A study on the agents that reduce the nicotine induced nicotinic receptor density in wistar rats

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ABSTRACT

The most important substance causing addiction towards cigarette is nicotine. Nicotine abstinence causes withdrawal symptoms in smokers. It is not just nicotine, along with it is the upregulation of nicotinic receptor density (NRD) that leads to addiction. All together makes nicotine deaddiction the most difficult aspect. Nicotine receptor density increases as long as the person is exposed to nicotine. When once the NRD is initiated by nicotine, later though you stop smoking, the increased nicotine receptors create an urge to smoke. Hence the person feels to smoke for satisfying the nicotine receptors. The smokers may attempt to quit smoking but the NRD will create an urge for nicotine again. One cannot completely quit smoking or cannot stop taking nicotine, until the NRD is reduced to normal. In our present study we have studied the effect of citric acid and tyrosine on decreasing nicotinic receptor density. We have induced the nicotinic receptor density to raise and studied the citric acid and tyrosine's effect in maintaining the NRD closer to normal. The study concludes that citric acid and tyrosine have reduced the NRD significantly. This can control withdrawal symptoms and can stop craving for nicotine and finally can lead to cessation of smoking and from taking nicotine therapy.

INTRODUCTION

Nicotine is obtained from tobacco. It acts on the CNS to stimulate pleasure. It acts as an acetylcholine agonist and triggers the acetylcholine nicotinic receptors which in turn releases the neurotransmitter dopamine. The feel-good hormone dopamine gives pleasurable feeling (Cherek et al., 1982). The continuous exposure to nicotine makes neuroadaptation. This leads to nicotine-mediated desensitization of receptors. Unresponsiveness of receptor causes tolerance and dependence. When the nicotinic cholinergic receptors become responsive, this leads to the symptoms of craving and withdrawal. During this process, the nicotinic receptor density increases, as long as the density does not decreases the person finds it difficult to quit smoking (Lukas et al., 1999).

Few earlier studies have shown the effectiveness of the CA and TY Against nicotine toxicity and dopamine levels, Hence, present study was designed. CA a week acid present abundantly in citrusous fruits. There are many studies in 80’s saying the effect of CA on urine and blood alkalinity in making the person stop smoking. Similarly, there is scientific evidence about the effectiveness and role of

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TY in the synthesis of dopamine. A study explains how certain drugs affect the autonomic nervous system and TY in the presence of tyrosine hydroxylase producing dopa and dopamine in the brain (Chiara and Imperato, 1988). There is an interaction of nicotine with nicotinic acetylcholine receptors, activating dopamine pathway leading to the positive reinforcement (Pontieri et al., 1996; Lipovac et al., 2001).

There is a relation between the upregulation of nicotinic receptor density and quitting smoking. A study says that smokers with less nicotinic receptor density are more likely to quit smoking with treatment that smokers with higher nicotinic receptor density. The same study also suggests that there should be nicotinic receptor density testing equipment made available to test clinically. This will help to determine which smoker need intensive treatment (Brody et al., 2014).

Increased NRD is one of the most critical parameters to assess the changes that occur in the brain of a smoker. Single-photon emission computed tomography studies and positron emission tomography studies have shown the increased NRD in smokers than in non-smokers (Mamede et al., 2007; Staley, 2006).

The chronic smokers who smoke since a very long period will have more NRD than that of the smoker who has quit and non-smokers. This was a human postmortem brain tissue studies observation. The NRD will be reverted to normal during abstinence and will be easy to de addict.

**Aims**

1. To study the substance that can make easy to quit smoking without withdrawal symptoms
2. To study the substance that can decrease the nicotine receptor density

**Objectives**

1. To study the effect of CA on nicotine receptor density
2. To study the effect of TY on nicotine receptor density

**MATERIALS AND METHODS**

After getting institutional animal ethical committee (IAEC) approval from Saveetha University, 36 rats were procured from Biogen Bangalore. The animals were divided into six groups. Group 1 as control, group 2 only nicotine, group 3 nicotine and CA100 mg/kg bw, group 4 nicotine and CA200 mg/kg bw, group 5 nicotine and TY100 mg/kg bw and group 6 nicotine and TY 200 mg/kg bw.

The study was conducted for 26 days.

Group 1 received only sterile water for 21 days.

Group 2 received nicotine at a dose of 600 micrograms/kg bw subcutaneously for 21 days.

Group 3 received nicotine (600 μgms/kg bw subcutaneously) for 21 days and CA 100 mg/kg bw orally for 21+5 days.

Group 4 received nicotine (600 μgms/kg bw subcutaneously) for 21 days and CA 100 mg/kg bw orally for 21+5 days.

Group 5 received nicotine (600 μgms/kg bw subcutaneously) for 21 days and TY100 mg/kg bw orally for 21+5 days.

Group 6 received nicotine (600 μgms/kg bw subcutaneously) for 21 days and TY200 mg/kg bw orally for 21+5 days.

On the 26th day, all the animals were euthanized and the brain was dissected and used for nicotinic receptor density analysis.

**Immunostaining of neuronal nicotinic acetylcholine receptor expression in brain tissue**

**Procedure**

Immunohistochemical analysis was performed by assessing nAChR immunoactivity in brain tissues. The tissues were immersed in the fixative solution for 4h.

The tissues were cryoprotected in 30% sucrose, embedded in tissue-freezing medium with liquid nitrogen, and cut into frozen sections (3-5μm) using a cryostat. The Sections were stored under anti-freeze buffer.

Parallel free-floating sections were subjected to endogenous peroxidase quenched with 1% H₂O₂ in PBS, followed by treatment with blocking buffer (5% normal chicken serum in PBS and 0.3% Triton X-100 for overnight at 4°C) and incubated with nAChR primary monoclonal antibody. After washing with PBS, tissues were incubated with a biotinylated goat antimouse secondary antibody.

The tissues were subsequently exposed to an avidin-biotin-peroxidase complex for two h. The peroxidase activity was visualized using a stable diaminobenzidine solution.

The sections were viewed through light microscope, and these results were quantified using the Image analysis J 1.46 software.
RESULTS AND DISCUSSION

The comparison of NRD was done by one-way analysis of variance. The control, only nicotine, N+CA100, N+CA200, N+TY100 and N+TY200 groups were compared. These groups mean and standard error of mean are represented in Table 1 and in Figure 1. The statistical values showed significant difference among these groups.

From the Table 1 and Figure 1, it is clear that there is a significant elevation in the NRD in only nicotine treated group when compared with the other groups. On the other hand, the NRD of the test groups i.e. N+CA100 N+CA200 N+TY100 N+TY200 has decreased significantly. This suggests that in spite of unaltered nicotine supplementation in the CA and TY groups, the NRD is not elevated as that of the only nicotine group.

The overall results suggest that NRD us reduced by CA and TY. If the NRD is controlled than the person can easily get deaddicted from the nicotine.

Figure 1: Effect of CA & TY on NRD

Nicotine from different sources is a very dangerous and most addictive alkaloid. It is present in the tobacco plants, specifically from Nicotiana tabacum species of Solanaceae family. The leaves of this plant are used in a different form to burn and inhale the smoke. Nowadays, nicotine is extracted and is encapsulated as patches and in chewable tablet forms, to treat the nicotine-addicted patients who smoke tobacco in different forms. The intention behind this is to make those addicted people away from the much dangerous and life-threatening habit of smoking or chewing tobacco.

But nicotine is the important substance that is making this addiction, several studies were put forth to solve this problem, but they could not succeed because of the complication in the process of dead diction. Studies suggest that the nicotine receptor density is the key culprit in holding the nicotine addiction. A study that focused on nicotine placebo patch revealed that NRD is directly proportional to chances of quitting smoking (Brody et al., 2014).

It is evident that by controlling the NRD, we can treat nicotine addiction. A study indicates that by determining the brain nicotine, plasma and salivary markers associated with greater nicotine exposure would be much useful in predicting the adverse effects of nicotine that may lead to the addiction (Paoletti et al., 1996; Norregaard et al., 1993).

The mechanism by which the NRD increases is not known, although some studies indicate that nicotine exposure increases the receptor function and the receptor gets sensitive to nicotine, and this may lead to the increase in the NRD, moreover due to the continuous nicotine exposure, the receptor on the cell surface increase concomitantly (Govind et al., 2009).

Earlier studies on CA states that it is a week organic acid found in all the citrus fruits like lemon, grapefruit and orange. The rich source of CA is lemon and lime juice (Penniston et al., 2008).

Another study stated that citrate decreases lipid peroxidation and down-regulates inflammation by reducing polymorphonuclear cell degranulation and attenuating the release of myeloperoxidase, elastase, interleukin and platelet factor 4 (Gritters et al., 2006). Citric acid has been proven to reduce the hepatocellular injury evoked in rats by carbon tetra-chloride (Tiranathanagul et al., 2011).

There are very few attempts made to reduce the nicotine NRD scientifically, which is a crucial way to de addict the person from nicotine. They are many other alternative methods studied and proposed in the field of nicotine deaddiction. The outcomes of almost all the studies ended up in side effects or perhaps cost ineffective. Our study handles both side effects and costs as well since we are using a commonly available substance.

CA is a much-neglected substance in terms of its action on the nicotine deaddiction. Very few studies are proving the effects of CA on smoking. One study states that the aerosol spray of citric acid stimulates the tracheal sensations produced by cigarette smoke and satisfies smokers desire for a cigarette. Smokers rated the respiratory tract sensations produced by citric acid aerosol equal to or better than that of low tar and nicotine cigarettes in terms of liking, similarity to their brand, and reduction in cigarette craving (Rose and Hickman, 1987). A similar study on CA
Table 1: Effect of CA & TY on nicotinic receptordensity different groups

<table>
<thead>
<tr>
<th>Group Name</th>
<th>N</th>
<th>Missing</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>0</td>
<td>1.367</td>
<td>0.611</td>
<td>0.250</td>
</tr>
<tr>
<td>Nicotine</td>
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<td>0</td>
<td>7.445</td>
<td>1.361</td>
<td>0.556</td>
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<tr>
<td>N+CA100</td>
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<td>0</td>
<td>2.702</td>
<td>0.815</td>
<td>0.333</td>
</tr>
<tr>
<td>N+CA200</td>
<td>6</td>
<td>0</td>
<td>4.135</td>
<td>1.261</td>
<td>0.515</td>
</tr>
<tr>
<td>N+T100</td>
<td>6</td>
<td>0</td>
<td>3.148</td>
<td>1.848</td>
<td>0.755</td>
</tr>
<tr>
<td>N=T200</td>
<td>6</td>
<td>0</td>
<td>2.352</td>
<td>1.389</td>
<td>0.567</td>
</tr>
</tbody>
</table>

Inhaler says that the citric acid aerosol may promote the successful smoking reduction or cessation in a subgroup of smokers and relieves withdrawal symptoms like craving for cigarettes (Behm et al., 1993).

Previous studies reported that smoking does not rely upon the mechanism action of nicotine. On the other hand, the smoke inhalation scratches the tracheobronchial, which leads to sensory provocation. The researchers appraised unique portable-sized inhaler machine for distributing the citric acid aerosol and verified it is a fulfilling alternative for cigarettes. The study supported that citric acid aerosol is beneficial for smokers who are trying to drop out smoking (Levin et al., 1990).

Similarly, TY is known to have its effect on catecholamine synthesis. There is a lot of information about the role of TY in dopamine synthesis, but unfortunately, there are no studies on TY effect tested in nicotine deaddiction. A study on TY says that the TY transiently and significantly increases the extracellular concentration of striatal dopamine, the peak increase in ECF dopamine occurred 60 mins after rats received the highest 200mg/kg TY dose. Another study on TY says that administration of tyrosine (100mg/kg i,p) elevated retinal dihydroxyphenyl acetic acid among light-exposed animals, but failed to affect dopamine release among animals in the dark. And it also adds that the physiological stimulus-light exposure can cause catecholaminergic neurons to become tyrosine – dependent, they also suggest that food consumption may affect neurotransmitter release within the retina (Gibson et al., 1983). One more similar study states that the dopamine rise is short-lived suggesting that receptor – mediated feedback mechanism responded to the increased dopamine release by diminishing neuronal firing or sensitivity to tyrosine (Acworth et al., 1988). Another study says that older adults showed a dose-dependent increase in plasma tyrosine concentrations, and the plasma response was higher than for young adults with the same dose.

A dietary amino acid - L-phenylalanine absorbed from the gut, and it is converted to L-tyrosine using phenylalanine hydroxylase which presents in the liver. The dispersed L-tyrosine is hydroxylated by tyrosine hydroxylase to L-dopa in neuronal cytoplasm of the noradrenergic neuron. In the final step of the biosynthesis of dopamine, L-dopa is switched to dopamine by cytoplasmic L-amino acid decarboxylase in dopaminergic neurons. The nicotine levels of only nicotine administered group didn't alter compared with test groups. It concluded that the CA and TY might be meddling in the nicotine metabolism (van de Rest et al., 2017).

A very recent study indicated that CA and TY were effective in limiting the nicotine levels despite normal nicotine intake levels. It also proves that the dopamine levels are unaltered. The present study has also proved that CA and TY affect NRD. CA and TY groups showed significant decrease in NRD as compared with the nicotine treated group. The exact mechanism by which the NRD decreased has to be studied further (Brody et al., 2013).

CONCLUSION

Nicotinic receptor density increases when a person or an animal is exposed to nicotine. It plays a vital role in the addiction and withdrawal symptoms. It takes almost 6 to 8 weeks of abstinence to bring back the NRD to normal. If a person stops smoking for a few days, it is the NRD which craves or urges the person for another cigarette. Hence without decreasing the NRD, we cannot make the person quit smoking. On the other hand, the increase in NRD will eventually addict the person. If we can stop the elevation of NRD despite nicotine intake, we can stop or control the person from getting addicted. We conclude in our study that CA and TY have controlled the NRD significantly.

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Conﬂict of Interest
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