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Pharmacoperones: Targeting Therapeutics Toward Diseases Caused by Protein Misfolding

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ABSTRACT

Pharmacoperones are hydrophobic molecule drugs that enter cells and serve as a molecular framework to cause misfolded mutant proteins to fold properly and adopt a stable conformation compatible with proper intracellular trafficking. Pharmacoperones have successfully been used experimentally to rescue function of some misfolded proteins (enzymes, receptors, channels) that lead to disease. Identification of pharmacoperones by high-throughput screens of drug libraries will likely provide new molecules that may be potentially useful to treat diseases caused by protein misfolding. (REV INVEST CLIN. 2015;67:15-9)
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Proteins play important roles in the structure of cells and are key regulators of the function of all the body's tissues and organs as enzymes, receptors, and ion channels. The synthesis of proteins occurs in the endoplasmic reticulum (ER), where correct folding and conformation are monitored by a stringent quality control system. Proteins that fail to fold properly are retained in the ER and are subsequently degraded through the polyubiquitination/proteasome pathway and by other mechanisms. By monitoring the structural and folding correctness of newly synthesized proteins, the ER quality control system prevents accumulation of defective proteins that may aggregate in a highly crowded environment, thereby interfering with normal cell function. The mechanisms that operate at the ER to identify and sort proteins according

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Table 1. Examples of misfolded, trafficking-defective G protein-coupled receptors, diseases caused, and pharmacoperones tested *in vitro* and/or *in vivo*[1,2,6,8]

GPCR	Disease or variant	Pharmacoperones
Rhodopsin	Retinitis pigmentosa	Retinoids (9-cis-retinal, 11-cis-retinal, 11-cis-7-ring retinal, vitamin A palmitate)
V2R	Nephrogenic diabetes insipidus	Satavaptan, relcovaptan, VPA-985, YM087, tolvaptan, OPC31260
GnRHR	Hypogonadotropic hypogonadism	IN3, IN30, Q89, A177775, TAK-013.
MC3R, MC4R	Obesity	ML00253764
MC1R	Red head color phenotype and propensity to skin cancer	NBA-A
CaR	Familial hypocalciuric hypercalcemia	NPS R-568
FSHR	Premature ovarian failure	Org 41841
LHR	Leydig cell hypoplasia	Org 42599

GPCR: G protein-coupled receptor; V2R: vasopressin V2 receptor; GnRHR: gonadotropin-releasing hormone receptor; MC3R: melanocortin-3 receptor; MC4R: melanocortin-4 receptor; MC1R: melanocortin-1 receptor; CaR: calcium-sensing receptor; FSHR: follicle-stimulating hormone receptor; LHR: luteinizing hormone receptor.

to their maturation status include members of major molecular chaperone family, and ER-resident proteins that assist in folding and assembly of the polypeptide chain for efficient ER export. Properly folded proteins are eventually allowed to enter the pathway, leading to their final destination within the cell, e.g. the plasma membrane (PM)[1].

Conformational diseases are disorders of protein misfolding, often due to mutation, that compromise protein structure and/or function. Many conformational diseases are associated with failure of the defective protein to traffic to the cell surface plasma membrane. Examples include prion-related and other neurodegenerative diseases (e.g. Alzheimer's and Parkinson's disease), familial hypercholesterolemia, retinitis pigmentosa, cystic fibrosis, nephrogenic diabetes insipidus, congenital hypothyroidism, and hypogonadotropic hypogonadism, to mention a few (Table 1)[1,3]. In nephrogenic diabetes insipidus, for example, urine is not concentrated due to arginine vasopressin resistance of the kidney or to defects of the arginine vasopressin-responsive aquaporin-2 water channel. When expressed *in vitro*, most (~ 70%) arginine vasopressin-2 receptor (V2R) mutations show intracellular retention of the receptor molecules, blocking their traffic to the PM and thereby impeding the binding of agonist. Likewise, loss-of-function mutations of the thyrotropin receptor can cause destabilization of the newly synthesized receptor and prevent its cell-surface expression, leading to hypothyroidism due to thyrotropin resistance[1]. Misfolding may yield proteins that *retain function* but, for reasons of mislocation only, cease to function normally, leading to disease. The fact that some misfolded proteins (receptors, enzymes or channels) may retain function offers the therapeutic opportunity to directly correct misrouting and rescue, either partially or completely, the location and function of the mutant proteins, potentially curing disease.

Several *in vitro* approaches to correct folding and promote trafficking of the protein from the ER to their correct destination within the cell have been tested. These include physical (e.g. lowering incubation temperatures), genetic (by introducing or deleting specific sequences into the abnormal protein: genetic rescue), chemical (i.e. incubation in the presence of chemical chaperones, such as polyols and sugars, that act as stabilizing agents), and pharmacological (employing pharmacoperones) approaches[1,2]. Although chemical chaperones can rescue some misfolded proteins, they are nonspecific and might potentially increase secretion or intracellular retention of many different proteins in different cellular compartments, provoking inappropriate changes in the levels and/or secretion of many proteins, thereby compromising cell function.

Pharmacoperones are among the most promising therapeutic strategies to treat conformational diseases due to protein misfolding. Pharmacoperones

(from "pharmacological chaperone") are small, hydrophobic molecule drugs that enter cells and serve as a molecular scaffold to promote otherwise-misfolded mutant proteins to fold properly and adopt a conformation compatible with ER export[1,2]. In contrast with less specific chemical chaperones, pharmacoperones have the advantage of selective binding to the misfolded target. Examples of pharmacoperones include the competitive inhibitor 1-deoxy-galactonojirimycin, which facilitates the transport of mutant forms of α-galactosidase A (whose accumulation in the ER leads to Fabry disease in humans) from the ER to the lysosomes[4]; cyclosporin A (a substrate of the multidrug-resistant protein ATP-binding cassette transporter ABCB1), which may improve maturation and PM expression of the misfolded I541F mutant ABCB4 transporter linked to progressive familial intrahepatic cholestasis type 3[5]; and an array of ligands (e.g. agonists or antagonists) that bind misfolded mutant G protein-coupled receptors (GPCR) linked to diseases shown in table 1. In the case of particular proteins, e.g. the V2R and the gonadotropin-releasing hormone receptor (GnRHR), this approach has succeeded with a number of mutants leading to congenital nephrogenic diabetes insipidus and hypogonadotropic hypogonadism, underlying the view that pharmacoperones will become powerful therapeutic tools[6].

The feasibility (proof-of-principle) of the power of pharmacoperones as a therapeutic strategy for diseases caused by misfolded proteins is exemplified by *in vivo* studies in experimental animals and in humans bearing the above-mentioned diseases. The V2R and GnRHR are GPCRs, which is a superfamily of PM receptors that comprise the largest family of validated drug targets. The V2R is mainly expressed and localized in the basolateral membrane principal cells of the renal collecting duct of the kidney. Arginine vasopressin occupancy of the V2R promotes translocation and exocytic insertion of the specific water channel protein aquaporin-2 to the luminal membrane, resulting in water reabsorption in the kidney. In the case of misfolded V2Rs leading to nephrogenic diabetes insipidus, it has been shown that distinct hydrophobic, cell membrane-permeable antagonists can effectively rescue function of several misfolded, trafficking-defective mutants *in vitro*, an effect that cannot be mimicked by V2R impermeant agonists[6]. Further, in one brief clinical trial, administration of the peptidomimetic $V1_AR$/V2R antagonist SR49059 to patients with nephrogenic diabetes insipidus due to V2R mutations resulted in a significant drop in water intake and urine production as well as a significant increase in urine osmolarity[7]. Although the development of the drug used was halted due to interference with the cytochrome P450 metabolic pathway, this study represents a unique proof-of-principle, an example of the power and potential of pharmacoperone therapy.

The GnRHR is mostly expressed in the gonadotropes of the anterior pituitary; binding of its ligand (GnRH) leads to the release of the gonadotropins, which are key regulators of gonadal function. Mutations in the GnRHR lead to partial or complete hypogonadotropic hypogonadism, a failure of pituitary gonadotropes to respond to GnRH, resulting in decreased or apulsatile gonadotropin release and reproductive failure[8]. The prototype of a misfolded GnRHR that leads to the complete form of hypogonadotropic hypogonadism and that can be "rescued" by pharmacological means is the E90K mutation at the second transmembrane helix of the receptor. The GnRHR E90K mutant is retained within the cell by the ER quality control system and is sensitive to complete functional rescue by physical and genetic approaches *in vitro*[8]. It has also been shown that pharmacoperones of different chemical classes (indoles, quinolones, thienopyrimidinediones, and erythromycin-derived macrolides) rescue this and other mutants and revert its dominant-negative effect on the wild-type receptor[1]. Further, we elucidated the biochemical mechanism whereby the E90K mutant disrupts receptor structure, which has allowed to better understand the molecular mechanisms whereby the misfolded receptor is rescued by these pharmacoperones[8]. The genetic animal model bearing this mutation exhibits a phenotype very similar to that shown by its human counterpart, with some variations due to intrinsic species differences in the intracellular trafficking behavior between the human and the rodent GnRHRs; rodent GnRHRs traffic more effectively from the ER to the PM than the human receptor. Pulsatile administration of the pharmacoperone IN3, originally designed as an antagonist of the GnRHR, rescued the mutant receptor from the ER to the PM, restoring the response of the gonadotrope to endogenous GnRH as well as testicular weight and morphology, spermatogenesis (Fig. 1), proteins associated with steroidogenesis and serum androgen levels in mutant male mice[9,10].

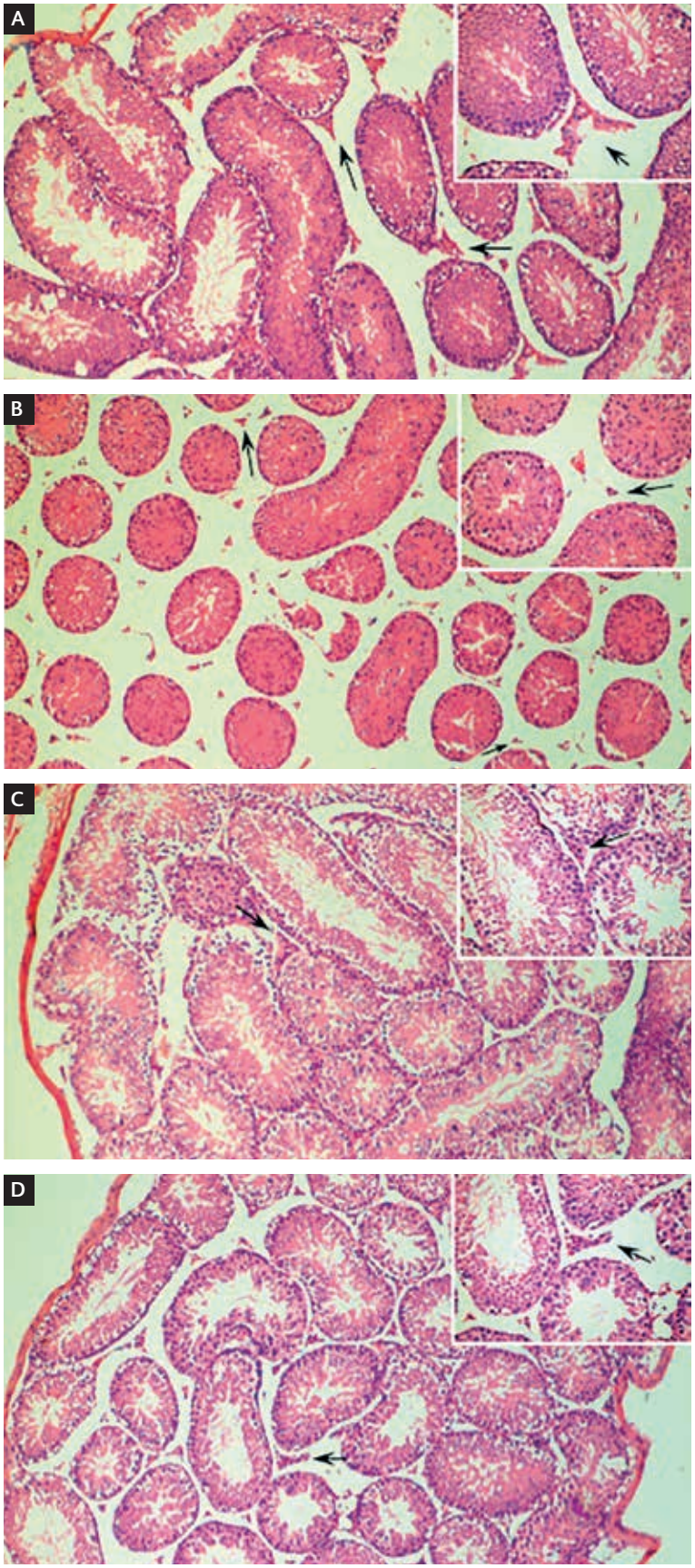

Figure 1. Testicular morphology (× 100; insets, × 400), from a wild-type mouse **(A)**, and from either untreated **(B)** or IN3-treated **(C and D)** transgenic mice harboring the homozygous GnRHR E90K mutation. Control animals **(A and B)** were age-matched to 30 days experimental animals **(C and D)**. Treatment (vehicle or IN3) was started at 60 days of age. IN3 was administered intravenously through an indwelling catheter placed in the left carotid artery. IN3 was administered every three days at a dose of 5 mg/ml, infused at 25 ml per hour for 8 **(C)** or 30 **(D)** days. At the end of the treatment, animals were euthanized and the testes were fixed in 4% paraformaldehyde solution. Testis sections (4 mm) were mounted on slices and stained with hematoxylin and eosin. Observe the differences between **A** (wild-type) and **B** (E90K mutant, untreated) in both Leydig cell clusters (arrows) and seminiferous tubules diameter. The mutant untreated animal exhibited scarce, small clusters of Leydig cells scattered in the interstitium; although the diameter of the seminiferous tubules is markedly reduced, some tubules exhibit some degree of spermatogenesis **(B, inset)**. In IN3-treated animals, the architecture of the testis regarding both seminiferous tubules and interstitial cell nests was similar among the wild-type, and the 8- and 30-days treated animals. It was surprising to find that only 8 days of IN3 treatment was sufficient to normalize the histological appearance of the testis in the E90K mutant animal shown in **C**.

A number of compounds obtained from high-throughput screening techniques are currently under study for their potential therapeutic application, not only as pharmacological chaperones to treat an array of diseases caused by misfolded proteins (including neurodegenerative and storage diseases as well as diseases caused by misfolding of membrane channels and GPCRs[10]), but also as modulators of function for proteins that traffic correctly but in which the mutation alters domains involved in function[11]. Thus, in our era of genomic technology we are now realizing that pharmacoperone treatment seems, in fact, less challenging to correct defective folding than replacing a mutant gene by a "healthy" one!

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