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Rhodium Interaction with Human NRG1 Gene of Schizophrenia

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Abstract - Rhodium (II) acetate [Rh2 (O2CCH3)4] could be used as an indicator for single nucleotide polymorphism (SNPs) involved in the onset of schizophrenia. Rhodium (Rh1) has affinity to make covalent interactions with neuregulin (NRG1) gene at SNPs mutation. Binding effects of Rh1 has been studied under different molar concentrations at different time periods. In this study we used Rh1 to evaluate its interaction with NRG1 gene in Schizophrenic patients of Pakistan. Rh-NRG1 adduct were amplified by PCR and visualized on agarose gel electrophoresis. Here

we show Rh1 binding with NRG1 gene was inhibited with increasing concentration ranges from 0.5 -3 μM. It has been noted that upon binding with NRG1 gene Rh1 decreased the mobility and intensity of the DNA bands. Noticeably Rh1 didn't inhibit the activity of Mun1 restriction enzyme having specific CAAA cleavage site. After the digestion of NRG1 gene having SNPs mutation combining with Rh1 proves its covalent binding only with Guanine or Thymine and not with Adenine or Cytosine. This is a novel study that shows rhodium can covalently binds with human dsDNA and can inhibit its amplification. The effect of Rh1 to target different SNPs mutations (normally occurs in genetic diseases such as schizophrenia) can be identified by using this technique. There are variations between human populations, so a SNP allele that is common in one geographical or ethnic group may be much rarer in another, and Rh1 can act as a useful tool to identify SNPs of schizophrenic genes.

Keywords - Pakistani Population, Schizophrenia, Single Nucleotide polymorphism (SNP), Neuregulin (NRG1), Rhodium (Rh)

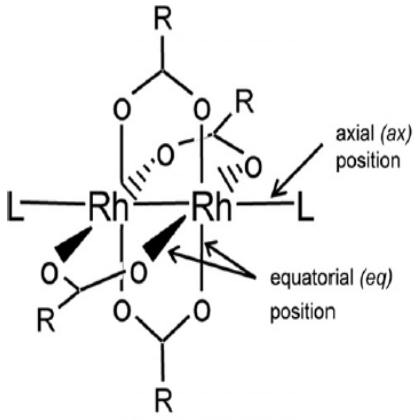
INTRODUCTION

Schizophrenia is a chronic, severe, and multifactorial brain disorder. NRG1 gene with SNPs mutation shows the increase risk for schizophrenia. Neuregulin (NRG1) is the strongest leading schizophrenia susceptibility gene. NRG1 was first implicated in schizophrenia in an Icelandic population (Stefansson, H, et al., 2002).Human NRG1 gene is located on chromosome 8p22-13 and is approximately 1.2 Mb long including more than 30 exons and several large introns (Paul J.H., Amanda, J.L., 2006). Recently 13 SNPs have been typed for schizophrenia in NRG1 gene(M Gardner, et al., 2006; Amanda J. Law et al., 2006) Previous studies have shown DNA

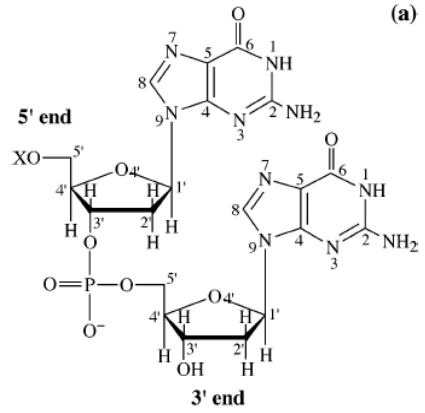
interactions with heavy metals such as zinc (Zn), rhodium (Rh) and copper (Cu) (Katsuyuki, A. Abdus, S., 2002; Rahman M.d.M, et al., 2008) Early studies have demonstrated that interaction of rhodium occurred only with poly Adenine but not with poly Guanine or poly Cytosine (J.L. Bear Jr, et al., 1975; J.L. Bear 1986; E. Tselepi-Kalouli, et al., 1990). Studies done on Rh using L121 cell line have revealed that rhodium could be used as an anti-tumor metal when they form adduct with carboxylate (R.A. Howard, et al., 1979).Metal based anti-tumor drugs play very beneficial role in identifying biological activities (Helen, T, et al., 2003), such as to detect mismatch base pairs, to identify single nucleotide polymorphism (SNPs) mutations and in cancers. Adducts are also important for understanding the mechanism of the biological activities of antipsychotic agents. Recently it has been shown that Rh has the ability to recognized mismatch base pairs in double stranded DNA molecule (dsDNA) and detected SNPs within the genome. This provided the general approach to the discovery of SNPs in amplified regions of the dsDNA (Jonathan, R.H, et al., 2004; Henrik Junicke, et al., 2003).

The purpose of this study was to explore rhodium's covalent attachment with NRG1 gene. Since rhodium has ability to target double stranded DNA by forming various covalent adducts, our study can be useful in the synthesis of rhodium based antipsychotics drugs for the treatment of schizophrenia. In this study we have selected the single nucleotide polymorphism (SNPs) primer (rs3924999, G38A) for NRG1 gene (Yang, J.Z, et al., 2003). Interaction between NRG1 and rhodium acetate were investigated under different molar ratio at different time durations As detect rhodium binding effect with NRG1 gene, Mun1 restriction enzyme was used to cleaved NRG1 gene and to check the binding of rhodium whether it bound with G, C or A.

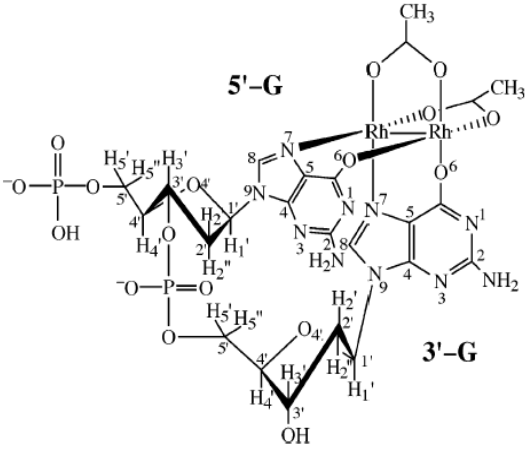
The purpose of this study is to explore rhodium's covalent attachment with NRG1 gene. As rhodium has ability to target double stranded DNA by forming various covalent adducts. Our study can be useful in the synthesis of rhodium based antipsychotics drugs for the treatment of schizophrenia.



Rhodium $Rh_2(O_2CR)_4L_2$



DNA Base (Guanine)



Rhodium Guanine Adduct

Fig. 1. Structure and atom numbering of Rhodium adduct (Helen, et al., 2003)

Experimental Method

DNA extraction

Venous blood samples werecollected from identified subjects after getting human ethic approval from Jinnah Post Graduate Medical Centre (JPMC) hospital. The samples were stored in 3 % EDTA solution in falcon tubes for 3 hrs. Genomic DNA was extracted using the phenol–chloroform method as described previously (Debomoy K. Lahiri and Bill Schnabel. 1993). Concentration of DNA was determined by using UV absorbance at 260 and 280 nm. The ratio of 260/280 nm was 1.76 which is close to 1.8.

Preparation of Rh (l) adduct with Human NRG1 gene

The isolated human DNA was combined with rhodium. Rhodium acetate was purchased from (MERCK& Co. Inc., U.S). NRG1 gene was prepared for Rh(1) binding at the concentration of μM (5, 3, 1, 0.5, 0.1) and nM (10, 5, 1, 0.5, 0.3 and 0.1) were dissolved in 1mM sodium phosphate buffer at PH 7 containing 3mM $NaCl_2$. The reaction mixture was incubated at 37 °C for 24, 48 and 72 hours in dark as to protect from light-induced disturbance during the incubation (Md. Masudur Rahman, et al., 2007).

Preparations of PCR mixture for Rhodium treated DNA adduct

We used two sets of DNA sample. One set was treated with rhodium but not used for PCR to serve as control. The sequence of the primer was as follows for the rs3924999: Forward primer sequence are 5`ACTGGTTTCACACCGAAGGAC 3`, and reverse sequence 5` CCAAGATGAGATCCATTTTCGC 3` (Yang et al., 2003)While other set was treated with Rhodium and used for PCR reaction, we have used SNPs primer (rs3924999) for PCR reaction. PCR reactions were performed in 0.2 ml PCR tubes contained a reaction volume of 50ul (1x PCR buffer, 1.5mM $MgCl_2$, 10mM primer forward and reverse, 100uM of dNTPs, 3 U of Taq polymerase and 200ng/ul template of human genomic DNA). PCR conditions used for amplification of NRG1

gene includes an initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, 55–62°C for 40 sec, 72°C for 1 min, and a final elongation at 72°C for 10 min. rs3924999 was designed to amplify 246bp. After the PCR amplification these samples were run on gel to determine the change in mobility and intensity of the DNA adduct. All the DNA samples were incubated with Rhodium adduct for 24, 48 and 72 hrs and the binding reaction were carried out with IμM DNA and plus Rh at different molar concentration as described earlier.

Digestion of the adducts by restriction enzymes

The digestion reaction was carried out by incubating the PCR adduct product SNP rs3924999) samples with MunI restriction enzyme at 37°C for 4 hrs and Tru1I at 55°C for 4 hrs, both enzymes were purchased from Fermentas (International Inc, Canada). The PCR adduct products were run in 2% (w/v) agarose gel in tris borate EDTA (TBE) buffer. The gel electrophoresis was carried out at 80 V for 1 hr and 45 minutes and washed with Milli Q water for 10 minutes to visualize the DNA The gel photographs were taken by Gel Doc system (Alpha Innotech Flour Chem™).

RESULTS AND DISCUSSION

Rhodium metal was used to make adduct by combining it with genomic DNA and NRG1 gene as to see whether these adducts changes their inhibitory properties (DNA replication, DNA band intensity and mobility) with time and concentration. The effects of these adduct with non-amplified, amplified and digested products were investigated. We observed slow DNA bands movement in non-amplified adduct with an increase rhodium concentration (from 10 nM to 5 μM). There were no changes found in the DNA band intensity after 24 hrs. incubation (Fig. 2a). However, longer incubation (72 hrs) with high levels of Rh-acetate leads to decreased band intensity (Fig. 2b). These results clearly illustrated the potency of Rhodium's inhibitory actions that start at 1nM and can reach maximally at 5μM. This indicates a time dependent Rh binding affinity towards the Neuregulin gene 1. On the other hand only decreased band intensity was observed in amplified

adduct after 24 hrs of incubation with Rh-acetate with no changes in band movements (Fig. 3a). Present result shows an inhibition of DNA bands with increasing concentration of Rh-acetate. For example bands strength decreases from 0.1 nM - 100 nM concentrations and gradually disappears at 0.5 μM – 5 μM after 48 hrs of incubation (Fig. 3b). Complete disappearance of the bands were observed after 72 hrs of incubation at 5 nM and onwards (Fig 3c). Amplified adducts were digested with restriction enzyme Mun1 that has a capability to cleave the DNA at CAAA sequence. The result showed bands after digestion with Mun1 from 0.1 nM to 3 nM confirmed the binding of Rhodium with G and not with C or A (Fig. 4).

DNA-Rhodium adduct samples (Without PCR)

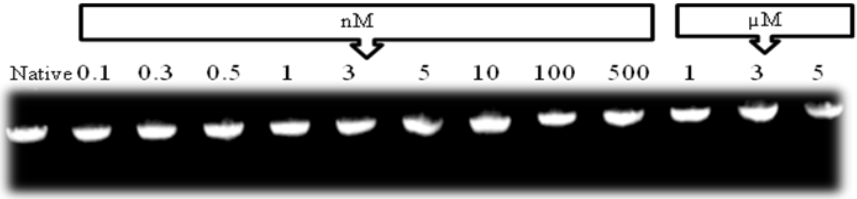


Fig. 2a. 24 hrs incubated samples without PCR

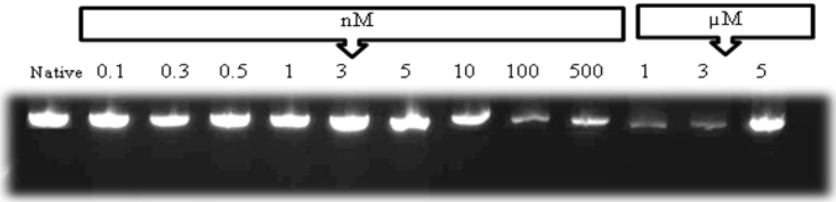


Fig. 2b. 72 hrs incubated samples without PCR
Native = DNA without treated Rhodium

Fig. 2(a & b). Agarose gel electrophoresis exhibit interaction between rhodium acetate and genomic DNA (non amplified) in TBE buffer at pH 8, incubated for 24hrs at 37°C. Lane 1 untreated (Native) DNA, lanes 2 to 12 are for rhodium DNA complex at different concentrations.

DNA-Rhodium adduct samples with PCR

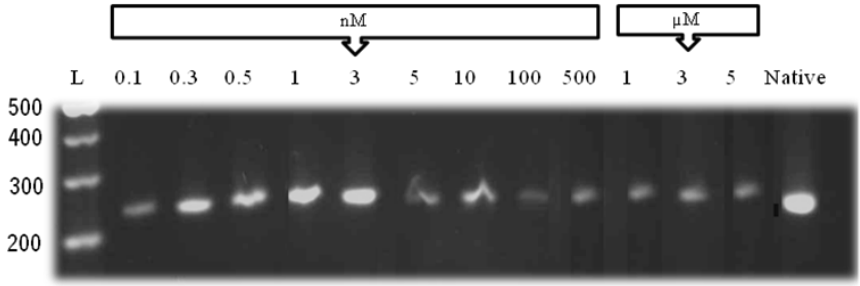


Fig. 3a. After 24 hrs incubation with PCR samples (SNP rs3924999)

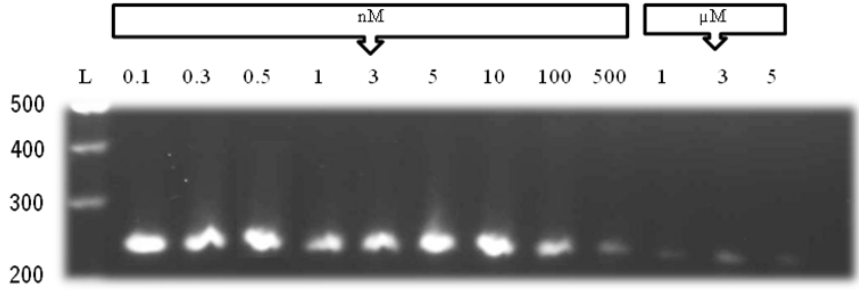


Fig. 3b. After 48 hrs of incubation

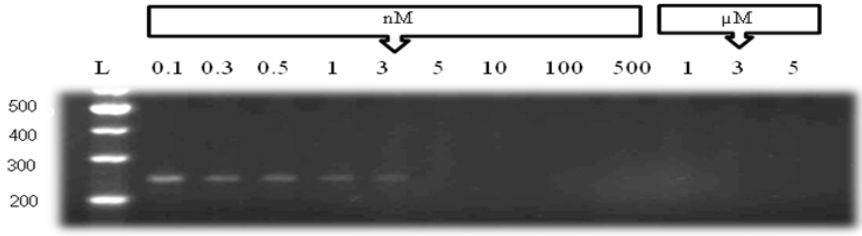


Fig. 3c. 72 hrs incubated PCR samples
L= ladder (Known sequence marker)
Native = DNA without treated with Rhodium

Fig. 3 (a, b & c) 2% agarose gel of the PCR product (246bp) of NRG1 gene. Lane L corresponds to molecular marker of 100 bp; Native lane indicates the amplified product of NRG1 without treated Rh. Lane 2 to 12 rhodium treated amplified product of NRG1 gene.

Restriction Digestion

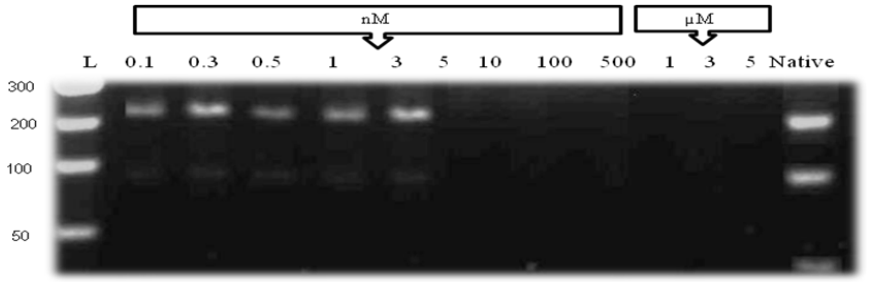


Fig. 4. Digested PCR product after 72 hrs incubation
L = ladder (Known sequence of DNA)
Native = DNA without treated Rhodium

In Fig.4 Rhodium treated amplified product (72hrs incubated) were digested with Mun1 restriction enzyme. Lane 1 to 12 exhibits rhodium acetate treated samples while lane 13 shows native sample (without rhodium).

Rhodium is a transitional metal and a member of platinum group. Previous studies have revealed the interaction of Rh1 form complexes with plasmid DNA and confirmed their binding effects on the mobility dependent on conformations (H.M. Ushay, et al., 1981; S.E. Sherman, S.J. Lippard., 1987; G.L. Cohen, t al., 1979). Rh upon binding with the plasmid DNA changes the circular closed confirmation (CCC) to the open confirmation (OC) with decreased mobility in gel electrophoresis. As Rh has ability to make covalent bond with the nucleotides many studies shows that Rh1 can be used as an anti-tumor agent for the treatment of cancer (R.A. Howard, et al., 1979). Jonathan and colleagues 2004 has revealed that Rh1 is the best target agent for the detection of mismatch base pairs in genomic DNA (Helen, T, et al., 2003). Earlier studies on Rh also revealed that Rh is used to induce inhibitory effects on DNA amplification by using plasmid DNA (Md. Masudur Rahman, et al., 2007). This can be taken in account for high binding affinity of the metal with DNA regardless of the source (plasmid, genomic DNA or oligonucleotides).

The present study emphasize on the time dependent reaction of Rh binding with human NRG1 gene period. Further study has

extended to visualize its binding effects on the replication of NRG1 and on endonuclease activity. Here two types of Rh-NRG1 adduct has been prepared. The first group contains a non amplified NRG1 gene whereas; the second group contains an amplified NRG1 gene. Both groups received same treatments including incubation times (24, 48 and 72hrs) and Rh concentration (0.1 nM – 5 μM).

Gel electrophoresis was carried out for the visualization of the Rh and NRG1 interaction at different concentratiheon and incubation time. Representative gel images shown are in Fig.2 in which the Rh -DNA adduct was allowed to move in electric filed toward anode, the mobility was slow (Fig. 2a and 2b) and band intensity decreased. These results indicated that Rhodium binding with genomic DNA increases with increase the Rh1 concentration and reaction time (24 and 72 hrs). This study suggests at Rhodium acetate complex has positive charge which neutralized the negatively charged PO_4 group of the DNA there by resulting in stable complex formation and decreased mobility as described in previous study (Ali Arslantas, et al., 2007).

Fig.3 shows that 246bp amplified product of NRG1 gene was inhibited by Rh1 at different concentrations. Amount of the amplified product of NRG1 gene decreased with increased the amount of Rh1 at different reaction times. Fig.3a 24 hrs incubated PCR adduct rhodium slightly affect the DNA replication but changes the mobility and intensity of DNA bands with increasing Rhodium concentration. Fig.3b shows the 48hrs incubation bands from 0.l up to 100nM concentration, which get lighter and gradually disappeared at 500nM onwards, indicating that DNA amplification is inhibited with increasing incubation time. In fig.3c after 72 hrs incubated samples the amplification was inhibited by rhodium from lower concentration of 5nM onwards and gradually disappeared. In this study we also investigated the interaction of human genomic DNA with Rhodium acetate and found covalent interaction with DNA at nucleotide mismatch site (cytosine and adenine) when digested with MunI enzyme which acts on cutting sites CAAA. Our results exhibited heterozygous mutation on Agarose gel showing 3 bands (one allele mutation) instead of 4 bans (two allele mutations) confirmed that Rhodium didn't have binding site for adenine and cytosine. It seems that rhodium bind with specific bases of the DNA and inhibited PCR amplification. These results confirm

earlier experiments indicating that rhodium acetate bind with guanine rather than adenine and cytosine (Mun1I has binding site at CAAA). Literature indicates when rhodium bind with DNA it prevents the DNA to replicate as it interacts with N7 and O6 of guanine forming the rhodium adduct. This adduct breaks the hydrogen bond especially at O6 position in the DNA structure (10).

This study clearly demonstrated that Rh complexes neutralized the negative charge DNA which further inhibited the DNA amplification due to covalent bond formation with guanine or cytosine bases at position N7 and O6.

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