Schizophrenia Research 108 (2009) 134–142

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Common effect of antipsychotics on the biosynthesis and regulation of fatty acids and cholesterol supports a key role of lipid homeostasis in schizophrenia

Mihael H. Polymeropoulos [a], Louis Licamele [a,b], Simona Volpi [a], Kendra Mack [a], Shruti N. Mitkus [a], Eugene D. Carstea [a], Lise Getoor [b], Andrew Thompson [a], Christian Lavedan [a,*]

[a] Vanda Pharmaceuticals Inc., 9605 Medical Center Drive, Rockville, Maryland, 20850, United States
[b] University of Maryland, Computer Science Department, AV Williams Bldg, Rm 3217, College Park, Maryland, 20742, United States

ARTICLE INFO

Article history:
Received 22 August 2008
Received in revised form 24 November 2008
Accepted 25 November 2008
Available online 15 January 2009

Keywords:
Antipsychotic action
Lipid homeostasis
Gene expression
Pathogenesis

ABSTRACT

For decades, the dopamine hypothesis has gained the most attention in an attempt to explain the origin and the symptoms of schizophrenia. While this hypothesis offers an explanation for the relationship between psychotic symptoms and dopamine kinetics, it does not provide a direct explanation of the etiology of schizophrenia which remains poorly understood. Consequently, current antipsychotics that target neurotransmitter receptors, have limited and inconsistent efficacy. To gain insights into the mechanism of action of these drugs, we studied the expression profile of 12,490 human genes in a cell line treated with 18 antipsychotics, and compared it to that of a library of 448 other compounds used in a variety of disorders. Analysis reveals a common effect of antipsychotics on the biosynthesis and regulation of fatty acids and cholesterol, which is discussed in the context of a lipid hypothesis where alterations in lipid homeostasis might underlie the pathogenesis of schizophrenia. This finding may help research aimed at the development of novel treatments for this devastating disease.

1. Introduction

The underlying molecular etiology of schizophrenia remains poorly understood although the dopamine hypothesis has been the most influential for decades. In the dopamine hypothesis, alteration of the homeostasis of neurotransmitters including dopamine and serotonin is believed to result in the production of the symptoms of the disease (Toda and Abi-Dargham, 2007). Other mechanisms of the pathophysiology of schizophrenia have been proposed including a neurodevelopmental hypothesis (Nasrallah, 1993) where alterations of membrane phospholipids could play a major part (Horrobin, 1998), a role for glutamate (Goff and Coyle, 2001) and the muscarinic cholinergic system (Raedler et al., 2007), and an inflammation of the microvasculature system (Hanson and Gottesman, 2005). There is also much evidence that schizophrenia is highly heritable and may be caused by several interacting susceptibility genetic loci and environmental factors (Van Os and Sham, 2003; Freedman et al., 2001; Tsuang et al., 2004). However, despite intense research efforts to identify genetic defects in the neurotransmitter systems in patients and families with schizophrenia, no consistent genetic alteration has been identified to date.

A number of pharmacological agents are available today in the class of antipsychotics which are used for management of the disease symptoms. They share the ability to block the effect of neurotransmitters through the binding of an array of receptors. The antipsychotics are often classified as typical and atypical agents. The typical agents have a primary affinity

* Corresponding author. Tel.: +1 240 599 4500; fax: +1 301 294 1900.
E-mail addresses: Mihael.Polymeropoulos@vandapharma.com (M.H. Polymeropoulos), Louis.Licamele@vandapharma.com (L. Licamele), Simona.Volpi@vandapharma.com (S. Volpi), Kendra.Mack@vandapharma.com (K. Mack), Shruti.Mitkus@vandapharma.com (S.N. Mitkus), Gene.Carstea@vandapharma.com (E.D. Carstea), Getoor@cs.umd.edu (L. Getoor), Andrew.Thompson@vandapharma.com (A. Thompson), Christian.Lavedan@vandapharma.com (C. Lavedan).

0920-9964/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.schres.2008.11.025

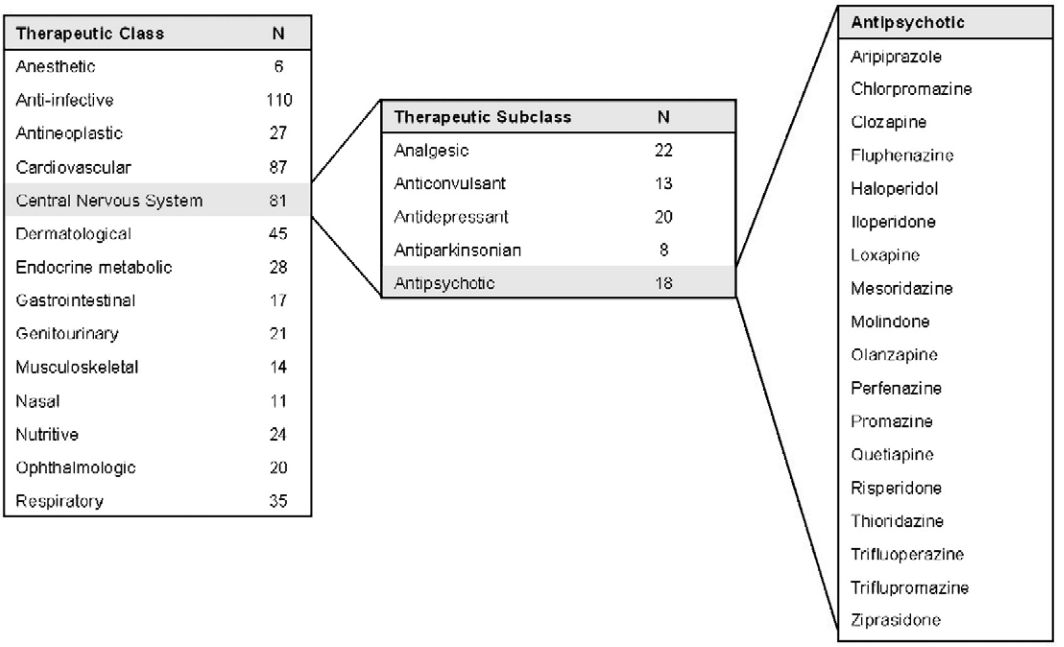

Fig. 1. Overview of compounds profiled across therapeutic classes and sub-classes. Therapeutic classes and subclasses are listed as defined by The Physicians' Desk Reference (PDR, Thomson Healthcare at Montvale, NJ, USA), with the number of drugs profiled (*N*). Some drugs are listed in more than one therapeutic class or sub-class.

for dopamine receptors whereas atypical agents bind both dopamine and serotonin receptors. It is believed that this dual receptor binding of atypical agents leads to a better tolerability profile, especially in the production of movement side effects that include extrapyramidal symptoms and akathisia. Unfortunately, patient response to treatment remains greatly variable and the discontinuation rate with antipsychotic treatment is high (Lieberman et al., 2005).

In an effort to discover molecular signatures of pharmaceutical agents, including antipsychotics, we have screened 466 compounds that belong to 14 different therapeutic classes (Fig. 1), in a human retinal pigment epithelia cell line (ARPE-19) and studied the resulting gene expression changes across 12,490 genes. The choice of the ARPE-19 cell line is particularly well suited for the study of compounds that affect neuronal type cells, in particular antipsychotics. It expresses a variety of cell surface receptors that include the dopamine receptor D2, the serotonin receptors 1A, 2A, and 2C, the muscarinic receptor M3, and the histamine receptor H1 (Dr. Maria A. DeBernardi, personal communication). Furthermore several antipsychotics have been associated with degenerative retinopathies (Fornaro et al., 2002).

We describe here the discovery of an "antipsychotic signature" which gives insights into the therapeutic effect of these drugs.

2. Experimental/materials and methods

2.1. Cell culture and drug treatment

The retinal pigment epithelia cell line, ARPE-19/HPV-16, was chosen to establish a database of drug profiles because it is from non-cancerous human origin, with a normal karyotype, and can easily be grown as monolayer in 96-well plates. H4 is a hypertriploid cell line from glioblastoma origin, which was used only for independent replication. Cell lines were propagated according to supplier's specifications (ATCC Manassas, VA). Compounds were obtained from Sigma (St. Louis, MO) or Vanda Pharmaceuticals (Rockville, MD). Cells were aliquoted to 96-well plates (~2×10e5 cells/well) and incubated for 24 h prior to providing fresh media with a drug, or the drug vehicle (water, dimethyl sulfoxide, ethanol, methanol, or phosphate-buffered saline solution). Drugs were diluted 1000 fold in buffered Advanced D-MEM/F-12 culture medium (Invitrogen, Carlsbad, CA) containing non-essential amino acids and 110 mg/L sodium pyruvate. In these conditions, no significant changes of pH were expected, which was confirmed by the monitoring of the pH indicator present in the medium. A final 10 μM drug concentration was chosen because it is believed to fit in the range of physiological relevance, and has been used in other cell line studies (Ferno et al., 2006; Lamb et al., 2006). Microscopic inspection of each well was conducted at the end of the treatment to discard any instance where cells had morphological changes consistent with apoptosis, and to verify that the drug had not precipitated in the culture medium.

2.2. Gene expression profiles

Cells were harvested 24 h after treatment and RNA extracted using the RNeasy 96 protocol (Qiagen, Valencia, CA). Gene expression for 22,238 probe sets of 12,490 genes was generated with U133A2.0 microarrays following the manufacture's instructions (Affymetrix, Santa Clara, CA). Antipsychotics were profiled in duplicate or triplicate, with multiple vehicle controls on each plate. A total of 708