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***rsfMRI* effects of KB220Z™ on Neural Pathways in Reward Circuitry of Abstinent Genotyped Heroin Addicts**

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Abstract

Recently Willuhn et al. reported that cocaine use and even non-substance related addictive behavior, increases, as dopaminergic function is reduced. Chronic cocaine exposure has been associated with decreases in D2/D3 receptors, also associated with lower activation to cues in occipital cortex and cerebellum in a recent PET study from Volkow's group. Therefore, treatment

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strategies, like dopamine agonist therapy, that might conserve dopamine function may be an interesting approach to relapse prevention in psychoactive drug and behavioral addictions. To this aim, we evaluated the effect of KB220ZTM on reward circuitry of ten heroin addicts undergoing protracted abstinence, an average 16.9 months. In a randomized placebo-controlled crossover study of KB220ZTM five subjects completed a triple blinded-experiment in which the subject, the person administering the treatment and the person evaluating the response to treatment were blinded as to which treatment any particular subject was receiving. In addition, nine subjects total were genotyped utilizing the GARS_{RX}TM test. We preliminarily report that KB220ZTM induced an increase in BOLD activation in caudate-accumbens-dopaminergic pathways compared to placebo following one-hour acute administration. Furthermore, KB220ZTM also reduced resting state activity in the putamen of abstinent heroin addicts. In the second phase of this pilot study of all ten abstinent heroin-dependent subjects, three brain regions of interest (ROIs) we observed to be significantly activated from resting state by KB220Z compared to placebo ($P < 0.05$). Increased functional connectivity was observed in a putative network that included the dorsal anterior cingulate, medial frontal gyrus, nucleus accumbens, posterior cingulate, occipital cortical areas and cerebellum. These results and other qEEG study results suggest a putative anti-craving/anti-relapse role for KB220Z in addiction by direct or indirect dopaminergic interaction. Due to small sample size, we caution definitive interpretation of these preliminary results and confirmation with additional research and ongoing rodent and human studies of KB220Z, is required.

Keywords

fMRI; Brain Reward circuitry; Dopamine; KB220Z; abstinent heroin addicts

INTRODUCTION

Previously others have found resting state functional connectivity patterns in heroin-dependent individuals that were significantly different from healthy subjects. There is evidence that such impairments in functional connectivity may negatively impact decision-making and inhibitory control.¹ Moreover, in earlier studies it was found that heroin addicts showed reduced activation in right amygdala in response to the affective pictures when compared to normal controls.² Other studies showed persistent abnormalities in brain function following one month of heroin withdrawal in the orbitofrontal cortex.³ Zijlstra et al. found lower baseline dopamine D2 receptor (D2R) availability in opiate-dependent subjects than controls in the left caudate nucleus.⁴ D2R availability in the putamen correlated negatively with years of opiate use. Opiate-dependent subjects demonstrated higher dopamine release after cue-exposure in the right putamen than controls. Chronic craving and anhedonia were positively correlated with altered DA release⁴ in psychoactive drug dependent subjects.

Understanding the true role of dopamine in escalated cocaine intake, for example, has been the subject of recent study by a number of investigators. Wise's group provided strong evidence that attenuated dopamine actions in the core (but not the shell) of accumbens results in greater cocaine intake and shorter inter-response times—during periods of self-administration.⁵ Conversely, they found that enhanced dopamine actions in the core (but not

the shell) resulted in decreased intake and longer inter-response times.⁶ In agreement with these findings Willuhn et al. surprisingly found that phasic dopamine decreased in ventro-medial striatum (VMS) as the rate of cocaine intake increased, with the decrement in dopamine in the VMS significantly correlated with the rate of escalation.⁷ Moreover, administration of the dopamine precursor L-DOPA, at a dose that replenished dopamine signaling in the VMS reversed escalation. Thus, this evidence demonstrates a cause and effect relationship between diminished dopamine transmission and excessive drug use. While this work is intriguing it may not be in conflict with surfeit rather than deficit theories of dopamine function^{8,9}. For example, Stewart suggests that the overall effect of widespread increases in phasic dopamine transmission is to precipitate drug-seeking behaviors, not to decrease it.⁸ In essence, these authors correctly suggested that during periods of abstinence dopamine receptors become supersensitive and if fact, relapse could even be linked to fatalities because of the higher euphoric response.

However it does seem to support dopamine-based agonistic modalities as suggested by Willuhn et al.⁷ Along these lines, Caprioli et al. correctly conclude that at present there is no FDA-approved medication for cocaine addiction.⁹ However, several clinical studies have suggested that agonist-based substitution treatment (for example, prescription oral amphetamine and methylphenidate) decreases illegal cocaine use.¹⁰ While we do not clinically agree with the amphetamine approach (chronically leading to down-regulation of D2 receptors as seen with other D2 agonists as well as the potential induction of relapse¹¹) it does point to the importance of dopamine agonist therapy compared to current therapies that utilize dopamine antagonistic therapy to treat cocaine escalation during protracted abstinence.

According to Volkow et al. drug addiction is characterized by a compulsive drive to take drugs despite serious negative consequences; it is a disorder that involves complex interactions between genetic and environmental variables.^{11,12} Undoubtedly heroin and other addictions are a complex phenomenon of the brain, involving both affective and cognitive processes.^{13,14} It has been found that in heroin-dependent individuals there is a decrease in white matter volume in the frontal area and decreased gray matter density in the bilateral prefrontal cortices and in the temporal regions compared to healthy subjects.¹⁵ In support, others consistently found differences in activation pattern between heroin-dependent subjects and healthy individuals during resting-state brain activities. These differences of activation patterns were found in the: orbitofrontal cortex (OFC); hippocampal/parahippocampal region; amygdala; caudate; putamen as well as the insula and thalamus.¹

Importantly, Elman et al. proposed a map consisting of four circuits involved in drug abuse and possibly reward behaviors: (1) reward, located in the nucleus accumbens (NAc) and ventral pallidum; (2) motivation/drive, located in the orbitofrontal cortex (OFC) and subcallosal cortex; (3) memory and learning, located in the amygdala and hippocampus; and (4) control, located in the prefrontal cortex and anterior cingulate gyrus (ACC).¹⁶ Simply stated but evidently even more complex, our current knowledge indicates that whereas aberrant craving behavior resides in the caudate-accumbens brain region, loss of control and thus relapse occurs in the cingulate gyrus as determined in cocaine abusers.¹⁷ Moreover,

Thanos et al. as well as Rothman et al. independently suggested that dopamine agonist therapy, by either increasing D2R availability and/or enhanced dopamine release respectively, could be useful therapeutic adjuncts for the treatment of cocaine and alcohol addictions,^{18,19} as well as for obesity, attention-deficit disorder, other Reward Deficiency Syndrome (RDS) behaviors and depression.²⁰ Indicating that higher dopaminergic activity could lead to reward seeking in the short-term, whereas, it must be noted that in the long term it is dopaminergic deficiency that can prevent recovery and lead to relapse.²¹

We hypothesized that the putative anti-craving/anti-relapse compound KB220ZTM as reviewed by Chen et al. may activate dopaminergic pathways as determined by *fMRI*.²² KB220ZTM is a neuroadaptagen consisting of amino-acid neurotransmitter precursors and enkephalinase-catecholamine-methyl-transferase (COMT) inhibition therapy called Neuroadaptagen Amino Acid Therapy (NAAT). Ongoing research on KB220ZTM repeatedly confirms the numerous clinical effects that ultimately result in significant benefits for victims having genetic antecedents for all addictive, compulsive and impulsive behaviors.²³ In earlier research using qEEG during protracted abstinence in psycho stimulant dependent subjects this compound showed an increase in both alpha and low beta bands in the OFC and cingulate gyrus overcoming qEEG abnormalities.²⁴

To this aim, we evaluated the role of KB220ZTM on reward circuitry in a triple-blinded-randomized-placebo-controlled-crossover study of 10 heroin addicts undergoing protracted abstinence (average 16.9 months). A triple blinded experiment is an experiment in which neither the subject, the person who administers the treatment nor the person evaluating the response to treatment knows which treatment any particular subject is receiving.

METHODS

IRB and Subjects

The experimental protocol was approved by Institutional Review Board of the Fourth Military University, China. Ten abstinent male heroin-dependent patients (HDP, right handed, age 33 ± 7.57 years, range 20–44 years) were enrolled from a local inpatient treatment research facility. All subjects signed an informed consent. All HDP volunteers met DSM-IV criteria for heroin dependence. They regularly used cigarettes and denied any psychotropic agent in the 3 months before *fMRI* scan. All HDP had a confirmed diagnosis of heroin dependency with mean abstinence from heroin of 16.0 ± 7.91 months (range 3–24 months), a negative test for morphine in urine analysis (reagent box produced by China Carrie City International Engineering Co.), and a negative HIV blood test. None of the HDP had a history of neurological illness or injury other than drug addiction. None of the subjects were taking psychoactive prescription drugs within 1 week of the *fMRI*. None of the subjects were previously exposed to a high magnetic field (Table 1).

Experimental Protocol

For the first part of the experiment we are reporting data on 5 of the 10 subjects who completed the cross-over study (Figure 1). The second phase analyzed data derived from all ten subjects (Figure 2) in terms of brain regions of interest (ROIs) relative to resting state

placebo and KB220ZTM treatment. The experimental protocol has been reported earlier by Zhang et al.¹ The *fMRI* research was carried out at Life Sciences Research Center, School of Life Sciences and Technology, Xidian University, Xi'an, Shaanxi, China.

Briefly, the experiment was carried out in a 3T GE (Medical Signa EXCITE) scanner. Prior to the functional run, a high-resolution structural image for each subject was acquired using three dimensional MRI sequences with a voxel size of 1 mm³ and with an axial Fast Spoiled Gradient Recalled (3D-FSGPR) (TR 500 ms, TE 7.7 ms, matrix 256×256, field of view 220 mm × 220 mm, 25 slices, 4 mm thickness, 1 mm inter-slice gap). A gradient echo T2*-weighted sequence with in-plane resolution of 3.75 mm×3.75 mm (TE 30 ms, TR 2 s, matrix 64×64, field of view 240 mm, and flip angle 90°) was also acquired. A total of 150 echo-planar volumes were acquired during the resting scan and functional image scanning lasted 5 min. Subjects were instructed to close their eyes but remain awake during the entire scanning procedure. After scanning, all of the subjects reported that they had remained awake during the full length of the scan.

Imaging data were preprocessed for motion and signal drift and analyzed using Statistical Parametric Mapping 5 (SPM5, <http://www.fil.ion.ucl.ac.uk/spm>). Images were first corrected for within scan acquisition time differences between slices (slice timing correction) and then realigned to the first volume to correct for interscan head motions. Upon reviewing the translation and rotation of the images, head movements greater than 1 mm or head rotations greater than 1° were discarded. The Multi-Voxel Pattern Analysis (MVPA) method is a straightforward application of pattern classification techniques to *fMRI* datasets, where the patterns to be classified are vectors of voxel activity values (BOLD signal).²⁵ In the current study, we used a novel variant of the “searchlight” (SL) approach (a form of decoding)²⁶ to select an appropriate set of voxels in order to define multivariate features as the input for the pattern classification analysis.²⁷ Following earlier reported procedures by Zhang et al., the training and test procedures were repeated 24 times.¹ Each test used feature vectors with k-1 different runs/subjects assigned as a training data set and a feature vector of a different run/subject assigned as the test dataset. The classifier accuracy was measured by the proportion of runs correctly classified for the central voxel (v_i). Thus, the mean classifier accuracy would yield vim by averaging all of the accuracies calculated for every fold of this k-fold cross-validation procedure. The same procedure was then repeated for the next spatial position at v_i . The mean for the decoding accuracy of each voxel was then used to create a 3-dimensional spatial map representing the decoding accuracy for each position v_i in the whole brain and would thus represent the statistical differences between the KB220Z compared to placebo groups.

It is noteworthy, that the SL procedure for the pattern analysis technique provides an indication of effect size independent of the amount of data, which in the case of the present work involves a preliminary set of test subjects. This means that the voxel activations are due to ‘activations’ across subjects and that they are statistically significant. Thus, from the maps themselves we obtain information regarding statistically significant spatial activation patterns. Since this is a pilot result, summary tables are not necessary and we prefer not to include these summary tables for now because they may even be misleading.

KB220Z™

Specifically, the patented product is comprised of the following ingredients in validated, evidence-based intake levels: Thiamine, 15 mg (1033% of Daily Value); Vitamin B6, 10 mg (500%); Chromium polynicotinate (as ChromeMate®) 200 mcg (166%); a fixed dose combination of amino acids and herbs called Synaptose™ (1.9grams), which contains DL-Phenylalanine, L-Tyrosine, Passion Flower Extract; a Metalloglycoside™ Complex containing Arabinogalactans, N-Acetylglucosamine, Astragalus, Aloe Vera, Frankincense Resin, White Pine Bark Extract, and Spirulina; Rhodiola (as RhodiGen™); L-Glutamine; 5-Hydroxytryptophan (5-HTP); Thiamine Hydrochloride; Pyroxidal-5-phosphate; Pyridoxine HCl. The matching placebo powder was manufactured by Cepham, Inc. (New Jersey). The product is safe and is exempt from IRB concerns. None of the patients took KB220Z or placebo during the 7 day period between testing.

Phase one of the *fMRI* experiment utilized a 2×2 design crossover comparing placebo against experimental KB220Z™ employing a randomized triple-blinded study design for 5 out of a total of ten HDP subjects undergoing protracted abstinence. Each of the five patients was randomly assigned to either the experimental or placebo group. The placebo was matched in cherry flavored inert powder that was subsequently mixed with Orange Juice (no Pulp). The KB220Z product was similarly mixed with Orange Juice. Each patient consumed the product (Placebo or Experimental) in the morning before breakfast and one hour prior to scanning. After 7 days the Experimental and the Placebo group were switched and *fMRI* tests were repeated on each patient. The acute dose for each patient was 24 grams in vehicle (before breakfast) and 1 hour prior to *fMRI* testing. In phase two of the experiment the remaining five subjects similarly participated in the crossover experiment. Like the first group they were tested then switched to either placebo or KB220Z and tested again after 7 days. The data collected was then sent blinded for statistical analysis. All patients were genotyped by means of a saliva genotyping collection tube.

Genotyping

A brief description of the genotyping methods for the polymorphisms to be assayed in this project follows. All methods are routinely performed in the Institute of Behavioral Genetics (IBG) laboratory at the University of Colorado, Boulder. One out of the 10 Chinese candidates was excluded from analysis due to poor Polymerase Chain Reaction (PCR) amplification. Details, including primer sequences and specific PCR conditions may be found in Anchordoquy et al.,²⁸ Haberstick and Smolen²⁹ and Haberstick et al.³⁰ Each patient was also genotyped for the following gene polymorphisms: **MAOA-VNTR**, **5HTTLPR**, **SLC6A3**, **DRD4**, **ANKKI- DRD2 TaqIA (rs1800497)** and the **COMT val¹⁵⁸met SNP (rs4680)**.

The dopamine transporter (DAT1, locus symbol SLC6A3)—This maps to 5p15.3 and contains a 40 base-pair Variable Number Tandem Repeat (VNTR) element consisting of 3–11 copies in the 3′ untranslated region (UTR) of the gene.³¹ The assay by Haberstick and Smolen is a modification of the method of Vandenbergh et al.^{29,31} Primer sequences were:

Forward- 5′-TGTGGTGTAGGGAACGGCCTGAG-3′; and

Reverse- 5'-CTTCCTGGAGGTCACGCT CAAGG-3'.

The dopamine D4 receptor (DRD4), which maps to 11p15.5, contains a 48 bp VNTR polymorphism in the third exon, which consists of 2–11 repeats.³²

The assay by Haberstick & Smolen is a modification of the method of Lerman, et al.^{29,33} Primer sequences were:

Forward- 5'-VIC -GCT CAT GCT GCT GCT CTA CTG GGC-3'; and

Reverse-5'-CTG CGG GTC TGC GGT GGA GTC TGG-3'.

Monoamine Oxidase A upstream VNTR (MAOA-uVNTR)—The MAOA gene, which maps to Xp11.3–11.4, contains a 30 bp VNTR in the 5' regulatory region of the gene which has been shown to affect expression; the MAOA-uVNTR assay is a modification of a published method of Sabol et al.³⁴ Primer sequences were:

Forward- 5'-ACAGCCTGACCG-TGGAGAAG-3'; and

Reverse- 5'-GAACGTGACGCTCCATTTCGGA-3'.

Serotonin Transporter-Linked Polymorphic region (5HTTLPR)—The serotonin transporter (5HTT, Locus Symbol SLC6A4), which maps to 17q11.1–17q12, contains a 43 bp insertion/deletion (ins/del) polymorphism in the 5' regulatory region of the gene.³⁵ Due to an error in sequencing this was originally thought to be a 44 bp deletion. The long variant (L) has approximately three times the basal activity of the short promoter (S) with the deletion.^{36,37} Primer sequences were:

Forward- 5'-6FAM - ATG CCA GCA CCT AAC CCC TAA TGT - 3';

Reverse- 5'-GGA CCG CAA GGT GGG CGG GA - 3'.

Hu et al. (2005) reported that a SNP (rs25531, A/G) in the Long form of 5HTTLPR may have functional significance.³⁸ The more common L_A allele is associated with the reported higher basal activity, whereas the less common L_G allele has transcriptional activity no greater than the S-form. The SNP rs25531 is assayed by incubating the full length PCR product with the restriction endonuclease MspI.

For all of the above VNTR and ins/del polymorphisms, PCR reactions contained approximately 20 ng of DNA, 10% DMSO, 1.8 mM MgCl₂, 200 μM deoxynucleotides, with 7'-deaza-2'-deoxyGTP substituted for one half of the dGTP, 400 nM forward and reverse primers and 1 unit of AmpliTaq Gold® polymerase, in a total volume of 20 μl.

Amplification was performed using touchdown PCR.³⁹ After amplification, an aliquot of PCR product was combined with loading buffer containing size standard (Genescan 1200 Liz) and analyzed with an ABI PRISM® 3130 Genetic Analyzer. Genotypes were scored by two investigators independently.

ANKKI-DRD2 TaqIA (rs1800497)—The gene encoding the dopamine D2 receptor maps to 11q23, and contains a polymorphic TaqI restriction endonuclease site in the 3' untranslated region of the gene. The A1 allele has been reported to reduce the amount of

receptor protein.⁴⁰ This SNP is done using a Taqman (5' Nuclease) assay.^{29, 30} Primer and probe sequences were:

Forward primer- 5'-GTGCAGCTCACTCCATCCT-3';

Reverse primer- 5'-GCAACACAGCCATCCTCAAAG-3';

A1 Probe- 5'-VIC-CCTGCCTTGACCAGC-NFQMGB-3';

A2 Probe- 5'-FAM-CTGCCTCGACCAGC-NFQMGB-3'.

COMT val¹⁵⁸met SNP (rs4680)—The gene encoding Catechol-O-methyltransferase (COMT) maps to 22q11.21 and codes for both the membrane-bound and soluble forms of the enzyme that metabolizes dopamine to 3-methoxy-4-hydroxyphenylethylamine.^{41,42} An A→G mutation results in a valine to methionine substitution at codons 158/108, respectively. This amino acid substitution has been associated with a four-fold reduction in enzymatic activity.⁴² The COMT SNP is assayed with a Taqman method.^{29,30} Primer and probe sequences were:

Forward Primer- 5'-TCGAGATCAACCCCGACTGT-3';

Reverse Primer- 5'-AACGGG-TCAGGCATGCA-3';

Val Probe- 5'-FAM-CCTTGTCCTTCACGCCAGCGA- NFQMGB-3';

Met Probe- 5'-VIC-ACCTTGTCCTTCATGCCAGCGAAAT- NFQMGB-3'.

Genetic Addiction Risk Score (GARS_{RX}TM) algorithm

In terms of genotyping data we have determined, based on literature review, that there are seven risk alleles involved in the six candidate genes studied in this patient population. To determine severity of the nine patients studied utilizing a modified genetic variants of the **GARS_{RX}TM** test (Dominion Diagnostics/IGENE) we calculated the percentage of prevalence of the risk alleles and provided an arbitrary severity score based on percentage of risk alleles present. Subjects that carry the following alleles: DRD2=A1; SLC6A3 (DAT) =10R; DRD4=3R or 7R; 5HTTLPR = L or L_A; MAO= 3R; and COMT=G. As depicted in table 2 Low Severity (LS) = 1–36%; Moderate Severity (MS) =37–50%, and High Severity (HS) = 51–100%.

RESULTS

Based on this analysis, all nine genotyped heroin subjects tested had at least one risk allele as represented by prevalence not frequencies: 11% (n=1) were HS, 56% (n=5) were MS, and 33% were LS (n=3). Therefore, using GARS_{RX}TM it was determined that 67 % of the Chinese HDP were at moderate-to-high risk for addictive behavior (Hardy-Weinberg Equilibrium). Of particular interest was the finding that that 56% of the HDP carried the DRD2 A1 allele (5/9) [see Table 2]. Our study revealed that all tested HDP's entering a residential treatment facility for Heroin addiction carried at least one risk allele.

Work in progress involves the exploration of *fMRI* research using KB220ZTM compared to placebo in a number of important studies. Figure 1 shows the resulting composite MVPA

maps from five subject scans. The additional five subjects were not averaged but showed similar results as evidenced from the individual subject maps. It is important to note here that the comparisons between groups rely on a MVPA searchlight strategy that identifies consistent features across subjects in the resting state datasets. Based on the five subjects, albeit a small sample size, the results suggest that compared to placebo KB220Z™ BOLD activates the accumbens–caudate of the abstinent heroin addict tested (see Figure 1). Moreover, we found that KB220Z™ might reduce the known hyper-excitability in the putamen as evidenced by the lower connectivity pattern including this region after KB220Z treatment (Figure 1). In preliminary data we have found that this complex activates brain reward pathways and compared to placebo produces significant differences in at least three brain regions (see Figure 2). The ROIs showing significant differences between placebo and KB220Z ($P < 0.05$) in *rsfMRI* included prefrontal, ventral striatal, occipital, posterior cingulate and cerebellar areas ($n=10$). We observed an increase functional connectivity in the nucleus accumbens and or surrounding striatal regions. Frontal regions showed an increased evidence of BOLD activity with KB220Z and occipital/cerebellar regions showed a reduction in BOLD activity (Figure 1 and 2). Specifically, prefrontal regions included dorsal anterior cingulate cortex (BA32), and bilateral (right and left hemisphere) portions of the superior and medial frontal gyrus (BA9). Occipital regions included bilateral middle occipital gyrus, lingual gyrus and cuneus (BA18). Cerebellar nuclei included BOLD voxels lying bilaterally in anterior and posterior lobes.

Moreover, voxels identified as active (or showing similar resting BOLD activation features) on maps are significant across subjects within each group. ROI analyses are based on registration to a standard atlas. As indicated in the text, the results are derived from a small number of subjects and therefore the data, while significant, are preliminary at this time.

DISCUSSION

Genotyping

It has been determined that when multiple RDS-associated genes are analyzed, such as the genes for serotonergic transporter (5HTTLPR), Dopamine D2 receptor (DRD2), Dopamine D4 receptor (DRD4), Dopamine transporter (DAT1), Catechol-o-methyl-transferase (COMT), and monoamine-oxidase (MOA), all subjects carried at least one risk allele [see figure 3]. It is important to realize that over the last two decades a major difficulty has been to eliminate spurious results due to poor unscreened controls for all RDS behaviors. Figure 3 at least shows the enormous amount of work reported in Pubmed in 2014. This however does not eliminate concerns regarding various associations but suggests that additional work in this area is warranted.

Blum et al.² in a paper related to the theories of “wanting” and “liking” for dopamine, discussed using Bayesian statistics showing that the DRD2 A1 allele had a predictive value of 74.4% for all Reward Deficiency Syndromes (RDS). Here, the subjects studied in this investigation had multiple drug abuse relapses and presented to in-patient residential treatment programs. The finding that 67% of these individuals had moderate-to-high GARS_{RX}™, whereas only 33% had lower positive GARS_{RX}™, indicates that pre-screening patients prior to enrolling in a treatment program could be beneficial for relapse-risk

assessment. Clinically, this will be important for understanding expectations of future success and the need for intensive treatment involving genomic solutions coupled with medical therapies, including bio-holistic therapies. It will also reduce guilt and denial when patients enter a chemical dependency program.

The GARS_{RX}TM experiment supports the understanding that identifying hypodopaminergic genotypes may be the best predictor of adult and adolescent drug abuse or other RDS behavior. These results are also consistent with a number of *fMRI* studies that show that the hypodopaminergic DRD2 A1 genotype leads to blunted responses that could lead to aberrant drug and/or food seeking behavior,⁴³ while the hyperdopaminergic A2 genotype serves as a protective factor against the development of drug disorders.⁴⁴

Albeit a small sample size, a further strength of this study is that only male subjects were used: males with hypodopaminergic functioning are more likely to abuse drugs that stimulate the mesocorticolimbic system than those with normal dopaminergic functioning. In contrast, females living in a negative environment (possibly irrespective of genotype) are at increased risk for using more drugs and even more types of drugs that increase their risk for RDS.⁴⁵ With this stated we must caution against any generalized interpretation in terms of gender.

This pilot study is in agreement with the work of Conner et al. confirming the importance of the cumulative effect of multiple genotypes coding for hypodopaminergic functioning, regardless of their genomic location, as a predictive method of drug use in males.⁴⁵ Moreover, it extends the current literature, by suggesting a simple method using genetic testing to classify risk behavior in male patients seeking in-patient residential treatment.

fMRI

Our present findings derived from this small pilot study shows a clear difference between placebo and KB220ZTM in terms of BOLD activation of the dopaminergic pathways of the caudate-accumbens area, which is encouraging. Due to the small subject number we cannot as yet determine statistical significance using more traditional approaches; as such the experiment will continue by adding additional heroin-dependent subjects. However, it is important that we did find statistical significance ($P < .05$) when we evaluated the ten subjects at baseline compared to KB220ZTM treatment in three important ROIs involved in addictive behaviors for both substance and non-substance seeking. This was in large part due to the use of the multivoxel pattern analysis approach that was able to identify similar BOLD activation patterns across subjects within each group.²⁶ In this pilot study we also observed an attenuation of the resultant hyperactivity in the putamen of abstinent heroin-dependent subjects. Currently, albeit knowing that there is a lower D2R availability in the putamen of abstinent heroin dependent subjects, we do not fully understand the mechanism by which KB220ZTM administration (post one-hour) induced an attenuation of this *hypo* state. This will be the subject of additional investigation and it may involve white matter synapses.

The present findings, although preliminary, speak to the ability of the KB220Z complex in modulating activity in a specific set of structures that are affected in drug dependence. Administration of KB220Z increased *rsfMRI* signal in a putative network involving dorsal

anterior cingulate cortex, medial prefrontal gyrus, accumbens and – somewhat surprisingly – the cerebellum. In addition to these regions, the posterior cingulate (retrosplenial cortex) was also significantly impacted by KB220Z. Based on the above structures it is likely that cognition and executive functions are modulated along with rewarding experiences with KB220Z. A search through the past and recent literature however will show that these same structures have been implicated in dependence and drug craving.⁴⁶ It is remarkable that in heroin dependent subjects there is a significant decrease in functional connectivity in dorsal anterior cingulate, medial frontal cortex, and bilateral cerebellum, and subregions of the occipital gyrus.⁴⁷ One could speculate, based on the present results, that administration of KB220Z counteracts reductions in connectivity in this specific network. In addition we find that BOLD activity in the accumbens is increased, which could provide the added effect of enhancing natural rewarding experiences (and diminishing the need for drug use). While not directly examined here, the role of the accumbens in rewarding experiences leads us to propose that this is a likely outcome. The assessment of reward efficacy should be included in the experimental design in future neuroimaging work.

In ongoing research we are also exploring the role of KB220Z compared to placebo in cue-induced craving behavior as well as the effect of KB220Z on white matter synapses in both rodents and humans. This additional experiment is important since the function and form of white matter synapses becomes increasingly important in disease. White vesicular neurotransmitter release has been known to be the preserve of gray matter. It is known that synaptic style release of glutamate occurs deep in white matter. As white matter is increasingly well-recognized as a substrate for disease, dysregulation of white matter synaptic transmission will play a role in RDS and a number of impulsive/compulsive/addictive behaviors.

Interestingly, current cocaine-dependent users show reductions in white matter (WM) integrity, especially in cortical regions associated with cognitive control that have been associated with inhibitory dysfunction. In a study by Bell et al. differences between the cocaine abstinent groups were observed bilaterally in the inferior longitudinal fasciculus, right anterior thalamic radiation, right ventral posterolateral nucleus of the thalamus, left superior corona radiata, superior longitudinal fasciculus bilaterally, right cingulum and the WM of the right precentral gyrus.⁴⁸ The findings suggest that specific white matter differences persist throughout abstinence while others, which are spatially distinct, discriminate as a function of abstinence duration. These differences may, therefore, represent brain changes that mark recovery from addiction. Similar findings have been found in PTSD-alcoholic veteran subjects from research in our laboratory.⁴⁹ Others have found that fractional anisotropy (FA) was significantly decreased in specific brain regions of the heroin-dependent patients ($P < 0.001$ uncorrected), including the frontal gyrus, the parietal lobule, the insula, and the corpus callosum. Thus, the presence of microstructural abnormality is found in the white matter of several brain regions of heroin-dependent patients.⁵⁰

Based on the current literature, and our pilot findings presented herein, we are poised to further evaluate the effect of KB220Z on microstructural disruption of white matter in heroin addicts revealed by diffusion tensor imaging (DTI). Certainly the coupling of BOLD activation of dopaminergic pathways in the caudate-accumbens; attenuation of abnormal

hyperactivity of the putamen in heroin-dependent subjects; and potential reduction of microstructural abnormalities in white matter by KB220Z should ultimately lead to a novel safe dopamine agonist for prevention, tertiary treatment and relapse attenuation in RDS victims, especially carriers of reward gene polymorphisms found in abstinent heroin addicts, having implications for both substance and non-substance seeking addictive behaviors.

It is known that dopamine agonist therapy has failed in treatment of addiction, because the administration of powerful D2 agonists like bromocriptine,⁵¹ apomorphine, as well as other D2 agonists will reduce D2 receptors chronically. This in turn is counterintuitive and will result in reinstatement of drug seeking behavior.^{23, 24, 52} The parsimonious approach would be to proliferate D2 receptors as well as regulating electrophysiology of the brain circuitry. Our earlier qEEG studies with KB220Z show this type of regulation in abstinent psycho stimulant abusers,²⁴ alcohol and heroin addicts.⁵³ The role of dopaminergic receptors as key in regulating a variety of RDS behaviors including cocaine and food has been further supported by work from Volkow's group.⁵⁴ Since 1978 we have been developing the concept of nutritional genetics with special emphasis on an amino-acid based enkephalinase approach to the treatment of alcohol [55],⁵⁵ cocaine,⁵⁶ opiates,⁵⁷ glucose,⁵⁸ and other addictions⁵⁹ pointing out the importance of dietary supplementation and the addictive brain which has served a basis for the current experiment.

CONCLUSION

Due to the pilot nature of this investigation, definitive interpretation must await larger triple blinded randomized placebo crossover controlled studies. Moreover, studies must include cue induced *fMRI* measurements to build an objective understanding of the mechanisms for the anti-craving actions of KB220ZTM, in support of 27 clinical trials confirming its effects.^{22, 23} Most importantly we are repeating this experiment in animal models particularly the evaluation of functional *rsfMRI* in rats utilizing a novel segmented atlas in both non-addicted and cocaine addicted rodents. KB220Z may have very complex effects in the reward circuitry of the brain. We have not teased out the entire mechanism of action as indicated previously. However, we are suggesting that following its interaction at multiple neurotransmitter signaling pathways, there is a putative net release of dopamine^{59, 60} which in the long-term provides benefit in attenuation of RDS behaviors⁶¹.

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Figure 1. Location of the nucleus accumbens in human CNS

Resting *fMRI* Data Analysis in the heroin users ($n = 5$) before and after KB220ZTM (SynaptoseTM) and placebo. Standard atlas shows the location of the nucleus accumbens (surrounding area) with significant increases in *rsfMRI* response with KB220Z. A modified figure was utilized in a review article by Blum et al.²³

Figure 2.
rsfMRI (1st Scan A – Before, the baseline) and *rsfMRI* (2nd Scan B - After); the circles defined the ROIs showing significant differences in the resting state (*rsfMRI*) brain activity.

Figure 3.
Reward Gene Publications as of 10/23/2014

Table 1

Demographic and clinical characteristics of male Chinese (n=10) heroin-dependent subjects.

Demographic	Heroin-dependent subject (mean/SD)
Age (years)	33.± 7.57
Duration of abstinence from heroin (months)	16. ± 7.91

Abbreviation: SD: standard Deviation.

Table 2

Genotyping data for each Chinese patient.

Subject	MAOAvNTR	5HTTLPR	5HTTLPR	SLC6A3	DRD4	DRD2	COMT	Any risk allele	SEVERITY * GARS
1	4R	S/L	S/L _A	10R/10R	4R/4R	A2/A2	A/A	POSITIVE	0.30-LS
2	3R	S/S	S/S	10R/10R	2R/4R	A1/A2	GAG	POSITIVE	0.38-MS
3	4R	S/S	S/S	10R/10R	3R/4R	A1/A2	G/G	POSITIVE	0.46-MS
4	3R	S/S	S/S	10R/10R	4R/6R	A2/A2	G/G	POSITIVE	0.38-MS
5	4R	S/S	S/S	10R/10R	4R/4R	A1/A2	A/G	POSITIVE	0.30-LS
6	3R	L/L	S/L _G	10R/10R	4R/4R	A1/A2	ND	POSITIVE	0.45 -MS
7	4R	L/L	L _A /L _G	10R/10R	4R/4R	A1/A2	A/G	POSITIVE	0.54 -HS
8	4R	S/S	S/S	10R/10R	4R/5R	A2/A2	A/G	POSITIVE	0.23-LS
9	3R	S/L	S/L _A	10R/10R	2R/4R	A2/A2	A/G	POSITIVE	0.46-MS