Review

Potential applications for sigma receptor ligands in cancer diagnosis and therapy ☆

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ABSTRACT

Sigma receptors (sigma-1 and sigma-2) represent two independent classes of proteins. Their endogenous ligands may include the hallucinogen N,N-dimethyltryptamine (DMT) and sphingolipid-derived amines which interact with sigma-1 receptors, besides steroid hormones (e.g., progesterone) which bind to both sigma receptor subpopulations. The sigma-1 receptor is a ligand-regulated molecular chaperone with various ion channels and G-protein-coupled membrane receptors as clients. The sigma-2 receptor was identified as the progesterone receptor membrane component 1 (PGRMC1). Although sigma receptors are over-expressed in tumors and up-regulated in rapidly dividing normal tissue, their ligands induce significant cell death only in tumor tissue. Sigma ligands may therefore be used to selectively eradicate tumors. Multiple mechanisms appear to underlie cell killing after administration of sigma ligands, and the signaling pathways are dependent both on the type of ligand and the type of tumor cell. Recent evidence suggests that the sigma-2 receptor is a potential tumor and serum biomarker for human lung cancer and an important target for inhibiting tumor invasion and cancer progression. Current radiochemical efforts are focused on the development of subtype-selective radioligands for positron emission tomography (PET) imaging. Right now, the most promising tracers are [^{18}F]fluspidine and [^{18}F]FTC-146 for sigma-1 receptors and [^{11}C]RHM-1 and [^{18}F]ISO-1 for the sigma-2 subtype. Nanoparticles coupled to sigma ligands have shown considerable potential for targeted delivery of antitumor drugs in animal models of cancer, but clinical studies exploring this strategy in cancer patients have not yet been reported. This article is part of a Special Issue entitled: Membrane channels and transporters in cancers.

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1. Introduction

Sigma receptors were originally described as a subtype of the opioid receptor family, but later shown to be unique proteins integrated in plasma, mitochondrial and endoplasmatic reticulum membranes of several organs including liver, kidney and brain. Two subtypes of sigma receptors have been identified, termed sigma-1 and sigma-2 [1]. As discussed previously [2], the endogenous ligands for these receptors have not been identified with certainty but may include steroid hormones (particularly progesterone), sphingolipid-derived amines and *N,N*-dimethyltryptamine (DMT).

Recent evidence has been presented in support of the hypothesis that DMT, a well-known hallucinogen, may in fact be an endogenous sigma-1 agonist. DMT is a substrate for the serotonin transporter (with even higher affinity than serotonin itself) and is also a substrate for the vesicular monoamine transporter 2. These transporter proteins may allow the accumulation of DMT (and other tryptamines) in neurons to the micromolar levels needed for sigma-1 receptor activation [3]. Consistent with this hypothesis, radiolabeled DMT enters the brain of living rabbits very rapidly (10 s) and is retained there in intact form for at least 7 days, whereas the compound is cleared from the rest of the body *via* the renal route [4]. Although the authors labeled DMT with ^{131}I and thus modified its structure, the *in vivo* behavior of the 2-iodo derivative is expected to be similar to that of the parent indolealkylamine. Persistence of DMT in the mammalian brain can be explained by the fact that DMT is stored in vesicles and thereby protected from degradation by monoamine oxidase. Using immunocytochemical techniques, indole-*N*-methyl transferase (INMT), the enzyme that converts tryptamine to DMT, was shown to be localized to postsynaptic sites of C-terminals of mouse motoneurons in close proximity to sigma-1 receptors which are enriched at these sites [5]. Moreover, DMT inhibits INMT non-competitively by binding to an allosteric site on the enzyme molecule. DMT formation may therefore be regulated *via* a negative feedback loop [6]. Interestingly, downregulation of INMT has been associated with tumor recurrence (*e.g.*, of malignant prostate and lung cancers) and *inmt* was identified as a candidate gene in the prevention of cancer progression [7].

Molecular biology techniques have indicated that sigma-1 receptors play critical roles in the mammalian nervous system. As discussed previously [2], sigma-1 knockout (KO) mice are viable and fertile and do not display any overt phenotype, although the response of such animals to painful stimuli is strongly suppressed and KO mice display depressive behavior under certain forms of stress. A more recent study has reported gender-related alterations in KO mice. Male knockouts show signs of increased anxiety in the open-field, passive avoidance and elevated plus-maze tests. They also show increased depressive-like behavior in the forced swimming test, but no memory changes. Female knockouts show deficits in spontaneous alternation or water maze learning, and avoidance escape latency. These symptoms of impaired memory appear to be related to changes in steroid tonus, since female KO mice have decreased plasma levels of 17β-estradiol compared to wild-type mice and treatment with 17β-estradiol reverses their memory deficits [8].

By the transduction of cultured rat hippocampal neurons with siRNA for the sigma-1 receptor and gene expression analysis using a rat genome cDNA array, knockdown of the sigma-1 receptor was shown to impair many cellular functions, including steroid biogenesis, protein ubiquitination, organization of the actin cytoskeleton and Nrf2-mediated responses to oxidative stress [9]. Several studies have shown that sigma-1 receptors play an important role in the protection of retinal cells against various forms of damage. KO mice suffer from late onset inner retinal dysfunction [10]. They show accelerated retinal ganglion cell death after optical nerve crush [11] and a more rapid loss of retinal function in diabetes [12].

In an article which preceded the current overview [2], we wrote that the identity of the sigma-2 receptor was unknown and the existence of this subtype had only been proven pharmacologically. However, within one year a study was published [13] in which sigma-2 receptors were irreversibly labeled using WC-21, a ligand containing an azide moiety for photoaffinity tagging and a fluorescein isothiocyanate (FITC) group for visualization of the sigma-2 protein. By matrix-assisted laser desorption/ionization–mass spectrometry analysis, the membrane-bound protein which was labeled by WC-21 in rat liver was identified as progesterone receptor membrane component 1 (PGRMC1). Knockdown of the PGRMC1 protein with specific short interfering RNA (siRNA) reduced the binding of a radioiodinated sigma-2 receptor ligand in HeLa cells and of a fluorescent sigma-2 receptor ligand in human embryonic kidney 293T cells. Moreover, knockdown of PGRMC1 reduced the ability of sigma-2 agonists to induce caspase-3 activation in HeLa cells. Overexpression of PGRMC1 by transfection of HeLa cells with PGRMC1 cDNA was associated with a striking (60%) increase of the cellular binding of the radioiodinated sigma-2 ligand. Treatment of A549 lung cancer cells with a PGRMC1 ligand (AG-205) or a sigma-2 receptor ligand (WC-26) induced similar, dose-dependent upregulations of the PGRMC1 protein. Both the PGRMC1 ligand AG-205 and various sigma-2 receptor ligands (DTG, siramesine, SV119, and WC-26) displaced bound radioiodinated sigma-2 receptor ligand in tumor cell membrane homogenates in a concentration-dependent manner. Thus, these ligands appeared to bind to the same site. Confocal microscopy indicated that PGRMC1 and the sigma-2 receptor protein had the same intracellular localization, *viz.* in mitochondria and endoplasmatic reticulum [13]. Based on this combined evidence, the PGRMC1 protein complex was identified as the putative sigma-2 receptor binding site.

It had already been known for a long time that sigma-2 ligands inhibit high-affinity progesterone binding to a microsomal fraction of porcine liver, suggesting that high-affinity progesterone binding sites are part of a complex including sigma receptors, if not themselves sigma receptors [14]. But the important study of Xu et al. [13] provided actual proof for the identity of the sigma-2 receptor and PGRMC1. Since PGRMC1 is a known protein, the cDNA sequence of the sigma-2 receptor gene may in fact already have been determined, both in porcine [15] and human [16] tissues.

Although the close identity of the sigma-2 receptor and PGRMC1 appears to have been established, some questions remain unanswered. First, the molecular masses of both proteins appear to be different. Values of 22 to 28 kDa have been reported for PGRMC1 [17–19] whereas a value of only 21.5 kDa has been determined for the sigma-2 receptor protein [20]. These differences may be related to post-translational processing or splice variants of a single protein. Second, inhibition of tumor cell proliferation has been reported to require the agonist action of anti-cancer drugs at sigma-2 receptors [21,22] but an antagonist action at PGRMC1 [23,24]. This contradiction may be more apparent than real, since agonist or antagonist actions at sigma receptors have not been well-defined. Compounds which were originally classified as sigma agonists may in fact be antagonists, and *vice versa* [25–27]. Caspase-3 activation by sigma-2 receptor ligands has been proposed to serve as a functional assay for differentiating sigma-2 agonists, partial agonists and antagonists [27]. Finally, PGRMC1 is known to bind to P450 resulting in the stimulation of its activity and increased cholesterol synthesis [28], but for the sigma-2 receptor such binding has not been reported. Future research in this exciting field may resolve these discrepancies and may result in further confirmation of the identity of PGRMC1 and the sigma-2 receptor protein.

Although sigma-1 and sigma-2 receptors are usually treated as members of a common sigma receptor "family" and share affinity for certain artificial ligands and steroids like progesterone, the structures of the sigma-1 and sigma-2 receptor proteins are in fact unrelated. While the sigma-2 receptor seems to belong to a progesterone receptor complex, the sigma-1 receptor has been characterized as a chaperone protein [29,30]. The function and intracellular location of this protein are altered by ligands but the protein can be active even in the absence of ligands. Thus, the notion of "agonists" and "antagonists", which is