Original Article

Effect of different drugs on Frog's Heart

A Comparative Study to Evaluate the Chronotropic Action of Citalopram, Fluoxetine and Paroxetine on Intact Frog's Heart

Ayesha Shahnawaz, Naila Abrar and Munir Ahmad Khan

ABSTRACT

Objective: To compare the chronotropic action of citalopram, fluoxetine and paroxetine on frog heart.
Study Design*:* Experimental animal study
Place and Duration of Study: This study was conducted at Yusra Medical and Dental College, Islamabad from October 2015 to February 2016.
Materials and Methods: Stunning and pithing of the frog was done following which the heart was exposed. The apex was attached to a force transducer. Heart rate readings were recorded on Power lab. Three groups were designed. In every set of experiments basal readings (without drug) were initially recorded that served as the control and then the tissue was treated with one antidepressant. In Group I we documented the effects of citalopram. In Group II we observed the effects of fluoxetine and in Group III we noted the effect of paroxetine. Statistical analysis was done using SPSS version 22. A p value of less than or equal to 0.05 was considered statistically significant.
Results: The isolated heart tissue sample was exposed to 0.5ml of drug. Citalopram at a concentration of 1.54mM reduced the heart rate from 30 to 19 beats/min. Fluoxetine at a concentration 1.6mM brought down the heart rate from 23 to 20 beats/min. Whereas, paroxetine at a concentration of 1.3mM increased the heart rate from 21 to 23 beats/min.
Conclusion: Citalopram out of the three chosen drugs caused the most marked reduction n in heart rate. Fluoxetine caused a subtle reduction in heart rate. Paroxetine on the other hand caused a mild increase in heart rate.
Key Words: SSRI's, Citalopram, fluoxetine, paroxetine, chronotropic, frog heart

Citation of article: Shahnawaz A, Abrar N, Khan MA. A Comparative Study to Evaluate the Chronotropic Action of Citalopram, Fluoxetine and Paroxetine on Intact Frog's Heart. Med Forum 2016;27(6):19-23.

INTRODUCTION

Depression contributes to a major global health burden; with over 150 million people being affected by it worldwide[1]. The mainstay of the treatment revolves around cognitive behavioral therapy and pharmacological intervention. For many years physicians relied on tricyclic antidepressants (TCAs) and Monoamine Oxidase Inhibitors (MAOIs). The TCAs presented with a myriad of adverse effects such as anticholinergic, cardio toxic and neurological effects[2]. Clinical data also revealed the large propensity with which the TCAs caused fatal outcomes in case of over dosage, owing to their low margin of safety[3].The MAOIs have also become an obsolete choice due to their adverse effects and potentially life threatening food and drug interactions[4].

Department of Pharmacology and therapeutics, Yusra Medical and Dental College, Islamabad

Correspondence: Ayesha Shahnawaz, Senior lecturer, Department of Pharmacology and therapeutics, Yusra Medical and Dental College, Islamabad
Contact No.: 0345-5192644
E-mail: Ayesha-usaph@hotmail.com

Received: March 18, 2016; Accepted: April 27, 2016

The Selective Serotonin reuptake inhibitors (SSRIs) were introduced in the 1980's, and since their advent the pharmacological choices for the treatment of depression have been revolutionized.

The SSRIs increase the availability of monoamine neurotransmitters in the synaptic cleft by,selective inhibition of the serotonin transporter (SERT) present on the presynaptic membrane[5]. TheSSRIs were instantaneously preferred over TCAs and MAOIs due to their greater efficacy, tolerability, limited deleterious effects and a safer profile even with over dosage of the drug. The SSRIs were developed with the objective of having a class of drugs with minimal interactions with other receptors,as; the TCAs had considerable unwanted interactions withreceptors like muscarinic, histamine, α adrenergic etc. Therefore, the narrow spectrum of adverse effects with this new class of drugs was fundamentally attributed to the drugs lack of affinity for miscellaneous receptors and specificity for the SERT receptor[2].

A lot of work has been done to undermine the toxic profile of the SSRIs. The SSRIs have been documented to cause adverse effects such as weight gain, sexual dysfunction and sleep disturbances[6]. They may also produce an assortment of other side effects such as gastrointestinal disturbances and CNS related

symptoms[7]. The adverse effects associated with the SSRIs differed from those produced by conventionally used TCAs and MAOIs. Also the adverse effects were milder and resulted in less fatal outcomes as compared to older therapeutic choices. One of the salient features distinguishing the TCAs from the SSRIs was the latter group's relative safe cardiac profile[2]. The TCAs were known to cause cardiac effects like tachycardia, intraventricular conduction delay and prolongation of the QT interval at therapeutic dosage and life threatening arrhythmias at over dosages[8].

Whereas the SSRI Citalopram, at therapeutic dosage has been shown to cause only a mild bradycardia with otherwise unappreciable effects on cardiac conductance[9]. With over dosage of greater then 400mg, the drug may result in a fatal outcome due to prolongation of the QTc interval[10]. Fluoxetine when studied for its cardiac profile, showed subtle changes in the cardiovascular profile, such as a decrease in heart rate and an increase in supine systolic pressure and ejection fraction in patients with compromised ejection fraction. However no significant changes on cardiac conductance were noted[11]. Paroxetine also causes a fall in heart rate and an increase in supine blood pressure[12].

Our study focuses on the comparison between the three SSRIs, Citalopram, Fluoxetine and Paroxetine with reference to their ability to cause bradycardia in the isolated frog heart. The aim of the present study was to compare the action of citalopram, fluoxetine and paroxetine on the heart rate of an intact frog heart preparation.

MATERIALS AND METHODS

The experimental work was carried out in the Laboratory of the Department of Pharmacology and therapeutics, at Yusra medical and dental college, Islamabad for a period of 5 months (October 2015-February 2015). Healthy frogsof both sexes weighing approximately 500gm were included. Animals with a resting heart rate of < 18 beats/min or > 35 beats / min were excluded from the study. Animals were kept in the animal house of the institution at room temperature and humidity of 60%. All animal handling procedures were conducted in accordance with the Guide for the care and use of Laboratory animals of the National institute of health, as well as the guidelines of the Animal welfare Act.After stunning and pithing of the frog, the precordium was dissected to expose the heart. The tissue was kept aerated and was intermittently bathed with amphibian ringer's solution. The frog was laid on a cardboard sheet. The heart was mounted on the force transducer (ML856). All observations were recorded on power lab machine.

The animals were divided in to three groups:

Group I. (n=6) Treated with Citalopram

Group II. (n=6)Treated with Fluoxetine

Group III. (n=6) Treated with Paroxetine

Baseline heart rate readings were recorded for each tissue sample. Next, the effect of drug was observed on individual groups. Group I, II and III were incubated with a single application of 0.5ml of citalopram, fluoxetine and paroxetine respectively. After an incubation period of 5 minutes the heart rate was recorded. Each tissue sample was only used once.

Citalopram, fluoxetine and paroxetine were purchased from Medizan laboratories (pvt) ltd Pakistan. Stock solutions for citalopram, fluoxetine and paroxetine were prepared at 1.5mM, 1.6mM and 1.3mM respectively. A single application of 0.5ml of the drug was used for each group.

RESULTS

The results were statistically analyzed by using SPSS version 22 and paired T test was used to evaluate the significance within a group. A p value of 0.05 or less was considered to be statistically significant.

Group I: The resting heart rate was documented. The tissue was next Incubated with 0.5ml of 1.54mM of citalopram. The application of citalopram lead to an appreciable negative chronotropic effect. The heart rate decreased from a mean basal value of 29beats/min to 19beats/min as shown in Table 1 and figure 1.

Table No 1: The effect of citalopram on heart rate.

Sr. No	Basal heart rate	Citalopram treated heart rate
1	27.27	20.47
2	25.64	18.51
3	33.89	20.76
4	33.33	19.60
5	33.14	19.23
6	24.79	17.80

Group II: After recording the resting heart rate, the tissue **samples** were preincubated with 0.5ml of 1.6mM of fluoxetine. The presence of the drug decreased the pacemaker activity of the heart from a mean value of 23beats/min to 20beats/min as shown in Table 2 and figure 1.

Table No 2: The effect of fluoxetine on heart rate.

Sr. No	Basal heart rate	Fluoxetine incubated heart rate
1	19.16	16.94
2	20.33	16.94
3	24	22.9
4	23.52	22.64
5	26.31	22.9
6	27.14	23.16

Group III: Intrinsic heart rate was noted. Subsequently the pretreatment of group III with 0.5 ml of 1.3mM of paroxetine was performed. The presence of paroxetine induced a positive chronotropic effect. The heart rate

increased from a basal value of 21 beats/min to 24beats/min as shown in Table 3 and figure 1.

Table No 3: The effect of paroxetine on heart rate.

Sr. No	Basal heart rate	Paroxetine incubated heart rate
1	21.34	23.48
2	20.40	22.74
3	21.58	24.51
4	22.13	23.38
5	19.54	22.50
6	23.87	25.63

Henceforth it was observed that citalopram brought the greatest change in the resting heart rate of the intact frog heart. It decreased the basal heart rate by 35%. The drug fluoxetine caused an 11% decrease in heart rate whereas paroxetine resulted in an 11% increase in heart rate as shown in figure 1 and 2.

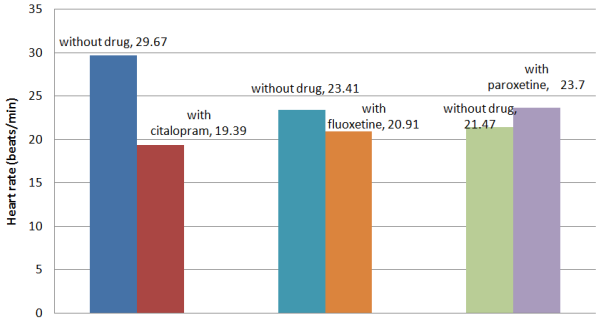

Figure No 1: A graphical representation of the effects produced on heart rate by citalopram, fluoxetine and paroxetine. Each drug is compared with its control values (without drug).

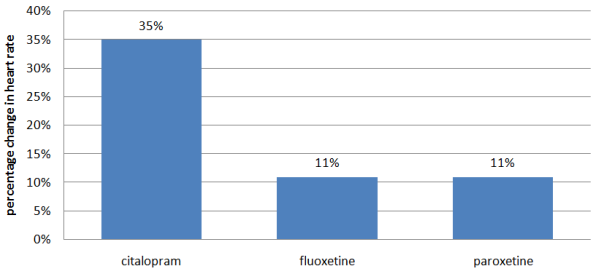

Figure No 2: a comparison of the percentage change in heart rate caused by citalopram, fluoxetine and paroxetine.

DISCUSSION

SSRIs have been documented to have a benign cardio toxic profile compared to the TCAs[7].Nevertheless they still exert a mild effect on the chronotropic action as seen in our experimental design.

In our study we compared three commonly used SSRIs in terms of the changes in the cardiac pacemaker activity caused by them. It was seen that the resting heart rate of the frog was influenced by all the three antidepressants. Citalopram induced the greatest change by decreasing the resting heart rate (30±4 to 19±1 beats per minute; p=0.001). Fluoxetine also resulted in a similar effect but produced less pronounced bradycardia (23±3 to 20±3 beats per minute; p=0.05). Paroxetine on the other hand, exhibited a mildly positive chronotropic effect and increased the basal heart rate (21±1 to 23±1 beats per minute; p<0.05) as shown in figure 1a.

The Sino atrial node maintains the auto rhythmicity of the heart. The establishment of an action potential depends on ion conductance through the voltage dependent sodium, calcium and potassium channels. Any changes in ion conductance would result in a change that may be appreciated on an electrocardiogram. Citalopram has already been documented to produce electrophysiological effects on isolated heart tissue samples obtained from rabbit, canine, rat and guinea pig. It has shown to alter ion conductance through sodium and Calcium channels[13,14].

In our study 1.54mM of citalopram produced a significant decline in heart rate. Pacher *etal*have similarly demonstrated that citalopram caused inhibition of the L-type calcium channels in guinea pig myocytes at a concentration of 100 μM[15].The decrease in the heart rate observed in our study is also hypothesized to be attributable to the ability of the drug to inhibit the long lasting Calcium channels of the Sinoatrial node.

Citalopram is generally considered a safer choice for depressed patients. On the other hand clinical studies conducted by Geoffrey *etal* demonstrated that over dosage of citalopram not only causes bradycardia but also leads to pronounced prolongation of the QT interval[16].

Amongst all the SSRIs, so far the most well studied is Fluoxetine. It has been observed to influence electrophysiological parameters on animal cardiac tissue samples. It has shown to havean inhibitory action on cardiac sodium and Calcium channels[13,14].Our study validated the drugs negative chronotropic action on frog heart. The proposed mechanism of action of fluoxetine is suspected to be through inhibition of the cationic channels. Although in comparison with citalopram the amplitude of the depressor effect was relatively subtle.

In the study conducted by Pacher *etal* it was corroborated that fluoxetine has no significant influence on the amplitude of potassium currents. The cardiac effects observed with Fluoxetine were also due to the inhibition of sodium and Calcium channels, as with the case of citalopram[17]. Studies have shown the drug's enhanced ability at inhibiting the L-type of Calciumchannels as compared to the other ion channels involved in the generation of an action potential[15].When compared with citalopram, Fluoxetine proved to be a more potent inhibitor of these channels[18].

Apart from the action on Calcium channels, fluoxetine was also shown to have an inhibitory action on hERG K^+channels resulting in a prolongation of the QT interval in HEK cells[19].Contrary to this, clinical studies

have proven that fluoxetine has a very feeble role in influencing heart rate or repolarization[20].

Relatively sparse data is available on the cardiac profile of paroxetine. Paroxetine has so far been documented to exert a mild bradycardia[21]. Our study conversely showed that paroxetine caused a mild tachycardia. Pollock *etal* demonstrated that the subtle change in heart rate could be attributed to the weak antimuscarinic action of the drug[22]. In this respect paroxetine may have a similar profile to that of the tricyclic agents that also cause profound tachycardia due to their anticholinergic activity. As we used a single concentration of the drug in our experiment, the dose related adverse effects in the case of over dosage cannot be quantified from our study. The toxic profile of conventionally used TCAs corroborate that over dosage results in sinus tachycardia and marked ECG changes, such as prolongation of the QRS complexes[23]. Such evidences have postulated grounds for us to speculate similar effects with paroxetine.

CONCLUSION

When comparing the three SSRIs we used in our experimental protocol it may be concluded that, citalopram results in profound bradycardia. This outcome may pose complications in patients with preexistingbradycardia occurring as a result of either metabolic disturbances like hypothyroidism or due to diseases directly affecting the heart's rhythm like sick sinus syndrome and other arrhythmias. Also, the concurrent administration of SSRIs with other drugs producing bradycardia, like the Beta blockers and calcium channel blockers, may precipitate the complication. Thus, the use of citalopram should be rationalized in such cases, and monitoring of cardiac indexes should be undertaken.Over dosage of the drug as mentioned earlier may also result in a deleterious outcome[16].

Fluoxetine, also resulted in a slight decrease in heart rate. Although the change in this case was about one third to that observed with citalopram. Thus, it may be suitable to assume that fluoxetine would serve as a safer choice in depressed patients with concomitant bradycardia.

Paroxetine shared congruency with the TCAs with respect to the influence on heart rate. Paroxetine brought a negligible increase in heart rate. It may be contemplated that the anticholinergic activity of the drug may give rise to complications in case of drug toxicity. However, the translation of our experiment in to clinical subjects may be quite different from what we have observed.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. World Health Organization. [homepage on the Internet]. 2015 [cited 2016 Mar 21]. Available from:http://www.who.int/mediacentre/factsheets/fs369/en/
2. John H, Citalopram — Pharmacological profile of a specific serotonin uptake inhibitor with antidepressant activity, Progress in Neuro-Psychopharmacology and Biological Psychiatry 1982; 6(3):277-295.
3. Mann AM, Catterson AG, Macpherson AS. Toxicity of imipramine: report on serious side effects and massive overdosage, Canad MA J 1959;81(23-27).
4. Livingston MG, Livingston HM. Monoamine oxidase inhibitors, drug safety 1996;14(4):219-227.
5. Bertram G. Katzung. Basic and Clinical Pharmacology. Antidepressant agents. 13th ed. 2016.p.514-515.
6. Ferguson JM, SSRI Antidepressant Medications: Adverse Effects and Tolerability. Prim Care Companion J Clin Psychiat 2001;3(1):22–27.
7. Tripathy KD. Essentials of medical pharmacology. Drugs used in mental illness: Antidepressant and antianxiety drugs. 6th ed.2008. p.445.
8. Roos JC, Cardiac effects of antidepressant drugs. A comparison of the tricyclic antidepressants and fluvoxamine Article first published online: 26 JUL 2012, Bri J Clin Pharmacol 1983;15(53): 4395-4455.
9. Ramussen SL, Overo KF, Tanghoj P. Cardiac Safety of Citalopram: Prospective Trials and Retrospective Analyses. J Clin Psychopharmacol 1999;19(5):407-415
10. Catalano G, Catalano MC, Epstein MA, Tsambiras PE. QTc Interval Prolongation Associated with Citalopram Overdose: A Case Report and Literature Review. Clin Neuropharmacol 2001; 24(3):158-162.
11. Roose SP, Glassman AH, Attia E, Woodring S, Giardina EG, Bigger JT. Cardiovascular Effects of Fluoxetine in Depressed Patients With Heart Disease. Am J Psychiat 1998;155(5):660-665.
12. Roose SP, Laghrissi-thode F, Kennedy JS, Nelson C, Bigger T, Pollock BG, et al. Comparison of Paroxetine and Nortriptyline in Depressed Patients With Ischemic Heart Disease. Am J Med Assoc 1998; 279(4):287-291.
13. Pacher P, Ungvari Z, Nanasi PP, Furst S, Kecskemeti V. Speculations on difference between tricyclic and selective serotonin reuptake inhibitor antidepressants on their cardiac effects. Is there any? Curr Med Chem 1999; 6(6):469-480.
14. Hamplová-peichlová J, Krůek J, Pactl l, Slavice J, Lisa V, Vyskocil F. Citalopram Inhibits L-type

Calcium Channel Current in Rat Cardiomyocytes in Culture. Physiological Res 2002; 51:317-321.
15. Pacher P, Kecskemeti V. Cardiovascular Side Effects of New Antidepressants and Antipsychotics: New Drugs, old Concerns? Current Pharmaceutical Design 2004;10(20):2463–2475.
16. Isbister GK, Bowe SJ, Dawson A, Whyte IM. Relative Toxicity of Selective Serotonin Reuptake Inhibitors (SSRIs) in Overdose. J Toxicol: Clin Toxicol 2004; 42(3):277-285.
17. Pacher P, Magyar J, Szigligeti P, Banyasz T, Pankucsi C, Korom Z, et al. Electrophysiological effects of fluoxetine in mammalian cardiac tissues, Naunyn-Schmiedeberg's Archives of Pharmacol 2000; 361(1):67-73.
18. Witchel HJ, Pabbathi VK, Hofmann G, Paul AA, Hancox JC. Inhibitory actions of the selective serotonin re-uptake inhibitor citalopram on HERG and ventricular L-type calcium currents. FEBS Lett 2002; 512(1-3):59-66.
19. Rajamani S, Eckhardt LL, Valdivia CR, Klemens CA, Gillman BM, Anderson CL, et al. Drug-induced long QT syndrome: hERG K+ channel block and disruption of protein trafficking by fluoxetine and norfluoxetine. Bri J Pharmacol 2006;149(5):481–489.
20. Baker B, Dorian P, Sandor P, Shapiro C, Schell C, Mitchell J, et al. Electrocardiographic Effects of Fluoxetine and Doxepin in Patients With Major Depressive Disorder, J Clin Pharmacol 1997; 17(1):15-21.
21. Erfurth A, Loew M, Dobmeier P, Wendler G. ECG changes after paroxetine. 3 case reports. Der Nervenarzt 1998; 69(7): 629-631
22. Pollock BG, Mulsant BH, Nebes R, Kirshner MA, Begley AE, Mazumdar S, et al. Serum Anticholinergicity in Elderly Depressed Patients Treated With Paroxetine or Nortriptyline, Am J Psychiat 1998; 155:1110-1112
23. Harrigan RA, Brady WJ. ECG abnormalities in tricyclic antidepressant ingestion. Am J Emerg Med 1999; 17(4):387–393.