Open Access Journal of Toxicology
ISSN 2474-7599

Juniper Publishers
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Case Report
Volume 4 Issue 5 - February 2021
DOI: 10.19080/OAJT.2021.04.555650

Open Acc J of Toxicol

Are Salmonid Fish More Susceptible to the Class of Contaminants of Emerging Concern the Synthetic Glucocorticoid?

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Submission: February 02, 2021; **Published:** February 22, 2021

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Abstract

Synthetic Glucocorticoids (GC) are prescribed to treat a variety of medical conditions. Similar, to other pharmaceuticals they have been detected in aquatic environments and are contaminant of emerging concern. GCs act via binding to the Glucocorticoid Receptor (GR) to induce or repress target gene expression. In teleost fish a whole genome duplication event resulted in a duplication of the GR genes. These genetically distinct GRs have been retained in almost all fish studied to date. Functional transactivation analysis of teleost GRs has only occurred in a few species and reveal 3 groups: those species with startling divergent transactivation sensitivities with GR2, being hypersensitive, whilst GR1, hyposensitive; species with a moderate divergence in sensitivities and those species with no divergence. The salmonid rainbow trout is a fish that has a hyper and hyposensitive GR. The molecular trait that confers hyposensitivity in trout is found at the protein's N-terminus. Comparisons between other salmonid and related species GR sequences show that this group of fish possess a conserved 6 or 7 amino acid extension at the N-terminus. This is not present in the other ~70 species where genetic information is available. Thus, a hypothesis is that salmonid fish that are important recreational fishing and aquaculture species maybe more susceptible to synthetic GC contamination due to the retention of a hypersensitive GR. This is an example of the use of comparative genomics and in vitro gene functionality assays to predict species that may be vulnerable to classes of pollutants.

Keywords: Fish; Stress response; Endocrine disruption; Adverse outcome pathways; Glucocorticoid receptors; Predictive toxicology

Abbreviations: GC: Synthetic Glucocorticoids; GR: Glucocorticoid Receptor; CR: Corticosteroid receptors; MR: Mineralocortioid; MC: Mineralocorticoid; TALEN: Transcription Activator-Like Effector Nuclease; GRE: Glucocorticoid Response Element

Introduction

The steroid receptor-signalling pathway is an example of a complex physiological system whereby a ligand, its receptor and several co-activators or co-repressors interact to control a diverse array of cellular processes. Studies have shown how the steroid receptor family evolved from its simplistic deuterostome origins to the myriad of receptors and ligands of protostomes [1-3]. In the vertebrates, a whole genome duplication event in the early vertebrate lineage approximately 500MYA resulted in the duplication of an ancestral Corticosteroid Receptors (CR) gene [4]. Over time, the duplicated CR genes have evolved new functions, resulting in the Mineralocortioid (MR) and Glucorticoid Receptor (GR) which are found in extant tetrapods and sharks [5]. In landbased vertebrates, the MR and associated Mineralocorticoid (MC) hormone aldosterone are implicated in the maintenance of mineral homeostasis at a systemic and cellular level, whereas Glucocorticoids (GC), such as cortisol or corticosterone, influence a vast array of cellular and physiological functions, being involved in immune function, metabolism, cell growth, development, behaviour and the cardiovascular system [6]. A further WGD event occurred in the basal teleost lineage and the majority of teleost studied to date possess duplicated GRs, but only a single MR, with one duplicate MR being lost [4]. The exemption to this is the zebrafish which have lost the duplicated GR and thus only express one GR and MR [7]. The retention of 2 GRs potentially adds a layer of complexity in understanding the mechanisms by which these receptors regulate basic physiological processes in fish [3]. But it is still unclear whether the two fish GRs regulate different, or indeed the same, processes and there is no clear physiological role identified yet for the MR. In medaka knockout of the MR using Transcription Activator-Like Effector Nuclease (TALEN), suggests a novel role in recognition of visual motion stimuli suggesting the ancestral role for the MR may be linked to behaviour [8].

Due to the vast number of physiological processes the GC/GRs influence, they are targets for drug development. These include the synthetic GCs beclomethasone - used in a spray form to treat asthmas and in a cream for psoriasis - prednisolone used to treat allergies, blood disorders, skin diseases, infections and some cancers and dexamethasone, which has recently been used to help Covid-19 patients. GCs are thus widely prescribed for a variety of ailments [9]. Their structure is often modified to include chloride or fluoride atoms to increase stability and persistence and once ingested may pass through the body without being transformed [10]. Thus, they enter our sewage system through excretion or via disposal of unused medication down the toilet. Synthetic GCs are not readily removed by wastewater treatment plants and find their way into the aquatic environment. Predicted concentrations in the rivers of the Thames catchment in the United Kingdom, based on the number of prescriptions prescribed, are in the ng/L concentrations [11] and measured concentrations in other rivers are similar [12,13]. In the laboratory, exposure of fathead minnow to environmentally relevant concentrations (10ng/L) of the synthetic GC, Beclomethasone Dipropriate (BcD) causes increased gluconeogenesis and immunodepression [14,15]. Zebrafish embryos exposed to 0.1-1 µg/L prednisolone show perturbations to development and behaviour [16,17]. Thus, environmentally relevant concentrations of GCs cause adverse effects in non-target organisms and are contaminants of emerging concern and endocrine disrupting chemicals.

Current knowledge of the transactivational activity properties exists for only 7 fish species' CRs (Table 1). The transactivation assays involve insertion of the full-length coding region of each receptor into an expression vector. Cell lines are then transfected with the expression vector as well as a reporter plasmid containing the Glucocorticoid Response Element (GRE) upstream of a luciferase gene. Once the cells express the receptor to which they are exposed, GCs and the GC/GR complex bind to the GRE to induce luciferase synthesis. In this system, the teleost MR is activated at lower concentrations of the natural hormone cortisol than one or both GRs, similar to the scenario in mammals. Fish do not synthesise aldosterone and it has been proposed that 11-deoxycorticosterone is its natural ligand in fish (Table 1), however, the ancestral ligand for the MR is debated [18]. From this data the other 2 teleost fish CRs, GR1 and 2, can be placed into one of 3 categories based on their transactivation characteristics: Category 1, species with startling divergent transactivation sensitivities between the GRs, with GR2, being hypersensitive, whilst GR1, hyposensitive; Category 2, species with a moderate divergence in sensitivities and Category 3, those species with no divergence in sensitivities (Table 1). This hypo/hypersensitivity trait has emerged in two unrelated species, whose lineages are separated by approximately 90 million years ago [19], the rainbow trout and Japanese medaka, belonging to the Salmoniformes and the Beloniformes, respectively.

Table 1: Cortisol induced transactivational activity for the known teleost CRs.

Species	Cortisol EC50 (nM) for teleost CRs			Sensitivity Category
	GR1	GR2	MR	
Rainbow trout (*Oncorhynchus mykiss*)	46nM	0.72nM	1.1 nM	1 [22,24]
Japanese medaka (*Oryzias latipes*)	57nM	0.85pM	12.05PM	1 [23]
Common carp (*Cyprinus carpio*)	7.1nM	2.4nM	4.1nM	2 [25]
Butterflyfish (*Pantadon buchholzi*)	10.4nM	2.7nM		2 [26]
Burton's mouthbrooder (*Astatotilapia burtoni*)	5.4nM	3.6nM	0.02nM	3 [27]
Marine Medaka (*Oryzias dancena*)	21.8nM	9.9nM		2 [28]
Zebrafish (*Danio rerio*)	10.1nM		0.59nM	[18]

We have previously identified regions of the GRs that conferred GR hypo- and hypersensitivity in rainbow trout [20]. The C-terminus of the rainbow trout GR1 possesses an additional 6 amino acids as well as two amino acid substitutions QK to AL (Figure 1) [20]. The C-terminus β-strand forms a conserved β-sheet with a β-strand between helices 8 and 9 of the GR protein, suggesting it is important in stabilising the active ligand/GR conformation [21]. Thus, a change from a charged (K) to a hydrophobic (L) side chain or the additional C-terminal amino acids may be a contributing factor to hyposensitivity [20]. The other Pro acanthopterygian, which includes the salmonids, GR1s also possess these substitutions and the additional amino acids at the C-terminal and in the Japanese medaka, there is only the substitution of QK to SS. These substitutions are not seen in other teleost GR sequences studied to date [4], and it is only in these two species where a hypersensitive GR has been observed.

In the study where physiological effects were observed in fathead minnow, plasma concentration of BcD reached a human therapeutic dose of between 2-28nM (1-15µg/L) [13]. The fathead minnow GRs transactivation properties have not been assessed, but this plasma concentration is between 2.7 and 38-fold higher than the BcD EC50 for the hypersensitive rainbow trout GR2 and

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22.4 to 1.6 lower than the EC50 for the hyposensitive rainbow trout GR1 [22]. The Japanese medaka's GR2 is incredibly ~1000 fold more sensitive to the natural hormone cortisol [23] compared to rainbow trout GR2, whilst the other Japanese medaka GR, GR1, is similar in sensitivity to the rainbow trout GR1 (Table 1). It can be hypothesised that species in category 1 are more susceptible to aquatic synthetic GC pollution because one of their GRs will be activated at a lot lower plasma GC concentrations [24-28].

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PikeGR1          LSVEFPDMLAEIISNQLPKFKDGSVKPLLFHNALNHDQMP
PikeGR2          LSVEFPEMLAEIISNQLPKFKAGSVKPLLFHQK
RtrouGR1         LSVEFPEMLAEIISNQLPKFKDGSVKPLLFHALNHDTMP
RtroutGR2        LSVEFPEMLAEIISNQLPKFKAGSVKPLLFHQK
AsalmGR1         LSVEFPEMLAEIISNQLPKFKDGSVKPLLFHALNHDTMP
AsalmGR2         LSVEFPEMLAEIISNQLPKFKAGSVKPLLFHQK
MarenaGR1        LSVEFPEMLAEIISNQLPKFKDGSVKPLLFHALNHDTMP
BtroutGR1        LSVEFPEMLAEIITNQIPKFKDGSVKPLLFHALNHDTMP

PerchGR1         FSVEFPEMLAEIITNQIPKFKDGSVKPLLFHQK
PerchGR2         LSVEFPEMLAEIISNQLPKFKAGSVKPLLFHQR
StickleGR1       LSVEFPEMLAEIISNQIPKFKDGSVKPLLFHQK
StickleGR2       LSVEFPEMLAEIISNQLPKFKAGSVKPLLFHQR
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Figure 1: The C-terminal of the Protacanthopterygii GRs (European Pike, Pike GR1 and GR2; Rainbow trout, Rtrout Gr1 and GR2; Atlantic salmon, AsalmGR1 and GR2; Marena whitefish, MarenaGR1 and Brown trout, Btrout GR1) and for perch and stickleback GR1 and GR2. Accession numbers: Northern pike, GR1 XP_010869409.1, GR2 XM_010871111.2; Atlantic Salmon GR1 XP_014053534.1, GR2 XP_014054152.1; Brown trout GR1 AY863149; rainbow trout GR1 Z54210.1, GR2 AY495372.1; Marena whitefish GR1 CEP28034; European perch GR1 EU861040.1, GR2 KC847473.1 and the stickleback, GR1 ENSGACT 00000027452, GR2, ENSGACT 00000024121.

Conclusion

Identifying the risks associated with exposure to emerging contaminants of concern to aquatic organisms is required to enable the appropriate regulatory measures to be implemented and mitigation to be prioritised. Pollution is one factor that is influencing the biodiversity loss [29] we are witnessing globally and combating pollution is an integral component of several the United Nations Sustainable Development Goals. The use of comparative genomics and in vitro gene functionality assays can be used to predict those species that may be vulnerable to classes of pollutants. Using this approach suggests that salmonids may be more susceptible to synthetic GCs than other fish species. However, without knowing the gene pathways the receptors control, we are unable to predict the physiological consequences of activations of the more sensitive GR. This is applicable to the health of both feral fish in rivers receiving wastewater treatment plant effluent and caged farmed fish located in waters close to large conurbation.

References

1. Thornton JW (2001) Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. Proc Natl Acad Sci USA 98(10): 5671-5676.
2. Thornton JW, Need E, Crews D (2003) Resurrecting the Ancestral Steroid Receptor: Ancient Origin of Estrogen Signaling. Science 301(5640): 1714-1717.
3. Baker ME (2019) Steroid receptors and vertebrate evolution. Mol Cell Endocrinol 496: 110526.
4. Bury NR (2017) The evolution, structure and function of the ray finned fish (Actinopterygii) glucocorticoid receptors. Gen Comp Endocrinol, 251: 4-11.
5. Bridgham JT, Carroll SM, Thornton JW (2006) Evolution of hormone-receptor complexity by molecular exploitation. Science 312(5770): 97-101.
6. Charmandari E, Tsigos C, Chrousos G (2005) Endocrinology of the stress response. Ann Rev Physiol 67: 259-284.
7. Schaaf MJM, Champagne D, Van Laanen IHC, Van Wijk DCWA, Meijer AH, et al. (2008) Discovery of a Functional Glucocorticoid Receptor β-Isoform in Zebrafish. Endocrinol 149(4): 1591-1599.
8. Sakamoto T, Yoshiki M, Sakamoto H (2017) Principal function of mineralocorticoid signaling suggested by constitutive knockout of the mineralocorticoid receptor in medaka fish. Sci Rep 6: 37991.
9. Schriks M, Van Leerdam JA, Van Der Linden SC, Van Der Berg B, Van Wezel AP (2010) Environ Sci Technol 44: 4766-4774.
10. Herberer T (2002) Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol Lett 131(2): 5-17.
11. Kugathas S, Williams RJ, Sumpter JP (2012) Prediction of environmental concentrations of glucocorticoids: The River Thames, UK, as an example. Environ Int 40: 15-23.

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12. Ammann AA, Macikova P, Groh KJ, Schirmer K, Suter MJF (2014) LC-MS/MS determination of potential endocrine disruptors of cortico signalling in rivers and wastewaters. Anal Bioanal Chem 406: 7653-7665.

13. Nakayama K, Sato K, Shibano T, Isobe T, Suzuki G, et al. (2016) Occurrence of glucocorticoids discharged from a sewage treatment plant in Japan and the effects of clobetasol propionate exposure on the immune responses of common carp (Cyprinus carpio) to bacterial infection. Environ Toxicol Chem 35(4): 946-952.

14. Kugathas S, Sumpter JP (2011) Synthetic glucocorticoids in the environment: first results on their potential impacts on fish. Environ Sci Technol 45: 2377-3383.

15. Margiotta Casaluci L, Owen SF, Huerta B, Rodríguez Mozaz S, Kugathas S, et al. (2016) Internal exposure dynamics drive the Adverse Outcome Pathways of synthetic glucocorticoids in fish. Sci Rep 6: 21978.

16. Mc Neil PL, Nebot C, Cepeda A, Sloman KA (2016) Environmental concentrations of prednisolone alter visually mediated responses during early life stages of zebrafish (Danio rerio). Environ Poll 218: 981-987.

17. Mc Neil PL, Nebot C, Sloman KA (2016) Physiological and Behavioral Effects of Exposure to Environmentally Relevant Concentrations of Prednisolone During Zebrafish (Danio rerio) Embryogenesis. Environ Sci Technol 50(10): 5294-5304.

18. Shaughnessy CA, Barany A, Mc Corminck SD (2020) 11-Deoxycortisol controls hydromineral balance in the most basal osmoregulating vertebrate, sea lamprey (Petromyzon marinus). Sci Rep 10(1): 12148.

19. Betancur RR, Broughton RE, Wiley EO, Carpenter K, López JA, et al. (2013) The tree of life and a new classification of bony fishes. Plos Curr 16: PP. 5.

20. Sturm A, Colliar L, Leaver MJ, Bury NR (2011) Mol Cell Endocrinol 333: 181-189.

21. Bledsoe RK, Montana VG, Stanley TB, Delves CJ, Apolito CJ, et al. (2002) Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. Cell 110(1): 93-105.

22. Bury NR, Sturm A, Le Rouzic P, Lethimonier C, Ducouret B, et al. (2003) Evidence for two distinct functional glucocorticoid receptors in teleost fish. J Mol Endo 31(1): 141-156.

23. Kai Ping Wen, Ying Chieh Chen, Chia Hui Chuang, Hwan You Chang, Chi Young Lee, et al. (2015) Accumulation and toxicity of intravenously injected functionalized graphene oxide in mice. J Appl Toxicol 35(10): 1211-1218.

24. Sturm A, Bury N, Dengreville L, Fagart J, Flouriot M, et al (2005) 11-Deoxycorticosterone Is a Potent Agonist of the Rainbow Trout (Oncorhynchus mykiss) Mineralocorticoid Receptor. Endocrinol 146(1): 47-55.

25. Stotle EH, De Mazon AF, Leon Koosterziel KM, Jęsiak M, Bury NR (2008) A differential role for corticosteroidreceptors in neuroendocrine-immuneinteractions in carp (*Cyprinus carpio L.*) J Endocrinol 198: 403-407.

26. Li Y, Sturm A, Cunningham P, Bury NR (2012) Evidence for a divergence in function between two glucocorticoid receptors from a basal teleost. BMC Evol Biol 12: 137.

27. Greenwood AK, Butler PC, White RB, De Marco U, Pearce D, et al. (2003) Multiple Corticosteroid Receptors in a Teleost Fish: Distinct Sequences, Expression Patterns, and Transcriptional Activities. Endocrinol 144(10): 4226-4236.

28. Kim MA, Kim DS, Sohn YC (2011) Characterization of two functional glucocorticoid receptors in the marine medaka Oryzias dancena. Gen Comparative Endocrinol 171(3): 341-349.

29. Otyero I, Farrell Pueyo S, Kallis G, Kehoe L (2020) Biodiversity policy beyond economic growth. Conserv Lett 13(4): e12713.

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