Effect of benzodiazepine addiction on genomes

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1. TITLE OF PROPOSED PROJECT

Genome wide study of transcriptional and epigenetic changes induces upon addiction to benzodiazepine and their inheritance.

2. RESEARCH AREA

Pharmacogenetics

3. PROJECT DIGEST

Some drugs that are available over the counter as well as those prescribed are known to produce side effects of addiction and dependency upon long-term use. Benzodiazepines are a class of drugs that have long been used for treatment of anxiety and sleep disorders. Since the start of their use, they have been known to produce dependency symptoms in patients that use it for more than a month. Upon withdrawal, they cause symptoms of anxiety, irritability, difficulty with concentration, sweating etc. Various studies have been directed to elucidate the mechanism behind this dependency and a number of reasons have been determined. However, a genome wide study for changes in the genome that result in this addiction has not been done yet. The purpose of this study will be to carry out a genome wide study to find out changes in gene expression induced by long-term use of these drugs and to identify the epigenetic factors leading to such changes. Carrying out such a study, will help us find new antagonists for these side effects. It will help us develop drugs that target and thus reverse this addiction in patients who have to use benzodiazepine for their medical condition. Moreover, the

inheritance of addiction to these drugs will be studied which will help vulnerable patients to take safety measures for their future generations.

1. Project Summary

The hypothesis of this study will be that addiction to benzodiazepine is caused by changes in gene expression due to epigenetics which can be transgenerational. The objectives will be to find changes in gene expression in mice exposed to benzodiazepine using microarray. These transcriptional changes will be correlated to epigenetic factors. Changes in DNA methylation of their promoters will be found using bisulfite sequencing. Various antibodies will be used against modified histones in ChIP analysis to determine chromatin remodeling around the differentially expressed genes. Moreover, heritability of these genomic changes will be analyzed for two generations of the treated mice.

2. Proposed Objectives

HYPOTHESIS/BASIS OF RESEARCH

Benzodiazepine addiction involves differential gene expression due to epigenetic variations which can be inherited to next generations.

RESEARCH OBJECTIVES

1. To find the changes in genome wide expression induced upon continuous use of benzodiazepine in mice.
2. To determine the epigenetic factors leading to the differential expression.

3. To identify the candidate genes that might be involved in addiction and further study their functions.
4. To observe the inheritance pattern of this addiction to next generations.

3. Introduction

Addiction to drugs as a result of over-dosage and repeated use is a common problem world-wide. Drugs that come under the class of benzodiazepine are commonly used to treat insomnia and anxiety [1]. Upon long-term use, they have been reported to produce adverse side effects of dependency and addiction [2, 3]. Some drug addictions have also been reported to be heritable. According to a survey conducted in Pakistan, benzodiazepine over-dosage was reported to be the most common reason for self-poisoning [4]. The exact mechanistic basis for its addiction and dependency is not yet known. Studying the mechanism and inheritance of this addiction will help vulnerable patients prevent and treat these side-effects and also to take precautionary measures for their future generations.

This study will help elucidate the mechanistic basis of the addiction towards benzodiazepine. The affects of addiction being persistent indicates that it may be due to genetic or epigenetic factors. A genome-wide study will be done to analyze changes in gene expression induced due to continuous consumption of benzodiazepine in mice. The inheritance pattern of its addiction will also be studied.

The objectives of this study will be to find the differential gene expression due to addiction to benzodiazepine and to identify the epigenetic factors

leading to these differences. The genes involved in addiction will be identified and further studied to understand how they actually function in causing addiction. Lastly, the inheritance pattern of this addiction to next generations will be determined.

4. A. Background

Benzodiazepine is one of the most frequently prescribed medication for anxiety and sleep disorders [5]. 25-76% of its users are estimated to be long-term users. Amongst them, 20-50% experience withdrawal symptoms when trying to cease its use after long-term use [6].

In a recent study done by Leonie and his colleagues, it was found that 50. 1% of the investigated 401 BZD users were long-term users and dependent on BZD[7].

Amongst a sample of 1079 patients in a survey conducted in New Zealand, 8. 1% of them were extensivebenzodiazepinesusers with a mean of about 3 years and 7 months. The users on a higher dose than the first prescribed dose were 39%. Out of 40 interviewed patients, 17% experienced withdrawal symptoms of varying severity [8].

Because physiological dependence can occur within 4-6 weeks of use, even in prescribed therapeutic dosage [9], prescription for longer than a month can pose the risk of developing dependency.

BZD with a short elimination half-life are known to cause more severe withdrawal and dependency symptoms than slowly eliminated BZD [10].

In a study by Carlos [11], 47% of the benzodiazepine users using the drug for more than a month developed dependence on it. Patients using short half-life benzodiazepines, higher doses and long-term users showed increased frequency of addiction.

Several aspects of benzodiazepine dependency have been studied and medicines have been developed to counteract these side effects. Inhibitory neurons in brain’s ventral tegmental area (VTA) down-regulate the firing rates of dopamine-producing neurons. Benzodiazepines inhibit and weaken these interneurons resulting in increased production of dopamine [12].

VTA interneurons contain a large number of receptors called GABA receptors. The effect of benzodiazepine on these interneurons is due to activation of a subtype of GABA receptors -alpha-1 subtype. Upon administration of benzodiazepine in two groups of mice, the firing rate of interneurons decreased, increasing the production of dopamine. Genetically modification inorder to prevent benzodiazepine from activating alpha-1 receptor prevented this neuron firing [13].

Administration on long-term induces changes in GABAA receptor complex, results in reduced sensitivity, thus requiring higher dosages [14]

Several antagonists have been developed to treat withdrawal syndromes associated with abrupt cessation of benzodiazepine. One such study on rat was done using 5-HT2 antagonists. Rats treated with diazepam for 14 days showed acute withdrawal symptoms, tested using social interaction paradigm and elevated puzzle. Pre-treatment with 5HT2 antagonists before

testing produced significant reduction in withdrawal anxiety levels which were comparable to control rats [15].

Administration of antidepressant agomelatine has been reported to reduce craving and improve relapse prognosis in benzodiazepine addicts. This effect may be due to anti-craving effects of agomelatine, or its property of receptor activation [16].

Antagonists for N-methyl-D-aspartate (NMDA), non-NMDA and metabotropic glutamate (mGlu) receptors have been shown to reduce addiction symptoms in rats. The expression NMDA receptors and phosphoinositide hydrolysis mediated by mGluR is increased in cerebrocortical area of these mice. Thus neuroadaptive responses are indicative of benzodiazepine dependence [17].

It has been hypothesized that abusive use of drugs leads to transcriptional changes in genes through different mechanisms. Modifications in the chromatin, actions of various transcription factors and function of non-coding RNAs are all thought to contribute to changes in the brain as a result of this exposure [18].

Repeated administration of cocaine induced differential gene regulation in mouse. In this study, the molecular pathways involved in this regulation were also found. Genome-wide study of the chromatin in mouse nucleus accumbens was done using CHiP and promoter microarray. ΔFosB and CREB were found to be two prominent cocaine-induced transcription factors, in this brain region. Moreover, the behavioural effects of cocaine were found to be significantly enhanced by Sirtuins (Sirt1 and Sirt2) [19, 20]

In another independent study, genome wide analysis (GWA) of SNPs associated with alcohol dependence was done by blah. Out of the 121 SNPS analysed, 19 were shown to be differentially expressed. Two closely related SNPs among them were found to be located on chromosome region 2q35 and have been linked to alcohol phenotypes. 9 SNPs were located in genes that have been reported to be associated with alcohol addiction [21].

A genome wide analysis of benzodiazepine dependent individuals has not been done yet. Knowing the differential expression in the entire genome will help us identify all the candidate genes that might be involved in causing dependence in patients after long-term use. This will direct us to the detailed mechanism of dependence and therefore its treatment in addicted patients. Moreover, drugs can be designed to inhibit the pathways that result in this addiction. Benzodiazepine can be administered along with these drugs thus preventing their side effects.

B. Research methodology: Phasing

Animals and reagents

12 C57 mice (6 males and 6 females) 10 weeks old will be obtained and housed for a week in three group. One group of mice will be injected intravenously with BZD with short half-life- alprazolam (Xanaxe). The second group with BZD with longer half-life- and the third control group with saline solution. This treatment will be carried out for 4 weeks.

Assay for withdrawal symptoms

After treatment, mice will be tested for symptoms of anxiety (indicative of addiction) by two methods:

- Social interaction test

In this test, mice will be placed in circular area. Their interaction with each other will be observed and given an interaction score based on time spent sniffing, grooming, touching, or crawling over each other.

- Elevated plus maze

A maze with two arms – open and close will be used. Anxiety in mice will be scored based on the time spent in each arm, exploring the risky open arm which will indicate their anxiety behavior.

Microarray analysis:

Mice from each group will be sacrificed and mRNA will be isolated from their brain tissue. Differently labeled cDNAs will be synthesized from control and treated mice. Differential expression of genes will be checked using microarray chip available for mouse genome. The differentially expressed genes will be studied computationally and their functions predicted. The candidate genes that might be involved in causing addiction in benzodiazepine treated mice will be identified.

Bisulfite sequencing:

The promoters of misregulated genes will be checked for changes in DNA methylation patterns using bisulfite sequencing. The results from treated and control mice will be compared.

Chromatin immunoprecipitation

Chip analysis will be used to determine whether changes in chromatin structure are involved in the differential regulation of the mice. Chip analysis will be done using antibodies against acetylated histone H3, H4, dimethylated H3K9 and H3K27. Immunoprecipitated DNA will be amplified and quantified by qRT-PCR using primers against the candidate genes.

RT-PCR

The expression of the candidate genes will be checked by isolating RNA from the treated and control mice followed by cDNA synthesis and thus quantification of gene expression.

Inheritance of differential expression

The male and female mice from the individual groups will be self-crossed and their offsprings will be analyzed for symptoms of anxiety as done for the parent mice. These symptoms will be correlated to changes in gene expression using microarray which might be inherited from their parents. This experiment will be done for two generations and thus inheritance pattern of benzodiazepine addiction will be analyzed.

C. References

1. http://www. ema. europa. eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003774. pdf
2. M Lader. Benzodiazepine dependence. Proq Neuropsychopharmacol Biol Psychiatry. 1984; 8(1): 85-95

3. CPO’brien. Benzodiazepine use, abuse and dependence. J Clin Psychiatry. 2005; 66 Suppl 2: 28-33
4. MMKhan, H Reza. Benzodiazepine self-poisoning in Pakistan: implications for prevention and harm reduction. J Pak Med Assoc. 1998; 48(10): 293-5
5. BPCharles, D Cristian. Pharmacotherapy of panic disorder. Neuropsychiatry Dis Treat. 2008; 4(4): 779-795.
6. L Michael, TG Marie, B Anna, C Carlo. High-dose benzodiazepine dependence: a qualitative studyof patients’ perception on cessation and withdrawal. BMC Psychiatry. 2015; 15: 116.
7. MLeoney et. Al. Correlates of benzodiazepine dependence in the Netherlands study of depression and anxiety. Addiction. 2012; 107(12): 2173-82.
8. AKhan, AR Hornblow, JW Walshe. Benzodiazepine dependence: a general practice survey. The New Zealand medical journal. 1981; 93(687): 19-21.
9. A David, MD Spiegel, JB Timothy. Benzodiazepine and exposure-based cognitive behavior therapies for panic disorder: conclusions from combined treatment trials. Am J Psychiatry. 1997; 154: 773-781.
10. WBarbara, RG Roland. Physical dependence on benzodiazepines: differences within the class. Drug and Alcohol Dependence. 1991; 29: 153- 156
11. C Carlos de las, S Emilio, F Juan de la. Benzodiazepines: more “ behavioural” addiction than dependence. Psychopharmacology. 2003; 167: 297-303.

12. KRTan, M Brown, C Yvon, C Luscher, et al. Neural basis for addictive properties of benzodiazepines. Nature. 2010; 463(7282): 769-74

13. Well-known mechanism underlies benzodiazepine addictive properties. Written by NIDA notes staff on April 19, 2012. Extracted on 25 May 2015 from: http://www. drugabuse. gov/news-events/nida-notes/2012/04/well-known-mechanism-underlies-benzodiazepines-addictive-properties

14. Feldman, B Lisa, Riva-Vazquez, A Rafael, Assessment and treatment of social anxiety disorder. Professional psychology: Research and practice. 2003; 34(4): 396-405.

15. DPBegg, KT Hallam, TR Norman. Attenuation of benzodiazepine withdrawal anxiety in the rat by serotonin antagonists. Behav Brain Res. 2005 Jun 20; 161(2): 286-90.

16. H Muller, F Seifert, JM Maler, J Kornhuber. Agomelatine reduces craving in benzodiazepine addicts: a follow-up examination of three patients. Singapore Med J. 2012 Nov; 53(11): e228-30

17. TMakoto, S Norifumi, S Tsutomu. Contribution of glutamate receptors to benzodiazepine withdrawal signs. Jpn. J. Pharmcol. 1999; 81: 1-6

18. JRAlfred, JN Eric. Transcriptional and Epigenetic Mechanisms of Addiction. Nat Rev Neurosci. 2011 Oct 12; 12(11): 623–637.

19. RWilliam, K Arvind, X guanghua, W Matthew, et al., Genome Wide Analysis of Chromatin Regulation by Cocaine Reveals a Novel Role for Sirtuins. Neuron. 2009 May 14; 62(3): 335–348.

20. TCBMathew, B Camilla, M Manuel, L Gwenael, et al., Drug-Driven AMPA Receptor Redistribution Mimicked by Selective Dopamine Neuron Stimulation. PLoS ONE. 2010; 5(12)

21. C Sven, et al., Genome wide association study of alcohol. Arch Gen Psychiatry. 2009; 66(7): 773-784

5. Impact

Benzodiazepine is a very commonly used drug for treating anxiety and sleep disorders. However, cases for addiction to these medicines have been reported very often. Elucidating the mechanism of addiction would help develop methods for preventing and treating it in vulnerable patients by synthesizing antagonists for the side effects of addiction.

Studying the inheritance of benzodiazepine addiction would help patients take necessary precautions for their next generations.