment.

OBJECTIFS ET MÉTHODE

It is in this context that an interdisciplinary coordinated program by Ineris involving different research teams has been carried out. The ANR project « PROOFS » (Occurrence and effects of environmental ligands of the progesterone

receptor on fish reproduction and neuro-development) aimed at acquiring new information on the modes of action and potential effects of progestins in the environment on neuro-development and the reproduction of fish. It also aimed at providing information on progestin activities in environmental samples and to identify the compounds responsible for these biological activities. For this, the strategy used relied on a panel of bioassays of the type gene-reporter *in vitro* and *in vivo* allowing to evaluate the capacity of substances to interact with signaling pathways mediated by nuclear steroid receptors and to deregulate the expression of target genes in different tissues of the zebrafish embryo (figure 1).

This strategy was applied to a set of 26 progestins and allowed to acquire new information on the interaction of these molecules with the progesterone receptor of zebrafish by demonstrating notably that the majority of these compounds behave as antagonists of the zebrafish PR receptor unlike the human receptor. In addition, Ineris demonstrated the multiplicity of actions of progestins which are capable of interfering with different nuclear receptors such as the receptors of androgens, oestrogens and glucocorticoids (figure 2). At the organism level, these molecular action mechanisms translate by precocious deregulations of the tissue-specific expression of genes involved in the biosynthesis of neuro-steroids (induction of the gene of the aromatase cerebral) and of corticosteroids (inhibition du

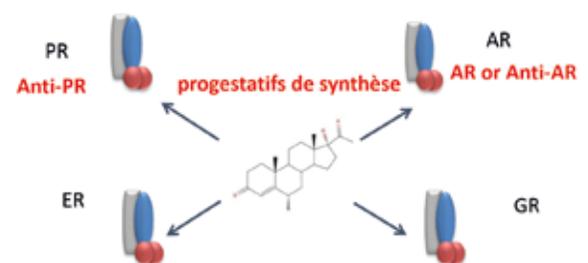
Figure 1 /

Stratégie développée pour renseigner des mécanismes et des effets PE d'un ensemble de progestatifs pharmaceutiques. Ces outils ont été combinés à de l'analyse chimique pour quantifier des activités progestagéniques dans les échantillons et identifier des substances chimiques.



Figure 2 /

Les progestatifs de synthèse se caractérisent par des profils toxicologiques complexes en raison de leurs capacités à interagir en tant qu'agoniste ou antagoniste de différents récepteurs nucléaires stéroïdiens chez le poisson zèbre.



gène de la 11 β -hydroxylase dans les cellules interrénales) (figure 3) [3; 4]. Une investigation plus poussée a permis de mettre en évidence que ces altérations s'accompagnent de modifications de la survie et/ou la prolifération neuronale ainsi que de la capacité des embryons à synthétiser du cortisol et à répondre à des stress. L'approche permet d'apporter des informations originales sur les dangers de ces molécules sur le système endocrinien d'un vertébré modèle. L'originalité de ce projet réside aussi dans l'utilisation intégrée d'outils *in vitro* et d'analyses chimiques au sein d'une démarche EDA (Effect Directed Analysis). Elle a permis de mettre en évidence, pour la première fois, des activités biologiques progestagéniques au sein de matrices environnementales (eaux usées, eaux de surface) en révélant des activités spécifiques des poissons et d'identifier des substances pharmaceutiques potentiellement impliquées dans ces réponses biologiques dont un métabolite dérivé d'un progestatif de synthèse.

CONCLUSION

Ce travail a permis l'acquisition d'un ensemble de données et de connaissances originales sur les dangers des progestatifs de synthèse sur le système endocrinien des poissons ainsi que sur l'occurrence environnementale d'activités biologiques associées à des ligands du récepteur de la progestérone. Ce projet souligne l'intérêt des stratégies combinant modèles *in vitro* et *in vivo* spécifiques du poisson que ce soit pour caractériser le potentiel perturbateur endocrinien des substances seules ou les matrices environnementales. À la lumière de ces travaux, il semble nécessaire de poursuivre des actions de recherche sur ces polluants aquatiques émergents afin d'en évaluer les risques pour les milieux aquatiques et adopter des actions de gestion adéquates.

Références

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ABSTRACT /

During the past twenty years, numerous studies have examined the effects of estrogenic compounds, notably 17 α -ethinylestradiol a potent synthetic steroidal estrogen used as pharmaceuticals in contraceptive pills, because these substances were among the first identified as endocrine disrupting compounds responsible for the feminization of wild populations of fish observed worldwide. There is now evidence of the occurrence of other natural and synthetic steroids in aquatic environment. Among steroidal pharmaceuticals, the risks encountered by synthetic progestins on aquatic species have been recently pointed out but data on their occurrence and effects on fish endocrinology and physiology are missing to properly assess their hazard and risk to aquatic species. The national project ANR "PROOFS" aimed at investigating the effect of progestins on key molecular and cellular targets of the endocrine system using zebrafish *in vitro* and *in vivo* reporter gene mechanism-based assays. It also aimed at providing data on the occurrence of progestagenic activity of environmental samples and to identify substances responsible for these activities through an effect-directed analysis (EDA) approach. Among the 26 pharmaceutical progestins investigated, our data revealed that they can act on several steroidal nuclear receptors. By using novel transgenic zebrafish models, we demonstrated for the first time their estrogenic activity in the developing brain and their capacity to disrupt corticosteroidogenesis in interrenal cells. Finally, we reported strong progestagenic activity in waste waters and identified several pharmaceuticals as potential candidate responsible for these activities. Overall, the ANR project "PROOFS" brings novel and relevant data on occurrence and endocrine potency of progestins stressing the need to further study these emerging contaminants.

Figure 3 /

Les progestatifs de synthèse sont capables d'altérer l'expression de différents gènes cibles dans le cerveau (aromatase cérébrale) et les cellules interrénales (11 β -hydroxylase) et altérer des processus clés du développement (neurogenèse) et le comportement.

