REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

Histological and Biochemical Changes Associated with Targeting of Adrenergic B3 receptor

Entedhar K. Hussain[1], Saraa H. Barsoom[2], Khesar H. Khalil[3], Ghayth M. Abdulrazzaq[4]*

[1]Department of Medical Laboratory Sciences, College of Medicals and Applied Sciences, Charmo University, Chamchamal, Iraq.
[2]Department of Clinical Biochemistry, College of Health Science, Hawler Medical University, Erbil, Iraq.
[3]Department of Anatomy, Histology & Biology, College of Medicine, University of Duhok, Duhok, Iraq.
[4]Department of Pharmacology and Toxicology, College of Pharmacy, University of Mosul, Mosul, Iraq.
*ghayth.abdulrazzaq@uomosul.edu.iq

Abstract

Overactive bladder (OAB) is regarded as a combination of symptoms associated with the urgency of urination, urinary frequency, with or without incontinence and nocturia which tends to increase with age in both males and females. Through this study, we aim to analyze the changes in liver enzymes and hepatic tissue histology after the use of mirabegron for three months. A double-blinded randomised control trial was held on the sample size of 10 rats which were divided into two groups, a controlled group and a mirabegron induced for three months. NaCl was used as a placebo. Biochemical analysis of liver enzyme was done through a blood sample taken from the femoral nerve followed by a histopathological examination. Glutamic oxaloacetic transaminase (GOT) level of the controlled group was 105 U/L which was reduced to approximately 87 U/L on the other hand, in the case of mirabegron induced group the levels reduced from 122.5U/L to 90 U/L, glutamic pyruvic transaminase (GPT) remained constant on controlled group 27U/L meanwhile in case of mirabegron group the level fell from 34U/L to 19U/L. the serum level of alkaline phosphates remained the same in the controlled group i.e. 300U/L but a reduction has been found in the mirabegron group from approximately 375U/L to 310U/L. These results indicate mirabegron as the best choice of drug for OAB with the hepatoprotective mechanism of action with better efficacy and tolerability.

Keywords: Mirabegron, B3-adrenergic receptor, overactive bladder, incontinence, liver enzymes.

Introduction

Overactive bladder (OAB) is regarded as a combination of symptoms associated with the urgency of urination, urinary frequency, with or without incontinence and nocturia which tends to increase with age in both males and females (Coyne *et al.*, 2002). Urge syndrome and urgency frequency syndrome are also interchangeable terms which can be used to state the following symptoms in the absence of any other associated pathological condition such as infections (Lee *et al.*, 2013). Frequency is characterized by more than seven voids per day, nocturia is more than one void per night and incontinence is defined as the unintentional leakage of urine (Gerig *et al.*, 2021). OAB is a stressful condition which has a significant negative impact on the individual's quality of life, social behaviour, mental health, and physical activities and it increases the prevalence of other associated factors including the high risk of falls and fractures, sleep disturbance, urinary tract infections, skin infections, anxiety and depression (Brown *et al.*, 2000). The symptoms of an overactive bladder can be managed by pharmacological treatment, bladder training, electrical stimulation, biofeedback and a combination of therapies (Wein and Rackley, 2006). There is a wide range of treatments and management which are categorized into 'lines of therapies' consisting of both invasive and noninvasive approaches, lifestyle modification including pelvic floor exercises, time voiding

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

techniques, avoidance of irritants etc. However, the efficacy of antimuscarinic agents as the first-line pharmacotherapy for relieving the urge syndrome is clinically proven and widely accepted (Kreydin *et al.*, 2021). Five different muscarinic receptors are found in humans (M1-M5), studies indicate that M2 and M3 receptors are present in the neuromuscular junction of detrusor muscle while M3 is dominantly responsible for its contraction (Sobhgol and Charandabee, 2008).

Antimuscarinics are thought to block the muscarinic receptors on detrusor muscle which are stimulated by bonding with acetylcholine causing the bladder to contract furthermore, they are primarily active during the storage phase by increasing the capacity of the bladder and decreasing the urge to urinate (Levin and De, 2008). Commonly used drugs amongst them to treat OAB are oxybutynin, darifenacin, solifenacin (Bolduc *et al.*, 2010) and tolterodine (Bolduc *et al.*, 2003). However, there are multiple potential side effects of these drugs which are reported. The anticholinergic and antimuscarinic drugs are reported to induce multiple adverse effects which include dry mouth symptoms following the hypofunction of the salivary gland (decreasing the production of saliva) and xerostomia (a subjective feeling of dry mouth), cogitation dysfunction in older adults, constipation and mydriasis (Mostafaei *et al.*, 2021, Pagoria *et al.*, 2011, Kay *et al.*, 2005).

Compared to the other beta-adrenergic receptors, b3-adrenoceptor (b3-AR) are predominantly found in the neuromuscular junction of the detrusor muscle of the human urinary bladder. B3-AR agonists are recommended as the latest class of drugs for the treatment of overactive bladder by inhibiting the effect of acetylcholine, relaxing the bladder and reducing the urge of micturition. Mirabegron is a newly discovered B3-AR compound which is proposed for the treatment of OAB with a different mechanism of action (Herschorn *et al.*, 2013). According to the efficacy and safety of the drug the initial recommended dose is 25mg, which can eventually be increased to 50mg as per the requirement and tolerability of the patient. The use of mirabegron is however not restricted to managing daytime incontinence but also to treating neurogenic disorders which compromise the bladder e.g. spina bifida (Ramlan *et al.*, 2021).

Through this study, we aim to analyze the changes in liver enzymes and hepatic tissue histology after the use of mirabegron for three months.

Methodology

Animal: Ten randomly selected Sprague–Dawley female rats were selected, weighing in the range of 200g to 300g approximately. The rats were kept under the standard laboratory condition at the controlled temperature of 23±3°C for three months with 12 hours of light and 12 hours of dark cycles. Free access to food and tap water was provided. All the rats were kept strictly under the protocols of the animal ethics committee. All the pharmacological experiments were approved and supervised by the institution.

Drug: Mirabegron is the selective and effective agonist b3 adrenoreceptor which is commercially available and it's the first licensed B3-AR to treat overactive bladder. It has been approved in Japan in 2011, the US, Canada in 2012 and Europe in 2013.

Experimental Design: A parallel-group, randomized, placebo-controlled, double-blind study was conducted amongst a sample size of ten rats which were divided into two groups with the use of simple probability sampling. Group1 was the controlled group which received the

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

placebo i.e. NaCl and group 2 the mirabegron group where rats received 0.3mg/kg mirabegron.

Histopathological Examination: For the examination of liver tissues of both groups, tissues were fixed for 24 hours in 10% formalin, dehydrated by passing in ascending grades of ethyl alcohol, cleared in xylene, and embedded in paraffin. Sections of microns were prepared by using a microtome and stained with hematoxylin and eosin by a method described previously by Bancroft and Gamble (2008). The stained sections were examined and photographed by the light microscope (Olympus CX41 microscope, Olympus, Tokyo, Japan).

Biochemical Analyses: At the end of the 12 weeks blood was taken from the femoral nerve of the rat to examine the liver function. After 10 minutes of centrifugation at 1000x the sample was separated at kept at − 20 °C. Glutamic oxaloacetic transaminase (GOT), serum glutamic pyruvic transaminase (GPT) and alkaline phosphate ALP were then analyzed.

Statistical Analysis: All the data is statically significant with the mean ± standard deviation (SD) $p \leq 0.05$. a comparison was established between the changes in liver enzymes of the controlled group and the mirabegron group using the Mann-Whitney test and group data was compared with one way ANOVA test on IBM SPSS version 22.0.

Results

The examination of liver sections forms the controlled group on magnification indicates the following structures; hepatic lobules composing hepatocytes cords radiating from the central vein which are separated by hepatic sinusoids however these hepatic sinusoids are seen dilated in the drug-treated group. The loss of normal morphology of hepatic lobules can be seen in the mirabegron group, additionally, no signs of inflammation were witnessed (Figure 1).

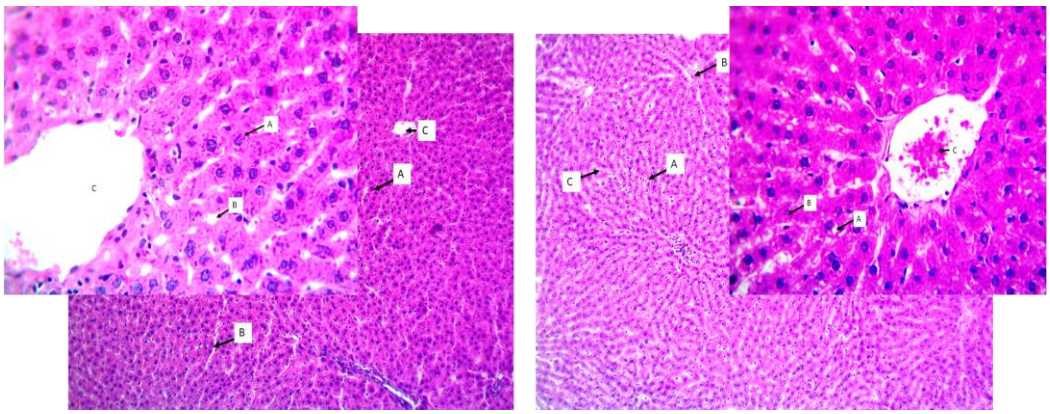

Figure 1. Representative image for liver (left) control, (right) after 3 month stimulation of B3 receptor with Mirabegron. H and E stain, 100X and 400X**.**

On the analysis of liver function enzymes Glutamic oxaloacetic transaminase (GOT) level of the controlled group was 105 U/L which was reduced to approximately 87 U/L on the other hand, in the case of mirabegron induced group the levels reduced significantly from 122.5U/L to 90 U/L. on close analysis of glutamic pyruvic transaminase (GPT) the graph of the controlled group represents no change in the levels and remained constant on 27U/L meanwhile in case of mirabegron group the level fell from 34U/L to 19U/L. the serum level of alkaline phosphates remained the same over 3 months in the controlled group but a

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

reduction has been found in the mirabegron group from approximately 375U/L to 310U/L (Figure 2).

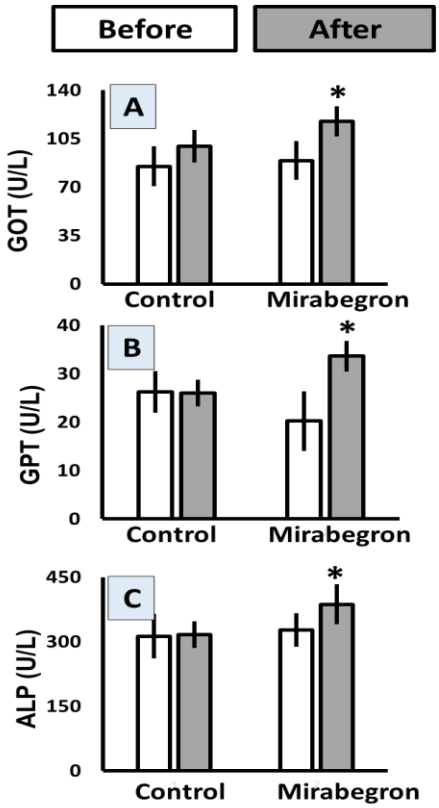

Figure 2. Elevation of liver enzymes [(a) GOT (b) GPT (c) ALP] after stimulation of B3 receptor. Data expressed as mean±SD, $p<0.05$ as compared to baseline before stimulation of B3 receptor or control group.

Discussions

Concerning the previous studies, it has been stated that the use of mirabegron on the chronically ischemic bladder of rats has protective and beneficial outcomes concerning the morphology and bladder function (Hatanaka, Ukai, Watanabe, Someya, Ohtake, Suzuki, Ueshima, Sato and Sasamata, 2013). The orally induced solution of mirabegron absorbs rapidly in the body and remains unchanged throughout circulation which is then defecated through urine and faeces.

Mirabegron follows the renal or metabolic route of elimination considering the hepatic or renal impairment after the use is clinically inconsiderable. Through the following trial, we have concluded that the decrease in hepatic enzymes GOT, GPT and alkaline phosphate highlights the hepatoprotective effect of the drug. Although the product label of mirabegron warns about the occasional elevation of liver enzymes particularly GOT and GPT no published reports have attributed hepatotoxicity to mirabegron. On the examination of histology of liver section in a few studies, mild inflammatory cells and dilated hepatic sinusoids are seen may be due to its metabolism but the evidence regarding liver injury is not known (Sui *et al.*, 2019).In humans, mirabegron is metabolized by an enzyme which is encoded by the CYP2D6 gene known as Cytochrome 2D6 (CYP 2D6) which is primarily expressed in the human liver, the slight inflammation might be seen as a result of metabolism.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 05 May 2022; Accepted: 30 May 2022; Publication: 27 August 2022

Compared to the other antimuscarinic drugs inducing adverse effects such as dry mouth, constipation and effects on the CNS mirabegron are proven to be more safe and efficient with the recommended dose of (25 and 50 mg/day) which is clinically approved (Chapple and Siddiqui, 2017). In a similar trial which compared the effect of mirabegron with another drug solifenacin used for the treatment of overactive bladder, it was found that fewer participants of mirabegron withdrew from the trial because of side effects compared to solifenacin (Vecchioli Scaldazza and Morosetti, 2016).

As per the results of our study mirabegron has controlled the elevation of liver enzymes and significantly reduced the levels over 3 months similarly in a trial the intravesical pressure in anaesthetized rats was examined, on the results, it presented that mirabegron depending on the dose decreased the frequency of nonvoiding contractions with significant effects noted at doses of 1 mg/kg i.v. or more and a dose of 3 mg/kg i.v almost completely diminished them (Hatanaka, Ukai, Watanabe, Someya, Ohtake, Suzuki, Ueshima, Sato and Kaku, 2013).

Conclusion

Mirabegron has significantly elevated liver enzymes and induced slight structural liver changes resulting in liver damage association, cautions need to be raised for use in liver diseases patients and we do recommend a clinical trial on a large sample to confirm the results of our findings.

Acknowledgement: The authors thank the College of Veterinary Medicine/University of Mosul for the facilities provided to accomplish this work.

Funding: Self-Funded

Conflict of Interest: The authors declare that no conflict of interest exists for this research.

References

[1] Bolduc S, Moore K, Nadeau G, Lebel S, Lamontagne P, Hamel M. Prospective open label study of solifenacin for overactive bladder in children. The Journal of urology. 2010 Oct;184(4S):1668-73. https://doi.org/10.1016/j.juro.2010.03.124

[2] Bolduc S, Upadhyay J, Payton J, Bägli D, McLorie G, Khoury A, et al. The use of tolterodine in children after oxybutynin failure. 2003;91(4):398-401. https://doi.org/10.1046/j.1464-410X.2003.04085.x

[3] Brown JS, McGhan WF, Chokroverty S. Comorbidities associated with overactive bladder. Am J Manag Care. 2000 Jul 1;6(11 Suppl):S574-9.

[4] Chapple CR, Siddiqui E. Mirabegron for the treatment of overactive bladder: a review of efficacy, safety and tolerability with a focus on male, elderly and antimuscarinic poor-responder populations, and patients with OAB in Asia. Expert Review of Clinical Pharmacology. 2017 Feb 1;10(2):131-51. https://doi.org/10.1080/17512433.2017.1275570

[5] Chughtai B, Levin R, De E. Choice of antimuscarinic agents for overactive bladder in the older patient: focus on darifenacin. Clinical interventions in aging. 2008 Sep;3(3):503. doi: 10.2147/cia.s3414.

[6] Coyne K, Revicki D, Hunt T, Corey R, Stewart W, Bentkover J, Kurth H, Abrams P. Psychometric validation of an overactive bladder symptom and health-related quality of life questionnaire: the OAB-q. Quality of life research. 2002 Sep;11(6):563-74. https://doi.org/10.1023/A:1016370925601

[7] Deepak Mathur, N. K. V. . (2022). Analysis & Prediction of Road Accident Data for NH-19/44. International Journal on Recent Technologies in Mechanical and Electrical Engineering, 9(2), 13–33. https://doi.org/10.17762/ijrmee.v9i2.366

[8] Gerig N, Cameron T, Arora S, Spear J, Lescozec L, Zhang M. Do we need more patient-friendly treatment options for overactive bladder (OAB)?. Neurourology and Urodynamics. 2021 Aug;40(6):1433-40. https://doi.org/10.1002/nau.24731

[9] Hatanaka T, Ukai M, Watanabe M, Someya A, Ohtake A, Suzuki M, Ueshima K, Sato S, Sasamata M. In vitro and in vivo pharmacological profile of the selective β3-adrenoceptor agonist mirabegron in rats. Naunyn-Schmiedeberg's archives of pharmacology. 2013 Mar;386(3):247-53. https://doi.org/10.1007/s00210-012-0821-4

[10] Hatanaka T, Ukai M, Watanabe M, Someya A, Ohtake A, Suzuki M, Ueshima K, Sato S, Kaku S. Effect of mirabegron, a novel β3-adrenoceptor agonist, on bladder function during storage phase in rats. Naunyn-Schmiedeberg's archives of pharmacology. 2013 Jan;386(1):71-8. https://doi.org/10.1007/s00210-012-0814-3

[11] Gill, D. R. . (2022). A Study of Framework of Behavioural Driven Development: Methodologies, Advantages, and Challenges. International Journal on Future Revolution in Computer Science &Amp; Communication Engineering, 8(2), 09–12. https://doi.org/10.17762/ijfrcsce.v8i2.2068

[12] Herschorn S, Barkin J, Castro-Diaz D, Frankel JM, Espuna-Pons M, Gousse AE, Stölzel M, Martin N, Gunther A, Van Kerrebroeck P. A phase III, randomized, double-blind, parallel-group, placebo-controlled, multicentre study to assess the efficacy and safety of the β3 adrenoceptor agonist, mirabegron, in patients with symptoms of overactive bladder. Urology. 2013 Aug 1;82(2):313-20. https://doi.org/10.1016/j.urology.2013.02.077

[13] Kay GG, Abou-Donia MB, Messer Jr WS, Murphy DG, Tsao JW, Ouslander JG. Antimuscarinic drugs for overactive bladder and their potential effects on cognitive function in older patients. Journal of the American Geriatrics Society. 2005 Dec;53(12):2195-201. https://doi.org/10.1111/j.1532-5415.2005.00537.x

[14] Kreydin EI, Gomes CM, Cruz F. Current pharmacotherapy of overactive bladder. International braz j urol. 2021 Oct 1;47:1091-107. https://doi.org/10.1590/S1677-5538.IBJU.2021.99.12

[15] Lee UJ, Scott VC, Rashid R, Behniwal A, Wein AJ, Maliski SL, Anger JT. Defining and managing overactive bladder: disagreement among the experts. Urology. 2013 Feb 1;81(2):257-62. https://doi.org/10.1016/j.urology.2012.09.028

[16] Mostafaei H, Salehi-Pourmehr H, Jilch S, Carlin GL, Mori K, Quhal F, Pradere B, Grossmann NC, Laukhtina E, Schuettfort VM, Aydh A. Choosing the Most

Efficacious and Safe Oral Treatment for Idiopathic Overactive Bladder: A Systematic Review and Network Meta-analysis. European urology focus. 2021 Sep 22. https://doi.org/10.1016/j.euf.2021.08.011

[17] Sharma, A. (2022). Some Invariance Results for Isometries. International Journal on Recent Trends in Life Science and Mathematics, 9(2), 10–20. https://doi.org/10.17762/ijlsm.v9i2.131

[18] Nasution R, Husein A, Adhyatma KP. Efficacy and safety of mirabegron in pediatric population: A systematic review. International Journal of Surgery Open. 2021 Dec 1;37:100412. https://doi.org/10.1016/j.ijso.2021.100412

[19] Pagoria D, O'Connor RC, Guralnick ML. Antimuscarinic drugs: review of the cognitive impact when used to treat overactive bladder in elderly patients. Current urology reports. 2011 Oct;12(5):351-7. https://doi.org/10.1007/s11934-011-0198-9

[20] Scaldazza CV, Morosetti C. Comparison of therapeutic efficacy and urodynamic findings of solifenacin succinate versus mirabegron in women with overactive bladder syndrome: results of a randomized controlled study. Urologia Internationalis. 2016;97(3):325-9. https://doi.org/10.1159/000445808

[21] Inna Rakhmawati, Anak Agung Ayu Mirah Adi, Ida Bagus Oka Winaya, Palagan Senopati Sewoyo. (2022). In Vivo Oncolytic Potency of Newcastle Disease Virus Gianyar-1/AK/2014 Virulent Strain Against Mice Fibrosarcoma Models. Revista Electronica De Veterinaria, 56 - 63. Retrieved from https://www.veterinaria.org/index.php/REDVET/article/view/139

[22] Sobhgol SS, Charandabee SM. Related factors of urge, stress, mixed urinary incontinence and overactive bladder in reproductive age womenin Tabriz, Iran: a cross-sectional study. International Urogynecology Journal. 2008 Mar;19(3):367-73. https://doi.org/10.1007/s00192-007-0437-2

[23] Sui W, Li H, Yang Y, Jing X, Xue F, Cheng J, Dong M, Zhang M, Pan H, Chen Y, Zhang Y. Bladder drug mirabegron exacerbates atherosclerosis through activation of brown fat-mediated lipolysis. Proceedings of the National Academy of Sciences. 2019 May 28;116(22):10937-42. https://doi.org/10.1073/pnas.1901655116

[24] Wein AJ, Rackley RR. Overactive bladder: a better understanding of pathophysiology, diagnosis and management. The Journal of urology. 2006 Mar;175(3S):S5-10. https://doi.org/10.1016/S0022-5347(05)00313-7