ORIGINAL ARTICLE

Electrophysiological Neuroimaging Using sLORETA Comparing 22 Age-Matched Male and Female Schizophrenia Patients

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ABBREVIATIONS
EEG = electroencephalogram
fMRI = functional magnetic resonance imaging
LORETA = low resolution brain electromagnetic tomography
MEG = magnetoencephalogram
MNI = Montreal Neurological Institute
MRI = magnetic resonance imaging
PET = positron emission tomography
PK = pharmacokinetics
sLORETA = standardized low resolution electromagnetic tomography
SnPM = statistical non-parametric mapping

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ABSTRACT

BACKGROUND: Standardized low resolution brain electric tomography (sLORETA) is a validated neuroimaging method for localizing the electric activity in the brain based on multichannel surface electroencephalogram (EEG) recordings, having the benefit of improved time resolution of EEG measurements, better than that of functional magnetic resonance imaging (fMRI), and with a spatial resolution similar to that of fMRI.

OBJECTIVE: The purpose of this electrophysiological neuroimaging study was to provide a deeper mechanistic understanding of both olanzapine and risperidone pharmacodynamics relative to gender. In doing so, we age-matched 22 men and women and initially evaluated their resting-state EEG recordings and later used sLORETA to visualize the differences in brain activity amongst the two patient groups.

METHODS: In this investigation, EEG data were analyzed from male and female schizophrenia patients treated with either olanzapine or risperidone, both atypical antipsychotics, during their in-patient stay at the Department of Psychiatry. Twenty-two males and females were age-matched and EEG recordings were analyzed from 19 silver/silver chloride electrodes. Thirty-seconds of resting EEG were spectrally transformed into sLORETA. Three-dimensional statistical non-paramentric maps for the sLORETA global field power within each band were finally computed.

RESULTS: The results indicated that, relative to males patients, females schizophrenia patients had increased neuronal synchronization in delta frequency, slow-wave, EEG band located in the dorsolateral prefrontal cortex, within the middle frontal gyrus (t= -2.881, p <0.03580). These findings suggest that females experience greater dopamine receptor and serotonin receptor neuronal blockade relative to age-matched males. Further, our findings provided insight to the pharmacodynamics of second-generation antipsychotics olanzapine and risperidone.

CONCLUSION: When compared to male patients, female patients suffering from schizophrenia have dopamine and serotonin receptors that are blocked more readily than age-matched male schizophrenia patients. Clinically, this may translate into a quicker time to treatment-response in females as compared to male patients.

INTRODUCTION

Men and women are alike in many ways. However, it is clear that there are gender differences in in the basic medical science areas of reproductive anatomy, physiology, and in bio-chemistry. From a clinical laboratory perspective, measures of hemoglobin, estrogen, testosterone and more provide recommended threshold levels, often based on both age and gender. However, often times, when prescribing medication, dosage recommendations are often not clearly defined. Moreover, in the area of psychopharmacology, clinicians often titrate doses in hopes of ameliorating symptoms of psychosis and to achieve therapeutic benefits for their male and female patents, pre-scribed the same drug. From a neurophysiological perspective, are these differences able to be visualized or even quantified in the brain? There has been mounting evidence during both resting-state and cognitive-task related electroencephalograms (EEGs), suggesting that several regions in the cerebral cortex have been identified as dysfunctional in schizophrenia and other psychotic disorders.[1,2] Moreover, there has been an increasing interest in understanding the foundations of the resting-state EEG networks due to evidence that the variations are highly sensitive for diagnostic associations, in genetic, and in different cognitive states.[3]

For several decades, investigators have consistently observed increases in delta frequency band and theta frequency band activity in patients diagnosed with schizophrenia.[4–6] However, in general pharmacology and therapeutics, women experience a higher frequency of adverse drug reactions than men.[7] Moreover, gender differences in the metabolism of anti-psychotic drugs have been reported potentially related to gender-specific genetic differences in cytochrome P450 enzymes which may point to marked genetic polymorphisms.[8] In this investigation, we control for both gender and age in patients diagnosed with schizophrenia to identify electrophysiological neuroimaging differences of atypical antipsychotic drugs blocking both serotonin 5HT2A and dopamine D2 receptors. In order to achieve this, we used the standardized low resolution brain electric tomography software (sLORETA), a validated method for localizing the electric activity in the brain based on multichannel surface EEG recordings.[9,10] sLORETA has the benefit of improved time resolution of EEG measurements of milliseconds, which is 3-fold better than that of functional magnetic resonance imaging (fMRI), with spatial resolution which is similar to that of fMRI (~7 mm).

METHODS

PATIENTS

All patient data were obtained from a large database of EEGs from the Department of Psychiatry's Neurophysiol-ogy Study Laboratory of the Medical University of Lublin in Poland. All patients were diagnosed by a board-certified Psychiatrist using the diagnostic criteria from the World Health Organization's International Statistical Classification of Diseases and Related Health Problems (ICD-10) section on Mental, Behavioral, and Neurodevelopmental Disorders. Our study included 22 male and 22 female schizophrenia patients who were age-matched (mean age = 22 ± 6.6 years). Patient EEG recordings were then classified and categorized by both gender and the ICD-10 diagnosis. Similarly, each patient's birth-month was recorded in an effort to identify any associations of seasonality of birth psychiatric illness based on gender. Pharmacologically, all patients were medicated with second generation, atypical antipsychotics, including olanzapine (dose 5 – 20 mg/day) or risperidone (dose 1 – 4 mg/day). Both of these medications are known to block dopamine D2 and serotonin 5-HT2 receptors in the central nervous system and have shown tremendous success in the management of patients with schizophrenia. This research protocol was approved by the Bioethical Commission of the Medical University of Lublin.

EEG RECORDINGS

All patients were comfortably seated at a semi-recumbent position in a sound and light attenuated room, while 20 minutes or more of routine eyes-open and eyes-closed resting EEG data were recorded using the 19-channel EEG Analysis Station (EL-MIKO Medical, Poland) and silver-silver chloride (Ag/AgCl) electrodes. Patient EEG recordings were in accordance to the *international 10/20 system* with electrodes placed at positions identified as Fp2, F8, T8/T4, P8/T6, O2, F4, C4, P4, Fp1, F7, T7/T3, P7/T5, O1, F3, C3, P3, Fz, Cz, and Pz; letters F, T, C, P and O standing for frontal, temporal, central, parietal, and occipital lobes, respectively (N.B.: there exists no central lobe, letter C is used only for identification purposes, and letter z, standing for zero, refers to an electrode placed on the midline). Even numbers (2, 4, 6, 8) refer to electrode positions on the right hemisphere, whereas odd numbers (1, 3, 5, 7) refer to positions on the left hemisphere. Letter codes A, Pg and Fp identify the earlobes, nasopharyngeal and frontal polar sites respectively. The *nasion* (depressed area between the eyes, just above the nose bridge) and the *inion* (lower point of the skull at the back of the head) were the two anatomical landmarks used for the positioning of the EEG electrodes.

Electrodes were referenced to linked earlobes and imped-ances were kept below 5 kΩ. The data sampling rate was 250 Hz, and the acquired signals were filtered with a band-pass filter of 0.15–70 Hz after sampling. Prior to data analysis, artifact detection was performed, visually, to exclude eye-movements, head-movements, muscle-movements, and segments of de-creased alertness. EEG recordings were then exported using ELMIKO's EEG DigiTrak Analysis software to the ASCII format for later processing.

NEUROIMAGING – LORETA

Following the export of the ASCII formatted data from the ELMIKO EEG acquisition system, 30-seconds of eyes-open EEG signals were recomputed to the average reference. Subsequently, spectral analysis was performed for the same 30-seconds of artifact-free data of each ICD-10 diagnostic group. The cross-spectra were averaged across the 50% overlapping windows, which yielded 7 EEG frequency bands: delta (1.5–6 Hz), theta (6.5–8 Hz), alpha-1 (8.5–10 Hz), alpha-2 (10.5–12 Hz), beta-1 (12.5–18 Hz), beta-2 (18.5–21 Hz) and beta-3 (21.5–30 Hz).[11] Lastly, low resolution brain electromagnetic tomography (LORETA) was used to estimate the 3-dimensional intracerebral current density distribution.[9,10]

The three-dimensional intracerebral neuronal source activity, illustrating statistical non-parametric maps of neuronal activity, are derived from the 19-channel electrodes were assessed using LORETA.[10] When considering the available methodologies of detecting intracerebral activity, it is important to note that of all available published three-dimensional, discrete, distributed, linear EEG/Magnetoecephalography (MEG) tomography methods for solving the classic EEG inverse problem, LORETA has been identified to report the lowest localization error (to within 1 voxel resolution on average).[10] The source localization results from LORETA, even without utilizing an individual patient's MRI anatomical scans, has demonstrated that with as many as 16-electrodes, and using the approximate three-shell head model registered to the Talairach human brain,[12] localization accuracy of EEG is 10 mm, for worst cases.[13] By adding the expected localization error, as a result of the head model, the average error is not expected to exceed 2 to 3 cm in the final LORETA source localization results. LORETA inverse solutions are a model of the 3D distribution of electric neuronal activity, represented by adjacent voxels, has maximum synchrony relative to the orientation and strength between neighboring neuronal populations.[10] LORETA inverse solutions are restricted to 2394 voxels (spatial resolution = 7 mm) within cortical gray matter and hippocampi, as determined by the digitized Talairach and probability atlases of the Brain Imaging Centre, Montreal Neurological Institute (MNI305).

EEG electrode coordinates are derived from cross-registrations between spherical and head geometry.[14] Concerning the validity of the neuronal activation results, currently, LORETA has received robust theoretical and cross-modal validation from localization studies combining this method with both structural and fMRI, positron emission tomography (PET), visual and auditory event-related potentials, and well as intracranial recordings.[11]

In our investigation of the gender-based diagnostic (ICD-10) group differences, standardized LORETA (sLORETA)[10] was used to evaluate the pharmacodynamic differences of risperidone and olanzapine, both atypical neuroleptics, in patients being treated for schizophrenia. The sLORETA inverse solutions are constrained to the MNI152[15] template composed of 6239 cortical gray matter voxels at 5mm spatial resolution and provides tremendous accuracy in source localization.

The sLORETA software package was used as a new improvement relative to LORETA. With sLORETA, realistic scalp electrode coordinates are adapted to a 10/5 electrode system[16] and are registered to the Montreal Neurological Institute's MNI152[15] scalp, with a 12-parameter transformation followed by a spline solution that projects the electrodes onto the scalp with minimal distortion.[17] By transforming the electrode system, sLORETA provides a much more realistic head-surface based coordinate system. Moreover, the sLORETA transformation matrix for the inverse solution uses the electric potential lead field computed within the boundary element method applied to the MNI152 digitized structural MRI template.[18] Further, the identified sLORETA inverse solutions are reported on MNI152 template which is composed of 6239 cortical gray matter voxels at 5 mm with anatomical labels as Brodmann areas are also reported using MNI space, with correction to Talairach space.[19]

STATISTICAL ANALYSIS

The demographic differences between the patient groups were graphically represented using Microsoft Excel. The localization of the differences in activity between the groups included 22 male schizophrenia patients and 22 female schizophrenia patients was assessed by voxel-by-voxel non-paired t-tests of the LORETA images, based on the log-transformed power of the estimated electric current density, which resulted in t-statistic 3-dimensional images. In these images, cortical voxels of statistically significant differences were displayed as statistical parametric maps (SPMs) using a randomization strategy that determined the critical probability threshold values for the actually observed statistic with corrections for multiple testing.[20]

To visualize the global distributions of the t-test differences, for each band we computed the location of the mean center of gravity of all voxels with positive and negative t-values. To correct for multiple comparisons, a nonparametric single-threshold test was applied on the basis of the theory of randomization and on permutation tests.[20] The omnibus null hypothesis of no activation anywhere in the brain was rejected if at least one t-value (i.e. voxel, t-max) was above the critical threshold for p = 0.05, determined by 5,000 randomizations.[21]

RESULTS

The results of the standard low resolution brain electrotomography (sLORETA) statistics are provided in Table 1. These results of the brain mapping pathway, which is illustrated in Figure 1, indicate that 22 female schizophrenia patients exhibited greater neuronal synchrony along both the dopamine

TABLE 1. Patient group results of sLORETA analysis between the age-matched 22 schizophrenia male versus 22 schizophrenia female patients.

| sLORETA Brain Mapping Results	
Patient Group with the Statistically Significant Neuronal Synchronization	22 Female Schizophrenia Patients
Whole Brain Voxel-wise t-test Result	t= -2.881
p-Value	p=0.0358
Frequency Band	Delta (1.5 Hz – 6 Hz)
Neuroanatomical Locations(s)	Right Middle Frontal Gyrus (Dorsolateral Prefrontal Cortex)
Lobe	Frontal Lobe
Brodmann Area	10
Montreal Neurological Institute Coordinates	X=30, Y=55, Z=25
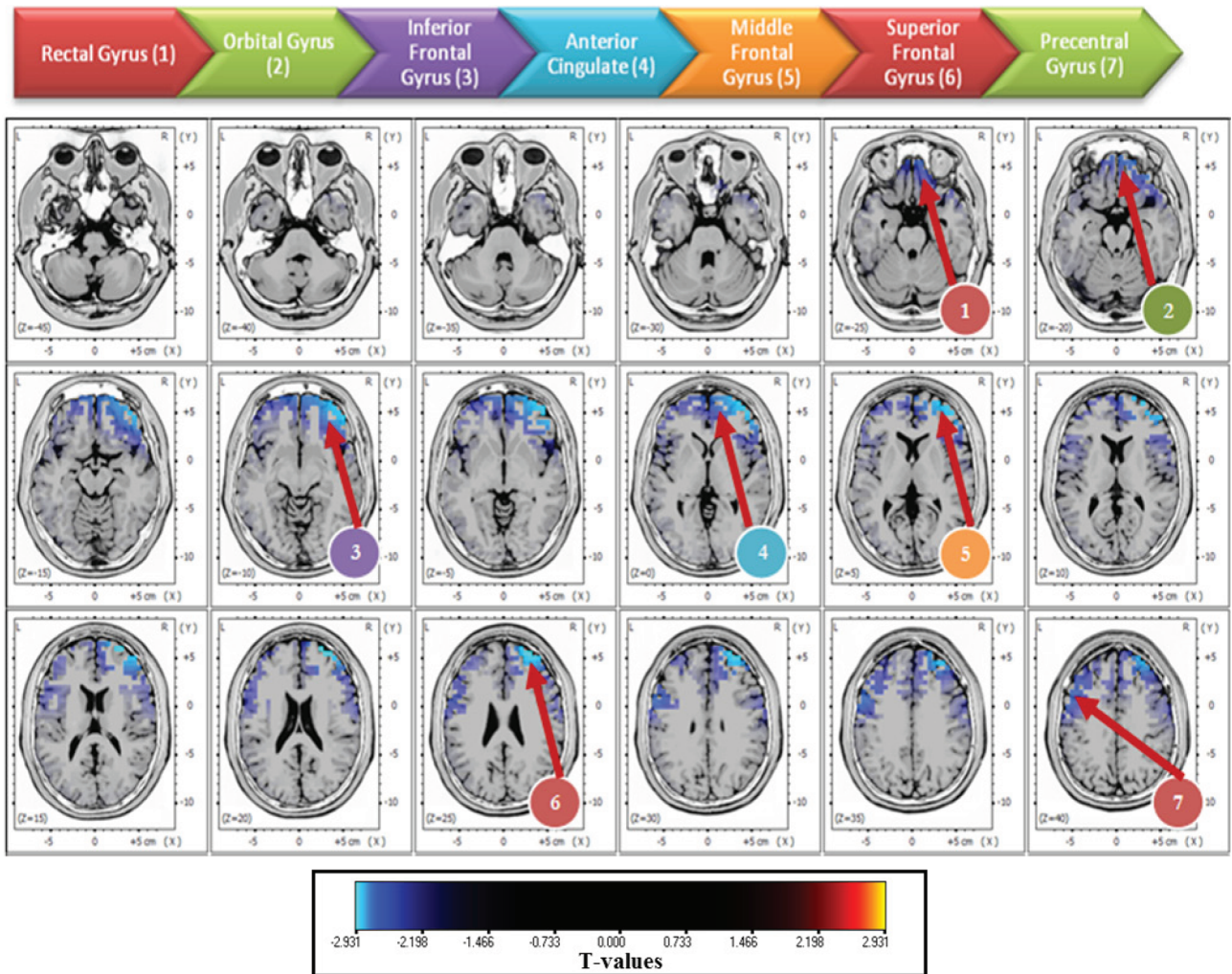
FIGURE 1. Atypical antipsychotic pathway of dopamine D2 and sertonin 5HT2 receptors. Blue colors indicate hypofunction of neurons in female schizophrenia patients compared to age-matched male schizophrenia patients.

(D2) and serotonin (5-HT2) containing neurons projecting from the rectal gyrus to the clinically relevant middle frontal gyrus. The neuroimaging findings, shown in Figure 2, provide axial, sagittal, and coronal neuroanatomical correlations for visualizing the pharmacodynamics of atypical antipsychotics in schizophrenia females versus males. Further evaluating the pharmacodynamics, relative to the 7 EEG frequency bands, the whole-brain voxel-by-voxel analysis provided statistically significant voxel differences, within the *delta frequency band* (1.5 Hz-6 Hz). Clinically, this illustrates a quieting/sedating effect of the atypical antipsychotics in the resting-state of the female patients more so than in the male patients. This provides further insight that men would generally require higher dosages, relative to age-matched females, to achieve similar pharmacodynamic results as would females at standard dosages.

The age-matched female patients showed slow-wave activation specifically in the dorsolateral prefrontal cortex (DLPFC), *right middle frontal gyrus* (t= -2.881, p=0.0358). The neuroanatomical location of the DLPFC provides tremendous clinical insight and activation along the dopaminergic and serotonergic pathway along the medial aspects of the brain to both clinical response and target locations during future drug discovery protocols. Generally, the results indicate that, relative to the age-matched males, females exhibited increased neuronal synchrony, to the same class of antipsychotics, when target-ing the same receptors (D2 and 5HT2), as shown in Figure 3. Moreover, our findings are fairly consistent with many studies
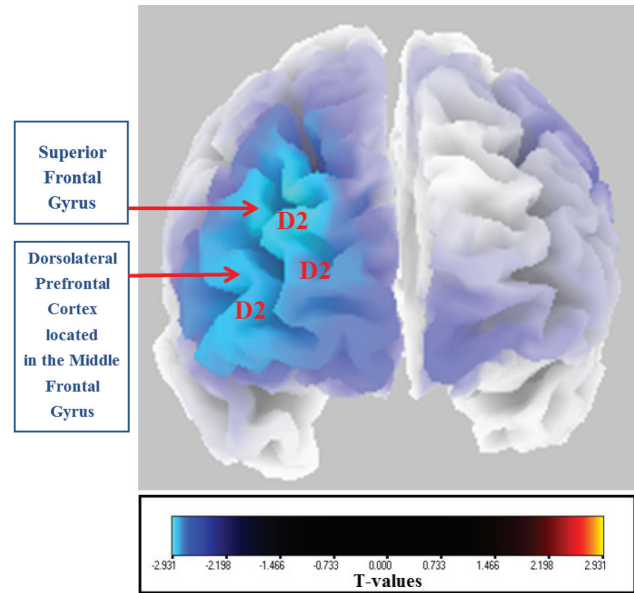

FIGURE 3. Resting state EEG neuroimaging findings illustrating dopamine D2 post-synaptic receptor blockade in female schizophrenia on medicated with atypical antipsychotics.
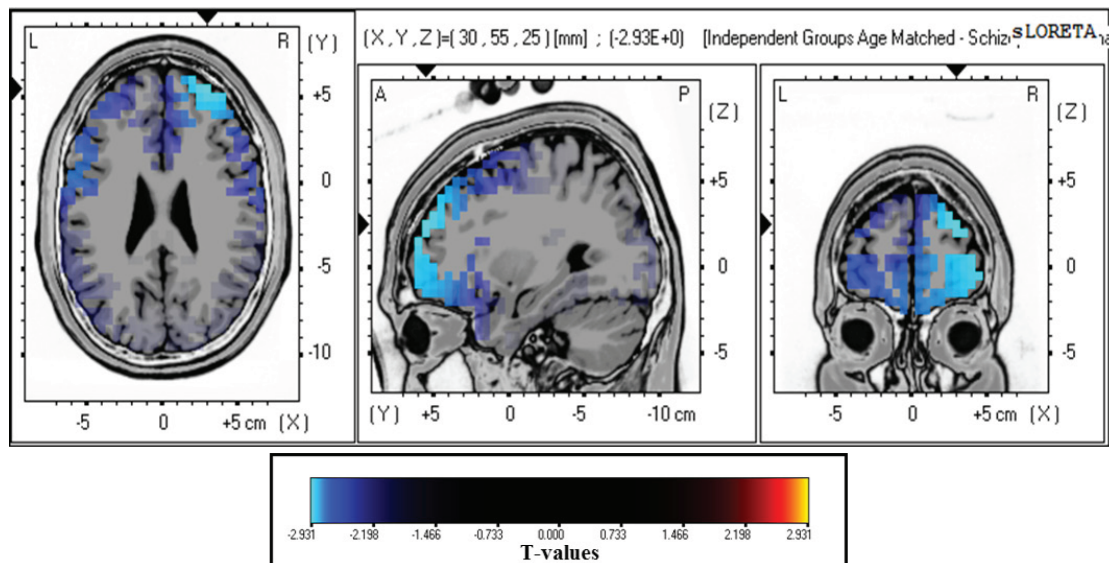

FIGURE 2. Resting state neuroimaging findings illustrating the action of atypical antipsychotics on post-synaptic dopamine D2 receptors following whole brain voxel-by-voxel non-paired t-statistic analysis that are based on the power of estimated electric current density statistical non-parametric map (SnPM) of sLORETA images in 22 schizophrenia females. The blue/cyan shades indicate decreased neuronal activity in the delta frequency band in the right middle frontal gyrus (Brodmann Area=10; MNI Coordinates (mm): X=30, Y=55, Z=25) along the dopamine mesocortical pathway. Structural anatomy is shown in grey scale (A: anterior; S: superior; P: posterior; L: left; R: right).

which have found an increase in current density in the delta frequency band in patients with schizophrenia in comparison to control groups, suggesting hypofrontality in female patients suffering from schizophrenia.[22–26] such as delta band activity, in specific brain regions are associated with psychotic symptoms. Data were obtained from 17 neuroleptic-naive patients with schizophrenia and age- and sex-matched 17 healthy control subjects. Low Resolution Brain Electromagnetic Tomography (LORETA Overall, the findings illustrate that second generation antipsychotic drugs block dopamine D2-receptors, more readily in female patients, relative to male patients, being administered similar dosing regimens in an inpatient psychiatric facility.

DISCUSSION

MALE VERSUS FEMALE PHARMACOKINETICS

In the National Institutes of Mental Health-funded Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) clinical trial that was focused on comparing the effectiveness of first-generation (available since the 1950s) antipsychotics to second-generation (available since the 1990s) antipsychotics used to treat schizophrenia found that in schizophrenia patients (n=406) treated with olanzapine, men cleared the drug 38% faster than women (P <0.0001).[27] These results were assessed using nonlinear mixed-effects modeling which determines population pharmacokinetics and suggests that our findings would provide a molecular imaging (D2 and 5-HT3 neuronal pathway) insight to the role of gender to the pharmacodynamics of second-generation antipsychotics.

In a another study conducted at two centers in Austria, comprising 129 patients, adjusted for 70-kg body weight, looking to evaluate the influence of age and gender on risperidone plasma concentrations, found that even when males where maintained at higher daily dosages, mean 5.3 + 2.0 mg/day (median = 6 mg/day) as compared to females dosed at 4.8 + 1.5 mg/day (median = 5 mg/day), plasma concentrations were nonetheless higher in female patients: male plasma risperidone levels 31.7 +18.8 ng/mL (median = 27 ng/mL) and female plasma risperidone levels 42.6 + 28.2 ng/mL (median = 35 ng/mL). These pharmacokinetic findings attest to the usefulness of sLORETA providing neuroimaging insight for when evaluating the pharmacodynamics of psychotropic medications.[28]

GENDER DIFFERENCES IN PHARMACODYNAMICS OF ATYPICAL ANTIPSYCHOTICS

In most studies using positron emission tomography (PET) scans for evaluating dopamine D2 receptor occupancy, clinical effects, therapeutic response, as well as extrapyramidal symptoms of atypical antipsychotic drugs, like risperidone and olanzapine, differences were found with that of first generation antipsychotic drugs.[29,30] Thus, in an effort to begin the next step in delivering personalized medicine based on pharmacodynamics and pharmacogenomics, a proper step forward would be to take gender into consideration in daily clinical practice as well as in therapeutic drug development.

As a group in schizophrenia patients, women have higher antipsychotic plasma levels than men after receiving the same dose of drug.[8] Moreover, based on our neuroimaging findings comparing male and female schizophrenia patients on similar atypical antipsychotic drug regimens, it appears that dopamine D2-receptor blockade is more pronounced in females as compared to males. These findings are consistent with previously published literature suggesting females requiring lower dosages of atypical antipsychotics drugs to suppress symptoms of psychosis.[31,32] Further, our findings may suggest that aims at drug discovery for new psychotropic medications may want to focus on brain activity localized to the middle frontal gyrus, in an effort of potentially identifying orphan-receptors from second/third generation neuroleptics.

TREATMENT MARKER OF THERAPEUTIC EFFICACY IN SCHIZOPHRENIA PATIENTS

A National Institutes of Mental Health (NIMH)-funded, double-blind randomized, clinical trial using fMRI evaluating 24 schizophrenia patients and 24 controls, identified that the right dorsolateral prefrontal cortex, as in our study, is a biomarker for improvement of psychotic symptoms.[33] A publication in Clinical Pharmacology & Therapeutics in 2001, with the lead author from the National Institutes of Health in Bethesda, Maryland defined a biological marker (biomarker) as, "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[34]

CONCLUSION

Based on the localization of brain activity relative to atypical antipsychotic drug therapy, we have identified a marker of therapeutic efficacy in patients with schizophrenia, corroborating the findings of several other studies. Use of EEG neuroimaging, provides cost benefit and broader mechanistic insight into the use and clinical utility of psychotropic medications and may be used in parallel with PET scans in both early and late phase clinical trials. EEG neuroimaging provides access to the special populations of people suffering from obesity, claustrophobia, or diagnosed with developmental delay or autism. Young children are able to move around with an EEG electrode cap without affecting the results. Moreover, patients with metallic particles from implants, tattoos, or war shrapnel who are often not able to have a fMRI may benefit, as well as those who cannot be exposed to the radiation of PET scans.

Electrophysiological neuroimaging may serve as a useful

tool for the pharmaceutical industry and academia when needing to add an extra dimensionality of evaluating the pharmacogenetic differences in patients who have are poor, intermediate, and rapid metabolizers based on cytochrome P450 single nucleotide polymorphisms. Lastly, from a clinical, daily practice, perspective mental health professionals may want to monitor female patients very closely for adverse effects, more specifically in the young and elderly populations. Likewise, clinicians may want to consider titrating dosages in male patients when symptomatic improvement is lagging relative to female patients. EEG neuroimaging may be utilized and databases of patient progress may be stored as an extra measure of research-based insight to the standard patient record. The overall efforts of the research and development arms of the pharmaceutical industry, mental health institutions, and academic medical centers globally are encouraging, all with the goal of improving the care and treatment of patients.

ACKNOWLEDGMENTS

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