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Dichlorophenyl piperazines, including a recently-approved atypical antipsychotic, are potent inhibitors of DHCR7, the last enzyme in cholesterol biosynthesis

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ABSTRACT

While antipsychotic medications provide important relief from debilitating psychotic symptoms, they also have significant adverse side effects, which might have relevant impact on human health. Several research studies, including ours, have shown that commonly used antipsychotics such as haloperidol and aripiprazole affect cholesterol biosynthesis at the conversion of 7-dehydrocholesterol (7-DHC) to cholesterol. This transformation is promoted by the enzyme DHCR7 and its inhibition causes increases in plasma and tissue levels of 7-DHC. The inhibition of this enzymatic step by mutations in the Dhcr7 gene leads to Smith-Lemli-Opitz syndrome, a devastating human condition that can be replicated in rats by small molecule inhibitors of DHCR7. The fact that two compounds, brexpiprazole and cariprazine, that were recently approved by the FDA have substructural elements in common with the DHCR7 inhibitor aripiprazole, prompted us to evaluate the effect of brexpiprazole and cariprazine on cholesterol biosynthesis. We report that cariprazine affects levels of 7-DHC and cholesterol in cell culture incubations at concentrations as low as 5 nM. Furthermore, a common metabolite of cariprazine and aripiprazole, 2,3-(dichlorophenyl) piperazine, inhibits DHCR7 activity at concentrations comparable to those of the potent teratogen AY9944. The cell culture experiments were corroborated in mice in studies showing that treatment with cariprazine elevated 7-DHC in brain and serum. The consequences of sterol inhibition by antipsychotics in the developing nervous system and the safety of their use during pregnancy remains to be established.

1. Introduction

Cholesterol is of critical importance in the brain during development and disruptions in its biosynthesis (Nes, 2011) have been associated with multiple disorders. Smith-Lemli-Opitz Syndrome (SLOS) is one such neurodevelopmental condition that arises from mutations in 7-dehydrocholesterol reductase (DHCR7),(Smith et al., 1964; Kelley and Hennekam, 2000; Dietschy and Turley, 2001; Dietschy and Turley, 2004; Witsch-Baumgartner et al., 2008; Porter and Herman, 2011; Kanungo et al., 2013) the enzyme that converts 7-dehydrocholesterol (7-DHC) to cholesterol, the last step in the complex biosynthesis. Scheme 1 shows the last steps in the pathway promoted by two reductase enzymes, DHCR7 that operates on the sterol B-ring double bond and DHCR24 that acts on the tail of the sterol at C24. SLOS is characterized biochemically by an increase of 7-DHC and a decrease of cholesterol in tissues and fluids.

Abbreviations: DHCR7, dehydrocholesterol reductase 7; DHCR24, dehydrocholesterol reductase 24; 7-DHC7, dehydrocholesterol; 8-DHC8, dehydrocholesterol; Des, desmosterol; Lan, lanosterol; Chol, cholesterol; SLOS, Smith-Lemli-Opitz syndrome; PTAD, 4-Phenyl-1,2,4-triazoline-3,5-dione; BSTFA, *N,O*-bis(trimethylsilyl)trifluoroacetamide; APCI, atmospheric pressure chemical ionization; SRM, selected reaction monitoring; AY9944, trans-1,4-bis(2-chloro-benzylaminomethyl); LC-MS/MS, liquid chromatography-tandem mass spectrometry; GC/MS, gas chromatography – mass spectrometry; NIH, National Institute of Health; FDA, US Food and Drug Administration; MeOH, methanol; TIC, total ion current; FBS, fetal bovine serum; DMEM, Dulbecco's Modified Eagle Medium; PBS, phosphate buffered saline; RCS, residual cholesterol synthesis; DMSO, dimethylsulfoxide; HF, human fibroblasts; m-CPP, meta-chlorophenyl piperazine; 2,3-DCPP, 1-(2,3-dichlorophenyl)piperazine

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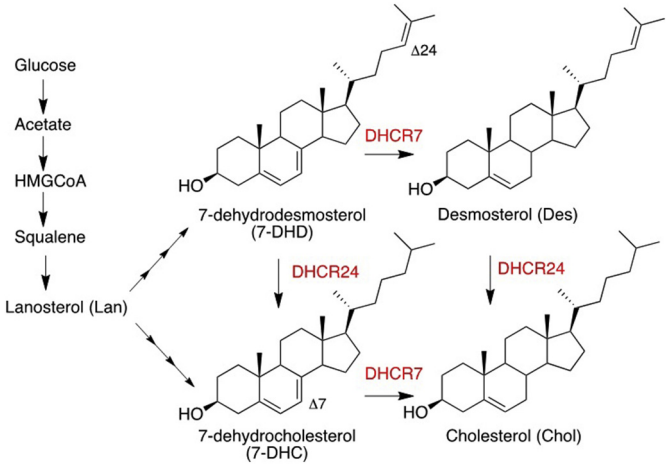

Scheme 1. Sterol Structures and Reductase Enzymes in the Last Steps of Cholesterol Biosynthesis. The reductase enzymes, shown in red, act on the Δ7 and Δ24 double bonds.

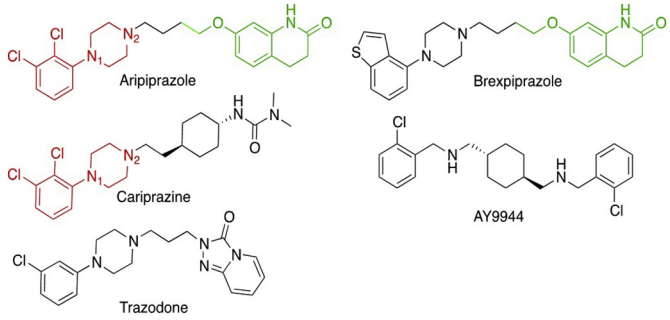

Fig. 1. Structures of the Compounds Investigated. Common substructure features of aripiprazole and cariprazine are shown in red, those of aripiprazole and brexpiprazole are shown in green. AY9944 is a teratogenic compound used to generated a pharmacological rodent model for Smith-Lemli-Opitz syndrome, trazodone is a commonly prescribed psychoactive piperazine. The piperazine nitrogens are labeled N_1 and N_2 for purposes of discussion. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A number of small molecules, including some widely prescribed pharmaceuticals, modulate the activity of DHCR7, increasing cellular levels of 7-DHC and reducing cholesterol levels in a way that parallels the perturbations of sterol homeostasis found in SLOS patients (Giera et al., 2007, 2008, Sanchez-Wandelmer et al., 2010, Canfran-Duque et al., 2013, Hall et al., 2013, Boland and Tatonetti, 2016, Korade et al., 2016, Korade et al., 2017a,b). Given the parallel cellular biochemistry found in SLOS and in human exposures to DHCR7 inhibitors, there has been increased interest in describing the set of DHCR7-active compounds(Horling et al., 2012) that constitute potential exposure threats during neurodevelopment.

We have recently used Neuro2a cells to screen an NIH clinical collection of small molecules and found that some 5% of the compounds in the library increased 7-DHC levels by inhibiting DHCR7 (Kim et al., 2016). Among the most active compounds identified in that study were the psychotropic medications haloperidol, aripiprazole and trazodone. In another report, Korade and co-workers reported elevated levels of 7-DHC and 8-dehydrocholesterol (8-DHC) in the blood of patients using these same drugs (Korade et al., 2017a, 2017b). Hall and co-workers also reported elevated levels of 7-DHC in 22 individuals who had not been diagnosed with SLOS, but were on treatment with either aripiprazole or trazodone (Hall et al., 2013).

Compounds that modulate the DHCR7 enzyme have been a particular focus of a recent review that examined the effects of different drugs on the cholesterol biosynthesis pathway (Boland and Tatonetti, 2016). The authors of this paper suggested that a higher incidence of fetal malformations, spontaneous abortions and intrauterine death was associated with the use of several DHCR7 inhibitors taken during the first trimester of pregnancy, a critical period when a decrease in cholesterol has been linked to teratogenicity. As a conclusion, the authors proposed that the use of such pharmaceuticals during pregnancy could lead to what is essentially a chemically induced SLOS, a disorder with similar negative outcomes whose hallmark is the accumulation of 7-DHC (Boland and Tatonetti, 2016).

With the increased use of atypical antipsychotics in recent years, new drugs have been approved by the FDA that bear a striking structural resemblance to aripiprazole, a highly-prescribed drug and potent inhibitor of DHCR7. Two such compounds, brexpiprazole and cariprazine appeared in 2015 and both of these drugs have sub-structures that are identical to elements present in aripiprazole, see Fig. 1. Because of this close structural relationship and the suggestion that DHCR7 inhibitors such as aripiprazole may have a negative effect on fetal outcomes, we have assessed the potency of brexpiprazole and cariprazine on cholesterol biosynthesis. We report here the results of this study and we include here a comparison of the effects on sterol homeostasis of these two new antipsychotic medications with the effect of AY9944, a teratogen known to inhibit DHCR7 (Roux et al., 1980). Indeed, AY9944 is a notable molecule for comparison since it has been used to generate a pharmacological rat model of SLOS (Kolf-Clauw et al., 1996, Fliesler et al., 2004, Fliesler and Bretillon, 2010, Xu et al., 2011a,b). Finally, we tested the ability of cariprazine to alter cholesterol synthesis in mice. Sterol levels were measured in blood and brain in order to confirm the *in vitro* effect and to establish a link between drug treatment and 7-DHC levels.

2. Experimental section

2.1. Chemicals

Unless otherwise noted, all chemicals were purchased from Sigma-Aldrich Co (St. Louis, MO). HPLC grade solvents were purchased from Thermo Fisher Scientific Inc. (Waltham, MA). All cell culture reagents were from Mediatech (Manassas, VA), Life Technologies (Grand Island, NY), and Greiner Bio-One GmBH (Monroe, NC). Cariprazine (marketed as VRAYLAR in the US and REAGILA in Europe), Brexpiprazole (marketed as REXULTI), Aripiprazole (marketed as ABILIFY) and AY9944 were dissolved in DMSO for the experiments. Ergosterol was purchased from TCI America. All sterol standards, natural and isotopically labeled, used in this study are available from Kerafast, Inc. (Boston MA). D-$^{13}C_6$-glucose was purchased from Cambridge Isotope Laboratories, Inc. Delipidated fetal bovine serum was prepared as described previously and did not have detectable cholesterol levels (Gibson et al., 1990).

2.2. Cell cultures

The neuroblastoma cell line Neuro2a was purchased from American Type Culture Collection (Rockville, MD). The A549 human lung carcinoma cell line was obtained from the European Collection of Authenticated Cell Cultures (ECACC). Control and SLOS fibroblasts were described previously (Korade et al., 2016; Korade et al., 2017a,b). All cultured control human fibroblasts used were passages of 5–18. All cells were subcultured once a week, and the culture medium was changed every two days. All cell lines were maintained in DMEM with high glucose (25 mM), 1 mM pyruvate, 1 mM L-glutamine, 10% fetal bovine serum (FBS; Thermo Scientific HyClone, Logan, UT), and penicillin/streptomycin at 37 °C and 5% CO_2*(medium 1)*. For the drug exposure experiments, human fibroblasts were cultured in DMEM with 10 mM $^{13}C_6$-glucose, 1 mM L-glutamine, 10% delipidated fetal bovine serum (FBS) and penicillin/streptomycin *(medium 2)*. Neuro2a and

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