This series of columns has 3 main goals: (1) to explain class warnings as used by the United States Food and Drug Administration, (2) to increase awareness of movement disorders that may occur in patients treated with antipsychotic medications, and (3) to understand why clinicians should refrain from immediately assuming a diagnosis of tardive dyskinesia/dystonia (TD) in patients treated with antipsychotics. The first column in this series began with the case of a 76-year-old man with major depressive disorder who developed orofacial dyskinesias while being treated with aripiprazole as an antidepressant augmentation strategy. It was alleged that a higher than intended dose of aripiprazole (i.e., 20 mg/d for 2 wk followed by 10 mg/d for 4 wk instead of the intended dose of 2 mg/d) was the cause of the dyskinetic movements in this man, and the authors were asked to review the case and give their opinion. The principal basis for this theory of causation was the class warning about TD in the package insert for aripiprazole. The rationale for concluding aripiprazole caused TD in the 76-year-old man led to this series of columns about aripiprazole, its potential—if any—to cause TD, and the presence of a class warning about TD in its package insert. The central point is to illustrate why class warnings exist and their implications for practice. The first column in this series focused on the historical background, incidence, prevalence, risk factors, and clinical presentations of tardive and spontaneous dyskinesias and concluded with a discussion of diagnostic considerations explaining why clinicians should avoid making a diagnosis of TD until a thorough differential diagnosis has been considered. This second column in the series reviews the pharmacology of aripiprazole and the preclinical and phase I translational human studies that suggest aripiprazole should have a low to nonexistent risk of causing TD compared with other antipsychotics. The third column in the series will review the systematic clinical trial data and “real-world” data on TD and the use of aripiprazole as adjunctive treatment with antidepressants for major depressive disorder to see whether these data support the conclusion of a low to nonexistent relationship between aripiprazole treatment and the development of TD. The fourth and final column in the series will consider the type of study that would need to be performed to avoid a specific class warning, focusing on the TD class warning as an example and discussing why such studies are rarely done.

MATTHEW MACALUSO, DO
ALEXANDRA FLYNN, MD, PhD
SHELDON PRESKORN, MD

MACALUSO and PRESKORN: Department of Psychiatry, University of Kansas School of Medicine-Wichita, Wichita, KS; FLYNN: Robert J. Dole VA Medical Center, Wichita, KS

Note: Dr. Preskorn is the primary author of this series of columns.

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Please send correspondence to: Matthew Macaluso, DO, Department of Psychiatry, University of Kansas School of Medicine-Wichita, 1010 W. Kansas, Wichita, KS 67228-8032 (e-mail: mmacaluso@kumc.edu).

Over his career, S.P. has worked with over 127 pharmaceutical companies in the United States and throughout the world. Over the past year, S.P. has received grants/research support from or has served as a consultant, on the advisory board, or on the speakers’ bureau for Alkermes, Assurex Health, Cerecor, Eisai, Johnson & Johnson, Merck, and Sunovion. Over the past year, M.M. has conducted clinical trials research as principal investigator for the following pharmaceutical companies: Alkermes, Allergan, AssureRx, Eisai, Forum, Janssen, and Naurex/Aptinyx; all clinical trial and study contracts were with and payments made to the Kansas University Medical Center Research Institute, a research institute affiliated with Kansas University School of Medicine-Wichita (KUSM-W). A.F. declares no conflicts of interest.

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This column, the second in a planned 4-part series, reviews the preclinical and translational science data suggesting that aripiprazole is associated with a low to nonexistent risk of tardive dyskinesia or dystonia (TD) and hence does not warrant a class warning for this adverse event. The first column in this series began by describing the case presentation of a 76-year-old man with major depressive disorder who developed orofacial dyskinesias while being treated with aripiprazole as an augmentation strategy and who was diagnosed with TD. It was alleged in this case that the higher than intended exposure to aripiprazole was the cause of the dyskinetic movements, a position based principally on the presence of a class warning about antipsychotics and the risk of TD in the package labeling for aripiprazole. The authors were asked to review the case and give their opinion. The first column then reviewed data concerning the historical background, incidence, prevalence, risk factors, and clinical presentations of TD and concluded with a discussion of why clinicians should avoid making a diagnosis of TD until a thorough differential diagnosis has been considered.

This second column in the series reviews the pharmacology of aripiprazole, including preclinical in vitro and in vivo studies and a phase I human translational study suggesting a reduced to nonexistent likelihood of the development of TD during treatment with aripiprazole. To provide context, this column begins by presenting a historical perspective on antipsychotics in general and aripiprazole in particular, including how this agent differs pharmacologically from other antipsychotics.

### HISTORICAL PERSPECTIVE ON ARIPIPRAZOLE

As discussed in the first column in this series and in several earlier psychopharmacology columns in this journal, the development of antipsychotics is better conceptualized as occurring in 4 generations, rather than the more commonly used 2-generation model (Fig. 1).
the criteria for being an “atypical” antipsychotic (Table 1). The second generation of antipsychotics in the 4-generation model includes haloperidol and fluphenazine, which differ from the first-generation antipsychotics because they are potent and selective dopamine-2 (D2) antagonists and are only “typical” antipsychotics or neuroleptics because of their greater risk of causing both acute extrapyramidal symptoms (EPS) such as Parkinsonism, acute dystonia, and akathisia early in treatment and TD with long-term administration.

Clozapine was the first of the third-generation antipsychotics, even though it is a derivative of chlorpromazine and was synthesized in the 1950s. The unique properties of clozapine led to its approval by the United States Food and Drug Administration 30 years after it was first synthesized. Clozapine was approved for the treatment of patients with schizophrenia that was not responsive to other antipsychotics and it is the only antipsychotic with such efficacy. Clozapine served as the “blueprint” that led to the discovery and development of all other “atypical” agents, beginning with risperidone and followed by olanzapine, quetiapine, ziprasidone, paliperidone, asenapine, iloperidone, and lurasidone. These agents were termed “atypical” because they were designed to be like clozapine in terms of having greater binding affinity for serotonin-2A receptors, generally abbreviated as 5-HT2A because serotonin is also 5-hydroxytryptophan, compared with their affinity for the D2 receptor. The higher binding affinity for 5-HT2A versus D2 receptors is believed to convey a lower risk of causing acute EPS, elevated prolactin levels, and TD compared with the D2 selective antagonists or “typical” antipsychotics (eg, haloperidol).

As shown in Figure 1, aripiprazole is a “fourth-generation” antipsychotic in this model due to the fact that it has a mechanism of action different from all of the preceding antipsychotics (ie, D2 partial agonism). The rest of this column will discuss the unique mechanism of action of aripiprazole and the preclinical and early phase 1 human translational studies suggesting it should have a low to nonexistent risk of causing TD.

**THE UNIQUE MECHANISM OF ACTION OF ARIPIPRAZOLE AND THE RISK OF TD**

Although often referred to as an “atypical” antipsychotic, the pharmacology of aripiprazole is different from that of other “atypical” antipsychotics because it is a D2 receptor partial agonist. Nevertheless, aripiprazole does meet the criteria for “atypicality” in terms of having a low risk of causing prolactin elevation, acute EPS, and TD. In contrast to the other “atypical” antipsychotics, aripiprazole has greater binding affinity for the D2 receptor than it does for the 5-HT2A receptor. More specifically, aripiprazole is a partial agonist at D2, D3, and 5-HT1A receptors and an antagonist at 5-HT2A receptors. It has high affinity for D4, D3, 5-HT1A, and 5-HT2A receptors but a low 5HT2A:D2 affinity ratio.

Even though aripiprazole is a D2 receptor partial agonist, it can antagonize the effects of a higher than normal concentration of dopamine, which can cause the psychotic symptoms characteristic of schizophrenia. Whether aripiprazole acts as an agonist or antagonist at the D2 receptor in a given individual at a given point in time depends on the synaptic concentration of dopamine. If the synaptic concentration of dopamine is low, then aripiprazole exerts agonism at the D2 receptor at approximately 30% of the maximum agonism of the endogenous neurotransmitter dopamine. If the synaptic concentration of dopamine is high, the addition of aripiprazole will act as an antagonist as it displaces dopamine from the receptor and brings the receptor’s activation down from 100% of dopamine to 30% of aripiprazole. The clinical implications of this pharmacology are illustrated in a study in normal volunteers presented later in this column.

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**TABLE 1. Definition of Atypicality**

| A drug with antipsychotic efficacy at a dose that has a low risk of also causing |
| Acute extrapyramidal symptoms |
| Elevated prolactin |
| Tardive dyskinesia/dystonia |
| And a drug with Greater binding affinity for the serotonin 5-HT2A receptor than for the dopamine D2 receptor |
| Superior efficacy in treating positive, negative, and cognitive signs and symptoms of schizophrenia |

*Source of information: Meltzer et al.*

Before discussing the preclinical and early human studies that evaluated whether aripiprazole can cause the degree of functional dopamine blockade (ie, approximately 80%) needed to cause acute EPS and TD, a brief summary of the pathophysiology underlying the development of TD will be presented.

PATHOPHYSIOLOGY OF TD

If the administration of a given dose of a second-generation antipsychotic (ie, a selective D₂ receptor antagonist such as haloperidol) results in >80% D₂ receptor occupancy (and hence >80% functional dopamine blockade) in the striatum, the brain will attempt to restore normal nigrostriatal function in 2 ways. First, the dopamine neurons in the substantia nigra will increase their firing rate to release more dopamine into the synapse in an attempt to displace the D₂ receptor blocker to restore functional blockade below 80%. This adaptive mechanism has been documented by electrophysiological studies conducted both in vitro (eg, in tissue slices) and in vivo (ie, in living animals). For example, Pucak and Grace demonstrated that addition of haloperidol to in vitro nigral-striatal slices increased the firing rate of dopamine neurons in the substantia nigra. On the basis of findings from in vivo studies, this adaptive mechanism lasts only a couple of weeks or less because dopamine firing is unable to displace sufficient amounts of a high potency and selective D₂ pure antagonist (eg, haloperidol) to bring the functional blockade below the 80% threshold which causes acute EPS and TD.

The brain’s next mechanism for overcoming excessive D₂ receptor antagonism and the most widely accepted mechanism underlying the development of TD involves a compensatory increased D₂ receptor synthesis and deployment to the dendritic surface of the cell. In the literature, this mechanism has been called either D₂ receptor upregulation or supersensitization. This theory has been supported by animal studies. Researchers observed rats develop spontaneous perioral movements as a result of long-term D₂ receptor blockade caused by administration of “neuroleptic” agents and hypothesized that such blockade was the initiating step underlying the pathophysiology of TD in humans. Parsons et al reported that rodents exposed to haloperidol, a full and selective D₂ receptor antagonist, had 24% and 33% increased density of striatal D₂ and adenosine A₂a receptors, respectively. Ginovart et al found that continuous blockade of D₂ receptors by haloperidol in cats led to upregulation (ie, increased density) of striatal D₂ receptors and is the pathophysiology underlying the development of TD. Silvestri et al were the first to use positron emission tomography (PET) to demonstrate the upregulation of D₂ receptors in living humans who had received long-term treatment with antipsychotic medications.

Using this information as background/context, this column will next examine the in vitro and in vivo studies that have evaluated whether aripiprazole can produce sufficient functional blockade of the D₂ receptor to induce the compensatory mechanisms that cause TD. The following caveat is critical to an understanding of this discussion. The percentage of receptor occupancy is equal to the magnitude of the functional blockade only when the drug is a D₂ full antagonist because in this scenario every receptor that is occupied is fully blocked. In contrast, the magnitude of functional blockade is only 70% of the percentage of receptor occupancy produced by a partial agonist with 30% intrinsic activity (eg, aripiprazole) because in this scenario every receptor that is occupied is turned on to 30% of its full activation by the partial agonist. Thus, the functional blockade is equal to the percentage of receptor occupied times 70% (eg, occupancy of 80% of striatal dopamine receptor by aripiprazole is equal to 56% functional blockade, ie, 80%×70%).

The studies presented here progressed from 2 preclinical models to a human study following the usual drug development process explained in 2 earlier series of columns. The 2 preclinical studies involved (1) an in vitro model of the dopamine synapse and (2) an in vivo study in rodents. The human study was a study of D₂ receptor occupancy and EPS using PET in normal volunteers.

IN VITRO DATA SUGGEST LOW LIKELIHOOD OF ARIPIPRAZOLE CAUSING ACUTE EPS AND TD

In a study published in 2002, Burris et al developed an in vitro model for what occurs in the dopamine synapse as a result of treatment with aripiprazole, as a prototypic partial D₂ agonist, compared with treatment with haloperidol, as a
prototypic pure and selective D2 antagonist. This model uses Chinese hamster ovarian cells transfecting with the human gene, which codes for the human D2 receptor. Using these cells, the investigators studied the effects of dopamine, haloperidol, and aripiprazole alone and in combination.28 In this study, Chinese hamster ovarian D2L cells were exposed to various concentrations of dopamine, haloperidol, and aripiprazole alone and in combination to measure the D2L receptor response to dopamine agonism or antagonism. (Parenthetically, the D2L receptor is a G-protein-coupled receptor linked to a second messenger system in which ATP is converted to cyclic AMP. That in turn produces a cascade of events within the postsynaptic cell that either cause changes in the cell that represent the end product of the message or transmit the message further forward.) The goal of the study by Burris and colleagues was to determine whether there is any difference in the second messenger response to treatment with aripiprazole versus haloperidol alone (ie, in the absence of dopamine) or in the presence of higher than usual intrasynaptic concentrations of dopamine.

By definition, incubation of these cells with high concentrations of dopamine alone produced a 100% response in the second messenger system. Conversely, incubation with haloperidol alone resulted in no second messenger system response, as would be expected from a selective D2 receptor full agonist. In contrast, incubation with aripiprazole alone produced approximately a 30% second messenger response consistent with the intrinsic activity of aripiprazole on the human D2 receptor.

In the next series of experiments, these cells were incubated with higher than normal intrasynaptic concentrations of dopamine (ie, 100 nM) alone and then in the presence of increasing concentrations of haloperidol or aripiprazole. As the concentration of haloperidol was increased, the response of the cells dropped to 0, consistent with full blockade of the D2 receptor. In contrast, increasing concentrations of aripiprazole brought the response down to 30% of the maximum response to dopamine alone. At those concentrations, aripiprazole was fully blocking the effect of dopamine but was still producing 30% activation of the D2 receptors consistent with its intrinsic agonism of the human D2 receptor.

On the basis of these results, aripiprazole cannot produce >70% blockade of D2 receptors, even at maximum in vitro concentrations (ie, 100% D2 receptor occupancy×70% functional blockade). Hence, aripiprazole should not be able to block D2 receptors to a point (ie, >80% functional blockade) that triggers increased D2 receptor synthesis as a compensatory mechanism, which is the pathophysiology underlying the development of TD. However, these results are from an in vitro study.

STUDY IN RATS COMPARING POTENTIAL FOR INCREASED D2 RECEPTOR PRODUCTION IN ARIPIPRAZOLE VERSUS HALOPERIDOL

To extend the in vitro findings of Burris and colleagues in humanized single cells to intact animals, Inoue et al29 compared the ability of haloperidol and aripiprazole to increase mRNA synthesis of D2 receptors as a necessary step to increase the postsynaptic density of D2 receptors in rat striata. In vivo administration of haloperidol was capable of increasing D2 mRNA synthesis consistent with D2 receptor upregulation or supersensitization, whereas in vivo administration of aripiprazole did not. This finding is consistent with a lower or even nonexistent risk of developing TD as a result of treatment with aripiprazole versus haloperidol.

PET STUDY EXAMINING RECEPTOR OCCUPANCY AND RISK FOR EPS

The findings from these 2 preclinical studies were followed by a human translational study that examined the potential for aripiprazole to cause acute EPS at D2 receptor occupancy well above 80%. As background critical to the interpretation of this human study, the antipsychotic effect of D2 antagonists is achieved when approximately 60% D2 receptor blockade is achieved, whereas EPS typically emerge when D2 receptor blockade exceeds approximately 80%.30 Using PET to quantify D2 receptor occupancy, Yokoi et al31 detected dose-dependent receptor occupancy between 40% and 95% with increasing doses of aripiprazole (up to 30 mg/d for 14 d) in healthy volunteers. Despite achieving >90% D2 receptor occupancy with the highest dose of aripiprazole used (ie, 30 mg/d), no EPS were detected.

This result is consistent with the in vitro findings of Burris and colleagues and the in vivo findings in rats of Inoue and colleagues. Taken together, these
3 studies are consistent with the conclusion that aripiprazole, even with 90% D₂ receptor occupancy, cannot produce greater than a 70% functional blockade of the D₂ receptor consistent with its 30% intrinsic D₂ partial agonism. Mathematically, 90% receptor occupancy multiplied by 70% (based on the 30% intrinsic D₂ receptor agonism of aripiprazole) produces <63% functional D₂ receptor blockade. That is well below the 80% functional blockade threshold usually required to produce acute EPS and hence increased D₂ receptor expression but still within the range necessary to produce antipsychotic efficacy. Taken together, these results suggest that the D₂ upregulation required for TD to develop does not typically occur even at the maximum recommended doses of aripiprazole.

On the basis of all of these results, aripiprazole was unlikely to have caused the dyskinetic movements in the case of the 76-year-old man presented in the first column in this series. Nevertheless, these results reflect preclinical testing and short-term treatment in healthy volunteers. For these reasons, the next column in this series will examine the clinical trial and naturalistic exposure data concerning aripiprazole to see whether those data are consistent with this conclusion.

**PHARMACOKINETIC OBSERVATIONS**

Before concluding this column, readers should be aware that, in addition to the pharmacodynamic findings reviewed here, there are also pharmacokinetic reasons to suspect that exposure to a higher than intended dosage of aripiprazole was not causatively related to the development of the dyskinetic movements in the case of the 76-year-old man. As discussed in an earlier series of columns on the pharmacokinetics of the newer antipsychotics, the half-life of aripiprazole is 75 hours (approximately 3 d) in individuals with normal cytochrome P450 2D6 (CYP 2D6) drug metabolizing enzymatic activity and 146 hours (approximately 6 d) in individuals genetically or phenotypically deficient in CYP 2D6 activity. (For more information on the role of CYP 2D6 enzymatic activity in drug metabolism, readers are referred to an earlier 2-part series on this topic.)

The general rule is that it takes 5 times the half-life to obtain 95% of the eventual steady-state concentration of the drug and a comparable amount of time to clear 95% of the drug. The genotype of the 76-year-old man in this case was not known. For this reason, the highest estimate (ie, half-life of 6 d) will be used to be conservative. Using this estimate, it would have taken 5×6 days to achieve a new and lower steady state concentration of the drug after a dose reduction.

As reviewed in the first column in this series, the research criteria for TD provided in Appendix B of the DSM-IV-TR indicate that TD typically occurs within 4 weeks of the discontinuation (or dose reduction) of the drug that was the putative cause. Four weeks is also consistent with the time course for individuals who are genotypically or phenotypically deficient in CYP 2D6 activity to develop a new steady-state concentration of aripiprazole after a dose reduction.

The dyskinetic movements in the case of the 76-year-old man presented in the first column in this series were attributed to either inadvertent exposure to 20 mg/d for 2 weeks or 10 mg/d for 4 weeks. If aripiprazole was the causative factor, then the dyskinetic movements should have occurred within 1 month of each dose reduction. Instead, the movements did not appear until 3 months after the reduction to 1 mg/d. That time-frame is inconsistent with the higher doses being causative in the case of that patient.

Although, based on this additional information, a good case can be made that the dyskinetic movements in the 76-year-old man were not causatively related to treatment with aripiprazole; this case involving aripiprazole and TD served as the impetus for this series of columns, the goal of which is to provide a broader discussion of the clinical relevance of class warnings in drug labels and what such warnings mean in terms of causation.

**CONCLUSIONS**

To summarize, aripiprazole has an intrinsic and unique ability to express “dopamine-serotonin stabilizing properties” on various brain receptors. This pharmacology may account for lower risks of EPS (including TD) and may also explain cases of improvement in TD following treatment with aripiprazole as will be discussed in the next column in this series. The first column in this series presented the case of a 76-year-old man who developed TD while being treated with aripiprazole and discussed
the incidence of TD and spontaneous orofacial dyskinesia as a function of age and treatment with selective D₂ receptor antagonists (eg, haloperidol). This column reviewed preclinical studies that suggest a low, if not absent, risk of TD with aripiprazole. These studies are not consistent with the development of TD in the 76-year-old man being the result of inadvertent treatment with higher than intended doses of aripiprazole. The next column in this series will review the systematic clinical trial and real-world data on aripiprazole to see whether they support the conclusion that aripiprazole has no appreciable risk of causing TD despite the fact that the package insert for aripiprazole contains the standard class warning for all antipsychotics which are almost all exclusively full D₂ antagonists in contrast to aripiprazole which is a D₅ partial agonist. The last column in this series will discuss the issue of class warnings and what they mean, as well as what type of study is generally required to waive a class warning and why such studies might not be done by the manufacturer of a drug.

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