Psychotropic Activity of Selective Tetracyclines

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Summary

Minocycline in addition to its neuroprotective and anti-inflammatory use in neurodegenerative diseases probably would be helpful in treatment of depression associated with these comorbid conditions. The findings of the present study in both the models of depression viz, forced swim test and tail suspension test indicate that minocycline has significant antidepressant activity, comparable to that of amitriptyline. If the findings of the present study are extrapolated to the humans, minocycline may not be a suitable drug to treat depression. Its co-administration with tricyclic antidepressants could add to the antidepressant activity of the latter. Whether such synergistic interaction will narrow the therapeutic window of tricyclic antidepressants is not known. To evaluate its role as an antidepressant and to establish safety of its co-administration with tricyclic antidepressants, further clinical studies are desirable.

Key Words: Psychotropic Activity, Tetracyclines, Minocycline, Doxycycline, Oxytetracycline

Introduction

Mood disorders like depression and mania form major component of psychiatric disorders and are usually associated with anxiety. These are also common psychiatric disorders that are encountered in day to day clinical practice. Though the exact pathology is not understood, various neurotransmitters like norepinephrine, serotonin etc and certain other factors like brain derived nerve growth factor (BDNF) have been implicated with pathogenesis of depression and anxiety. Logically various drugs that can favorably modify the neurotransmitters and other factors in the brain could be useful for the treatment of these mood disorders. In addition to currently used tricyclic antidepressants, selecte serotonin reuptake inhibitors etc antiepileptic agents like carbamazepine, sodium valproate have also been used in manic depressive illness as alternatives. Interestingly renin angiotensin antagonists like enalapril and losartan, through their effects on central neurotransmitters have been reported to possess antidepressant and anxiolytic action.

Surprisingly, minocycline, an antimicrobial has been recently reported to be useful in the treatment of schizophrenia, though its antidepressant activity was suggested in the year 1996. Obviously, the observed antidepressant activity of minocycline cannot be explained by its antimicrobial activity. The observed antidepressant activity could be attributed to its distinct pharmacokinetic features such as high lipid solubility and penetrability in brain.
Other tetracyclines viz. doxycycline, oxytetracycline in addition to their bacterial antiribosomal activity have been reported to possess antinflammatory activity. In view of other reported actions of these tetracyclines in addition to their antimicrobial action, they could also share the antidepressant actions of minocycline if any due to their structural similarity, which yielded scanty information in literature search. Therefore, it was decided to investigate antidepressant activity of two other commonly used tetracyclines viz. doxycycline and oxytetracycline along with minocycline, using anxiety and depression paradigms in Swiss mice.

**Materials and Methods**

**Animals:** Mice weighing 20-30g were obtained from the central animal house of the institute and were kept in the laboratory for about 10 days in 12:12 hr L: D cycle. Throughout the experiment the animals were fed with laboratory chow (Amrut Brand) and water ad libitum. All animals were fasted over night prior to the day of experiment and experiments were carried out between 09.00-14.00 hrs.

The study was approved by Institutional Animal Ethical Committee formed as per the guidelines of CPSCEA, New Delhi.

**Drugs and doses**- Amitriptyline (Inj. Typtin, sterfil Labs Ltd), Alprazolam (Tab. Alprax, Torrent Ltd), minocycline (Cap.Cynomycin), doxycycline (Tab.Doxt), and oxytetracycline (Cap.Oxytetracycline) were purchased locally.

Mice equivalent doses in mg/kg body weight of clinical doses were calculated as mg/kg body weight and were administered orally in case of alprazolam, minocycline, doxycycline, oxytetracycline. Amitriptyline was given intraperitoneally.

All the drugs were dissolved in distilled water while alprazolam was suspended in 2% gum acacia. All drugs were freshly prepared and were administered in a single oral dose in a volume of 0.5 ml. Equal volumes of normal saline were administered through oral route to the control group.

Behavioral studies- a) antidepressant activity studies were carried out in mice using forced swim test paradigm as described earlier.

Porsolt forced swim test Briefly, mice weighing 20-30g were pretreated with the drug/vehicle as per corresponding group and placed individually in a glass cylinder measuring 21x12 cm containing 10 cm of water at room temperature. The total duration of immobility in seconds was observed for a period of 6 min forced swimming test. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water making only those movements necessary to keep its head above water.

b) Tail suspension test was carried out in mice as described earlier.
The mouse pretreated with drug/vehicle was suspended from the hook hanging at the centre of a horizontal rod placed on metallic stand kept 35 cm apart. An adhesive tape stuck 2 cm proximal to the tail tip was used to suspend the animal through the hook hanging 35 cm distance from the ground. Immobility time in seconds was recorded by assessing motionless hanging of the mice over a period of 6 min.

c) Anxiolytic activity was studied using elevated plus maze as described earlier.\textsuperscript{7,10}

The elevated plus maze apparatus consists of two open arms (21.5 x 7.5 cm) and two closed arms (21.5 x 7.5 x 20 cm) without roof which extended from a central platform of 7.5 x 7.5 cms. The pretreated animals were placed individually for a 5 min at the centre of the elevated plus maze facing the head towards an open arm. The number of entries into open or closed arm and the time spent in each arm were recorded. At the same time, no of rears were recorded in each arm.

The percentage of number of entries (against the total number of entries both in open and closed arms) and percentage time spent in open arm (against total time spent both in open and closed arms) were calculated for each group. Similarly mean number of rears for each group was calculated.

d) Light-dark arena\textsuperscript{7,11}- It consists of a wooden box (50 x 30 x 35 cm) placed on a table, 1 cm above floor level. A partition with a gap of 7.5 x 7.5 cm at the centre of its lower border was fixed to separate $\frac{2}{5}$th of the base from the remaining $\frac{3}{5}$th. The smaller $\frac{2}{5}$th chamber was painted black and the other $\frac{3}{5}$th was painted white on all four sides. The chamber painted black was illuminated with dull red light while the other chamber was brightly illuminated with a 100 W light source located 17 cm above the box. Pretreated mice were placed in the centre of the bright area. The number of rearings, entries into and time spent in light area were recorded over a period of 5 min. the mean number of entries, and rears and percentage of time spent were calculated for each group.

The effect of all the drugs used in the present study, on locomotor activity was tested using open field test as described earlier\textsuperscript{7,8,9,12} and confirmed by actophotometer (M/S INCO).

Statistical analysis: The results were analyzed by one way ANOVA followed by Dunnet’s test using Graph pad prism software and $p \leq 0.05$ was considered significant.

Results

In the present study the three tetracyclines viz. minocycline, doxycycline, oxytetracycline were investigated for their antidepressant and anxiolytic activity in mice.

Amitriptyline as a standard antidepressant and alprazolam as a standard anxiolytic were also used for the sake of comparison.
Antidepressant activity study: this was carried out employing two models viz. forced swim test and tail suspension test.

Forced swim test:

The duration of immobility time in seconds was noted over a period of 6 minutes. The mean duration of immobility in the control group was 90.67±6.65 while it is 29.33±5.35, 29.83±9.34, 52.83±9.74 and 92.33±17.15 in the amitriptyline, minocycline, doxycycline and oxytetracycline groups respectively. There was significant change in the duration of immobility in the amitriptyline and minocycline treated groups when compared to that of control groups. (Table 1, Fig.1)

Tail suspension test:

The duration of immobility time in seconds was noted over a period of 6 minutes. The mean duration of immobility in the control group was 174.0±14.90 while it is 78.50±4.93, 137.5±2.86, 145.8±8.71 and 160.7±12.12 in the amitriptyline, minocycline, doxycycline and oxytetracycline groups respectively which differed significantly from the control group. There was a significant change (p<0.05) in the duration of immobility as compared to that of control group in the minocycline and amitriptyline treated groups. (Table 1, Fig.2)

Anxiolytic activity:

This was carried out employing two models viz. elevated plus maze and light dark arena.

Elevated plus maze:

In the elevated plus maze rearing behavior, number of entries and time spent in the open arm were observed.

The animals were placed in the maze for 5 minutes (300 seconds) and time spent in seconds in the open arm was noted for each animal to calculate the percentage of time spent in the open arm and mean was calculated. The mean percentage of time spent in the open arm in the control group was 3.07±1.01 while it is 16.02±2.39, 7.33±1.52, 0.61±0.15 and 8.48±3.26 in alprazolam, minocycline, doxycycline and oxytetracycline respectively, and showed significant change (p<0.05) in the time spent in comparison to control group observed only in alprazolam whereas other drug treated group of animals showed no significant change in percentage time spent in open arm. (Table 2, Fig.3)

The effect of various drugs on the number of entries into the open arm as well as closed arm (total entries) were noted to calculate percentage of entry into open arm for each animal and then mean was calculated. The mean percentage of entry into the open arms in the control group was 29.33±6.94 while it is 26.60 ± 7.13, 33.10±3.03, 13.72±1.76 and 38.47±6.69 in the alprazolam, minocycline, doxycycline and oxytetracycline respectively with no significant change observed statistically.(Table 2)
The effect of various drugs on the number of rears was noted over a period of 5 minutes. The mean duration of rears in the control group was 5.67±1.45 while it is 4.83±1.14, 9.83±1.42, 5.00±1.55 and 9.50±1.38 in the alprazolam, minocycline, doxycycline and oxytetracycline treated groups respectively, which showed no significant change in the number of rears as compared to that of control in all the drug treated groups of animals. (Table 2)

**Light dark arena:**

The individual animal was placed for 5 minutes in the light and dark arena. The rearing behavior, number of entries and time spent in the light arena was observed and noted.

The time spent in light arena was noted for each animal and the percentage time spent in the light arena to that of total time spent in light and dark arena was calculated and mean was tabulated. The mean percentage time spent in light arena in the alprazolam treated group is 46.85±3.69 which was significantly (p<0.05) more than the control treated group which was 33.93±1.53. The mean percentage time spent in the light arena in the minocycline, doxycycline and oxytetracycline are 42.73±2.05, 37.28±3.60 and 43.05±1.74 respectively and showed no significant (p>0.05) change in the percentage time spent in light arena in comparison to control group. (Table 2, Fig. 4)

Then mean number of entries into the light arena in the control group was 7.67±1.31, while it is 5.33±0.92, 11.00±0.73, 8.00±1.32 and 14.17±1.33 in alprazolam, minocycline, doxycycline and oxytetracycline treated groups respectively. The mean values showed no significant change (p>0.05) in the number of entries into light arena in all the drug treated groups compared to control group. (Table 2)

The mean duration of rears in the control group was 4.33±1.38 while it is 10.17±0.65, 6.67±1.17, 4.33±0.56 and 7.83±0.79 in the alprazolam, minocycline, doxycycline and oxytetracycline treated groups respectively, which showed significant increase in the number of rears as compared to that of control in the alprazolam drug treated group of animals. (Table 2)

**Locomotor activity:**

There was no significant change in the mean of the locomotor activity as compared to that of control whose mean was 207.2±7.79 while it is 201.3±4.72, 204.3±4.03, 180.7±5.21 and 242.2±14.48 in alprazolam, minocycline, doxycycline and oxytetracycline treated groups of animals respectively.
Table 1: Effect of various treatments on depression paradigms [values are mean ± SE from 6 animals in each group]

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose(mg/kg)</th>
<th>Immobility time(sec)</th>
<th>Forced swim test</th>
<th>Tail suspension test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>0.5 ml</td>
<td></td>
<td>90.67 ± 6.65</td>
<td>174.0 ± 14.90</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>39</td>
<td>29.33 ± 5.35*</td>
<td>78.50 ± 4.93*</td>
<td></td>
</tr>
<tr>
<td>Minocycline</td>
<td>13</td>
<td>29.83 ± 9.34*</td>
<td>137.5 ± 2.86*</td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>13</td>
<td>52.83 ± 9.74</td>
<td>145.8 ± 8.71</td>
<td></td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>65</td>
<td>92.33 ± 17.15</td>
<td>160.7 ± 12.12</td>
<td></td>
</tr>
</tbody>
</table>

Values are shown in mean ± sem and p<0.05* is significant

Figure 1: Effect of various treatments on duration of immobility (seconds)
Figure 2: Effect of various treatments on duration of immobility (seconds)

![Tail Suspension Test Graph]

Figure 3: Effect of various treatments on % time spent in open arm of Elevated plus maze

![EPM Graph]
Figure 4: Effect of various treatments on % time spent in light arena

Table 2: Effect of various treatments on anxiety paradigms [values are mean ± SE from 6 animals in each group]

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose (mg/kg)</th>
<th>% open arm entries</th>
<th>% Time spent in open arms</th>
<th>Rears in closed arm</th>
<th>% time spent in light area</th>
<th>No of entries to light area</th>
<th>Rears in light area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>0.5ml</td>
<td>29.33 ± 6.94</td>
<td>3.07 ± 1.0</td>
<td>5.67 ± 1.45</td>
<td>33.93 ± 1.53</td>
<td>7.67 ± 1.31</td>
<td>4.33 ± 1.38</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>0.65</td>
<td>26.60 ± 7.13</td>
<td>16.02 ± 2.39*</td>
<td>4.83 ± 1.14</td>
<td>46.85 ± 3.69</td>
<td>5.33 ± 0.92</td>
<td>10.17 ± 0.65</td>
</tr>
<tr>
<td>Minocycline</td>
<td>13</td>
<td>33.10 ± 3.03</td>
<td>7.33 ± 1.52</td>
<td>9.83 ± 1.42</td>
<td>42.73 ± 2.05</td>
<td>11.00 ± 0.73</td>
<td>6.67 ± 1.17</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>13</td>
<td>13.72 ± 1.76</td>
<td>0.61 ± 0.15</td>
<td>5.00 ± 1.55</td>
<td>37.28 ± 3.60</td>
<td>8.00 ± 1.32</td>
<td>4.33 ± 0.56</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>65</td>
<td>38.47 ± 6.69</td>
<td>8.48 ± 3.26</td>
<td>9.50 ± 1.38</td>
<td>43.05 ± 1.74</td>
<td>14.17 ± 1.33</td>
<td>7.83 ± 0.79</td>
</tr>
</tbody>
</table>

Values are shown in mean ± sem and p<0.05* is significant
Table 3: effect of various treatments on locomotor activity assessed by open field test [values are mean ± SE from 6 animals in each group]

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose (mg/kg)</th>
<th>No of boxes crossed by animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>0.5ml</td>
<td>207.2 ± 7.786</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>39</td>
<td>201.3 ± 4.723</td>
</tr>
<tr>
<td>Minocycline</td>
<td>13</td>
<td>204.3 ± 4.030</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>13</td>
<td>180.7 ± 5.213</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>65</td>
<td>242.2 ± 14.48</td>
</tr>
</tbody>
</table>

Values are shown in mean ± sem and p<0.05* is significant

Discussion:

The findings of the present study in both the models of depression viz, forced swim test and tail suspension test indicate that minocycline has significant antidepressant activity, comparable to that of amitriptyline. However, doxycycline and oxytetracycline though share basic structure of minocycline failed to show significant antidepressant activity. On the other hand all the selected tetracyclines failed to show any significant anxiolytic activity.

The antidepressant activity of minocycline as observed in the present study confirm the earlier clinical reports, where in, it was suspected to share antimaniac and antidepressant activity or to augment the effects of tricyclic antidepressants. Similar studies exploring antidepressant activity of doxycycline and oxytetracycline could not be traced in the available literature. Similarly no attempt has been made to investigate their effect on anxiety. Antidepressant activity of minocycline in contrast to other tetracyclines obviously is due to its high lipophilicity and easy penetration through blood brain barrier.

Observed antidepressant activity of minocycline is obviously not related to its antimicrobial activity. Minocycline has been reported to possess neuroprotective and anti-inflammatory activity. It has also been reported to inhibit caspase1 and caspase 3 and thereby inhibits disease progression in mouse model of huntington’s disease. Minocycline was shown to be effective in various animal models of neurological diseases like multiple sclerosis, spinal cord injury, parkinson’s disease and amyotrophic lateral sclerosis etc due to its neuroprotective and anti-inflammatory activity.
The present study was not planned to explore the mechanism of antidepressant activity of tetracyclines. However, based on the available literature it can be proposed that minocycline by modulating serotonergic and adrenergic neurotransmission through glutamatergic system is responsible for its antidepressant activity. Accordingly, several NMDA receptor antagonists have been reported to produce antidepressant like effects in experimental animals, and rise in extraneuronal levels of glutamate in selected brain areas of stress induced animals has been reported. Therefore glutamatergic neurotransmission is probably involved in depression.

There are reports showing involvement of metabotropic glutamate 5 receptors (mGlu5) in the pathogenesis of depression and anxiety, and anxiolytic and antidepressant like effects of mGlu5 receptor antagonists has been shown to facilitate neurogenesis and the release of serotonin, norepinephrine as well as dopamine. The role of noradrenergic and serotonergic system has been well established in the pathogenesis of depression. Several antidepressants act by increasing either noradrenaline or serotonin activity at central synapses. Therefore the observed antidepressant activity of minocycline could be due to its facilitatory action on serotonin and norepinephrine neurotransmission, secondary to reduced glutamate neurotransmission. Activation of p38 MAPK by glutamate in microglia cells has been implicated with excitotoxicity and minocycline has been reported to inhibit the enzyme in microglia cells, however to what extent this mechanism contributes for the observed antidepressant activity of minocycline is uncertain. Glutamatergic system has been implicated with pathogenesis of both depression and anxiety.

Minocycline by reducing glutamatergic neurotransmission could be expected to produce both antidepressant as well as anxiolytic activity. It is difficult to explain why minocycline failed to show significant anxiolytic activity in the present study. However, lack of anxiolytic activity could be explained by its predominant facilitatory effect on noradrenergic system, which contributes for its antidepressant activity and might add to anxiogenesis. Antidepressant activity of minocycline has been attributed for its predominant modulating effect on noradrenergic system. None of the treatments including minocycline failed to significantly change locomotor activity as compared to controls indicating that inhibitory neurotransmission like GABA are not affected.

If the findings of the present study are extrapolated to the humans, minocycline may not be a suitable drug to treat depression. Its co-administration with tricyclic antidepressants could add to the antidepressant activity of the latter. Whether such synergistic interaction will narrow the therapeutic window of tricyclic antidepressants is not known. To evaluate its role as an antidepressant and to establish safety of its co-administration with tricyclic antidepressants, further clinical studies are desirable.

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