FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2014/2015

Ana Sara dos Santos Ferreira

Mirabegron does not decrease urinary neurotrophins' levels in overactive bladder patients despite symptomatic improvement

março, 2015

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Mestrado Integrado em Medicina

Área: Urologia

Trabalho efetuado sob a Orientação de:
Prof. Doutor Carlos Martins Silva
E sob a Coorientação de:
Dr. Tiago Antunes Lopes

Trabalho organizado de acordo com as normas da revista:
The Journal of Urology®

março, 2015

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200905907	Julho/2015

DESIGNAÇÃO DA ÁREA DO PROJECTO

Urologia

TÍTULO DISSERTAÇÃO (riscar o que não interessa)

Mirabegron does not decrease urinary neurotrophins' levels in overactive bladder patients despite symptomatic improvement

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DEDICATÓRIA

Em primeiro lugar, dedico este trabalho ao Dr.Tiago Antunes Lopes a quem muito agradeço pelo tanto tempo e paciência dispendidos comigo ao longo do último ano, quer na realização das imensas consultas essenciais para este projeto de investigação, quer na escrita do artigo final. Muito devo às numerosas leituras, releituras, correções e dicas que fez ao longo dos últimos meses. Do mesmo modo, dedico também ao Prof. Doutor Carlos Silva pela sua infinita disponibilidade, pelo apoio e pela sugestão do excelente co-orientador que me pôde auxiliar mais de perto.

Dedico ainda, com um carinho muito especial, aos meus pais, que me possibilitaram a oportunidade de estar neste momento a terminar um curso como o de Medicina e que me têm sempre apoiado incondicionalmente.

Por último, e não menos importante, dedico às incríveis pessoas que cresceram comigo nos últimos 6 anos, que estão sempre presentes para me apoiar e alegrar nos bons e maus momentos e que foram também um importante alicerce durante a realização deste trabalho.

A todas estas pessoas o meu mais sincero MUITO OBRIGADO!

Mirabegron does not decrease urinary neurotrophins' levels in overactive bladder patients despite symptomatic improvement

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Runninghead

Mirabegron and urinary neurotrophins in OAB

Conflicts of interest

The authors report no conflict of interests in this work.

ABSTRACT

Purpose – NGF and BDNF play a key role in OAB. Their high urinary levels in OAB patients subsided after successful antimuscarinic treatment. In this study, we investigated, for the first time, urinary NGF and BDNF levels after mirabegron treatment, a β3-adrenoceptor agonist recently approved to treat this disease.

Materials and Methods – Twenty-two female OAB patients were enrolled. If they were taken antimuscarinics and not satisfied, a washout period of 4 weeks was required. As control, fifteen women without LUTS were used. At baseline, urine samples were collected from all participants and KHQ and PPBC questionnaires were completed. OAB patients were treated with mirabegron 50mg, once daily, and reevaluated at 4 and 12 weeks. At these time points, urine sampling and completion of the same questionnaires were carried out. Urine samples were processed for ELISA analysis of NGF and BDNF and the values were normalized against creatinine concentration. Neurotrophins' concentration values were logarithmized to improve distribution characteristics.

Results – At baseline, urinary NGF/Cr and BDNF/Cr were significantly higher in OAB patients compared to controls (NGF/Cr: 3.3±0.6 vs. 2.4±0.5, $p<0.01$; BDNF/Cr: 2.9±0.5 vs. 2.6±0.4, $p=0.028$). After taken mirabegron, NGF/Cr and BDNF/Cr values fell but without statistical significance. Eighteen participants reported marked symptomatic improvement, according to KHQ and PPBC scores.

Conclusions – Mirabegron does not change urinary neurotrophins' levels in OAB patients, despite symptomatic improvement. Since mirabegron acts on bladder smooth muscle, with a reduced effect on the urothelium, which is believed to release most of urinary neurotrophins, it could be a possible explanation.

Keywords

Overactive bladder syndrome, mirabegron, neurotrophins, nerve growth factor, brain-derived neurotrophic factor

INTRODUCTION

Overactive bladder (OAB) is a symptom complex defined by the International Continence Society (ICS) as the presence of urgency, with or without urgency urinary incontinence (UUI), usually associated with frequency and nocturia, in the absence of proven infection or other obvious pathology.[1]

With the continuous rise of life expectancy and consequent increase of the ageing population, OAB has reached an overall prevalence of 12%,[2] a number which may rise to 40% in the population aged more than 75 years old.[3] In addition, as a chronic, recurrent and progressive condition,[4] OAB greatly impairs quality of life and entails substantial economical and social costs.[5, 6]

The underlying pathophysiology of OAB remains elusive.[7, 8] Among the different mechanisms that have been forwaded, the sensitization of bladder primary afferents, leading to an excess of bladder sensory input in the central nervous system, as gained progressive interest.[8] As a consequence, neurotrophins, potent trophic factors, have been implicated in the emergence of OAB symptoms perhaps by changing the threshold of bladder sensory neurons and modulating their synaptic connections at the spinal cord level.[9] Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) constitute the most well-studied neurotrophins in lower urinary tract (LUT). Both neurotrophins[10, 11] are synthesized in the bladder, by urothelial and smooth muscle cells. This led to the investigation of urinary levels of NGF and BDNF in OAB patients.[12] Up till now, several authors have reported high urinary levels of NGF[13, 14] and BDNF[14, 15] in OAB patients that decreased following successful symptomatic treatment with either antimuscarinics[14, 16] or onabotulinum toxin A treatment.[17]

Recently, mirabegron, the first selective β3-adrenoceptor agonist, was approved by Japanese, American and European authorities for OAB treatment.[18] It is the leading agent of a novel class of oral drugs for OAB with a distinct mechanism of action. Mirabegron causes detrusor relaxation during the storage phase of the micturition cycle by activation of adenylyl cyclase, with the subsequent formation of cyclic adenosine monophosphate (cAMP).[19] To our knowledge, there are no studies addressing the effect of mirabegron on urinary levels of neurotrophins in OAB patients. Herewith, we intended to investigate this issue.

MATERIALS AND METHODS

The local ethics committee approved the study. Informed consent was obtained from all participants. Twenty-two female OAB patients with symptoms for at least 3 months were enrolled. Those medicated with antimuscarinics, but not satisfied with the treatment, completed a washout period of 4 weeks. An age-matched group of fifteen women without LUTS, including hospital and faculty employees and their relatives, were used as controls.

OAB followed the ICS clinical definition as a symptom complex marked by urgency, with or without urgency incontinence, usually with frequency and nocturia, in the absence of an underlying metabolic or pathologic condition.[1] Evaluation included anamnesis, physical examination, urinalysis, urine culture and cytology and bladder and renal ultrasound. Exclusion criteria were age less than 18 years, pregnancy, neurological disease, stress urinary incontinence, active UTI within 3 months, recurrent UTI, any systemic infectious or inflammatory condition, bladder outlet obstruction, significant pelvic organ prolapse, previous bladder or urethral surgery, urothelial neoplasms, previous pelvic radiotherapy, hematuria, urolithiasis and treatment with intravesical onabotulinum toxin A in the last 18 months.

At baseline, urine samples were collected from all participants and King's Health Questionnaire (KHQ)[20] and Patient Perception of Bladder Condition (PPBC) questionnaire[21] were completed. A higher score in KHQ indicates lower health-related quality of life and a higher PPBC score denotes more severe bladder problems.

OAB patients were treated with mirabegron 50mg, once daily, for three months, if it was well tolerated. During this period, the drug was provided by the local pharmacy without economical expenses for the patients. The following evaluations were performed after 4 and 12 weeks. At these time points, urine sampling and completion of the same questionnaires were carried out.

Urine collection was made in the outpatient clinic and samples were stored on an ice container at 4ºC for less than 2 hours. Afterwards, they were centrifuged at 3,000 rpm for 10 minutes. Supernatant was collected in 1ml aliquots and the remainder was used to determine the creatinine concentration. Aliquots were frozen at -80ºC until further processing. Samples were thawed and processed for enzyme-linked immunosorbent assay (ELISA) analysis of NGF and BDNF concentrations with the Emax® ImmunoAssay System (Promega, USA), following the manufacturer instructions. The amount of NGF/BDNF in the sample is measured at 450nm with a Synergy HT Microplate Reader (BioTek Instruments, USA). All samples were run in duplicate and values were averaged against a standard curve generated with known

amounts of NGF/BDNF. The urine NGF and BDNF content was normalized to creatinine (Cr) concentration (NGF/Cr and BDNF/Cr ratio – pg/mg). Neurotrophins' concentration values were logarithmized to improve distribution characteristics.

Data were expressed as the mean ± standard deviation (SD). The Mann-Whitney U test and Wilcoxon signed-rank test were used for statistical analysis between groups when considering nonparametric data, while paired samples t-test was used for analysis of parametric data. Statistical significance was considered at $p<0.05$.

RESULTS

In the group of OAB patients, the mean age was 62 ±14 years, while in control group was 41 ± 12 years. At baseline, urinary NGF/Cr and BDNF/Cr were significantly higher in OAB patients compared to healthy controls (**table 1**). During follow-up, two patients were excluded due to UTI, one at 4 weeks and the other at 12 weeks.

After treatment with mirabegron, there was a slight decrease in mean urinary NGF/Cr and BDNF/Cr ratios, which however did not reach statistical significance ($p>0.05$) (**Figure 1**). Eighteen out of twenty-two patients reported marked symptomatic improvement, reflected in the variations of KHQ and PPBC scores. It is important to notice that a higher score in KHQ indicates lower health-related quality of life and a higher PPBC score denotes more severe bladder problems. Data are summarized in **table 2**.

DISCUSSION

This is the first study investigating NGF and BDNF changes in the urine of OAB patients treated with mirabegron, the first β3-adrenoceptor agonist to enter clinical practice.

According to our findings, OAB symptoms are associated with high urinary levels of NGF and BDNF. Mirabegron does not significantly change urinary levels of both neurotrophins, despite causing marked symptomatic improvement. These results are in contrast with previously published observations carried out with antimuscarinics[14, 16] and onabotulinum toxin A treatment.[17]

In the bladder, muscarinic receptors M2 and M3 can be found in the urothelium, interstitial cells, afferent nerves and detrusor muscle.[22] In smooth muscle cells, the activation of these receptors involve different intracellular pathways and determine opposed effects. M1, M3, and M5 receptors are excitatory whereas M2 and M4

receptors are inhibitory. Although the M2 receptors predominate in the detrusor, the M3 receptors mediate the central part of the contraction.[23] Muscarinic receptors are functionally coupled to G proteins but the signal transduction systems vary. The M3 receptor-mediated contractile response in the bladder depends on intracellular calcium increase through activation of phospholipase C, nifedipine-sensitive channels and the Rho-kinase pathway.[22] Contrariwise, the functional role of M2 receptor is still not well clarified. They may enhance contractions mainly by inhibition of detrusor relaxation. M2 stimulation may reduce adenylyl cyclase activity, inhibit potassium channels and affect nonselective cation and transient receptor potential channels.[23]

In addition, it has been proposed that another mechanism underlying antimuscarinics effects in the treatment of OAB symptoms is the inhibition of afferent signaling from the bladder.[24, 25] Taking into account that both urothelial and detrusor smooth muscle cells express muscarinic receptors and are able to synthesize and release neurotrophins,[26] it is conceivable that antimuscarinics may hamper neurotrophins secretion, increasing the threshold of bladder sensory fibers[16] and causing the reduction of urgency episodes. In this particular, it should be noted that detrusor cells are situated deep within the bladder wall. Therefore, neurotrophins released by the muscle have smaller chance of reaching the bladder lumen than neurotrophins released from urothelial cells, as they would have to cross the entire lamina propria and urothelium before reaching the bladder cavity.[26] This means that the most probable source of urinary neurotrophins, which we can see decreasing in the urine of OAB patients after satisfactory antimuscarinic treatment,[14, 16] is thought to be the urothelium.

As an agonist of β3-adrenoceptor, it has been proposed that mirabegron induces bladder relaxation by increasing adenylyl cyclase, and subsequently cAMP, in detrusor smooth muscle cells, during urine storage. In addition, recent studies suggest that, in the bladder, potassium channels, and particularly big potassium (BK) channels, may be involved in β-adrenoceptor-mediated relaxation independently of cAMP.[27]

Although β3-adrenoceptors are known to be abundant in the detrusor smooth muscle cells, little is known about its expression in human urothelial cells. Some authors have demonstrated the presence of β3-adrenoceptors in urothelial cells, using RT-PCR and immunohistochemistry techniques.[28, 29] However, up to now, the functional role of β3-adrenoceptor in human urothelium, cannot be reliably concluded.[29] Functional studies suggested that urothelial β-adrenoceptors induce the release of a urothelium-derived factor which inhibits the β-adrenoceptor agonist-induced relaxation of the human detrusor smooth muscle.[29]

Thus, the capacity of mirabegron to influence the release of neurotrophins by urothelial cells may be substantial less than that of the antimuscarinic drugs, which might explain the lack of effect of mirabegron on the urinary levels of NGF and BDNF found in the present study. Interestingly, these findings may agree with the recent observation by Igawa and coworkers that mirabegron predominantly affects the Aδ population of bladder sensory afferents, which are known to be little sensitive to neurotrophins.[30] Nonetheless, it must be clear that, whatever the causes for the minor effect of mirabegron on urinary neurotrophins' levels, it does not impair the clinical efficacy of the drug. Our patients observed a remarkable symptomatic benefit and a relevant improvement in quality of life.

This study has some obvious limitations. Sample size was small, restricted to one center and no placebo arm was considered.

CONCLUSIONS

In contrast with other information about antimuscarinic agents, mirabegron seems not to produce significant changes in urinary levels of neurotrophins. However, this study confirms that it can cause marked symptomatic improvement. These data reduces NGF and BDNF utility in monitoring response to mirabegron in OAB.

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TABLES AND FIGURES

Table 1

		Controls (n=15)	**OAB patients** (n=22)	***p***
NGF/Cr	pg/mg	2,4 ± 0.5	3.3 ± 0.6	<0,01
BDNF/Cr	pg/mg	2.6 ± 0.4	2.9 ± 0.5	0,028

Table 1. Mean urinary NGF/Cr and BDNF/Cr ratios in female OAB patients and controls. NGF/Cr and BDNF/Cr were significantly higher in patients with OAB than in healthy women (p <0.05).

Table 2

			Baseline (n=22)	**4 weeks** (n=21)	**12 w**eeks (n=20)
NGF/Cr		pg/mg	3.3 ± 0.6	3.2 ± 0.5	3.0 ± 0.8
BDNF/Cr		pg/mg	2.9 ± 0.5	2.7 ± 0.6	2.7 ± 0.5
KHQ					
Part 1	General Health Perception Incontinence Impact	0 – 100%	58 ± 18 75 ± 21	56 ± 13 48 ± 27 *	55 ± 19 49 ± 31 *
Part 2	Role Limitations Physical Limitations Social Limitations Personal Relationships Emotions Sleep/Energy Severity Measures	0 – 100%	71 ± 17 66 ± 24 24 ± 23 13 ± 26 43 ± 21 49 ± 27 42 ± 23	38 ± 32 * 40 ± 30 * 7 ± 15 * 8 ± 20 15 ± 20 * 17 ± 21* 22 ± 21 *	32 ± 30 * 27 ± 27 * 7 ± 21 * 12 ± 24 9 ± 18 * 18 ± 18 * 24 ± 21 *
Part 3	Frequency Nocturia Urgency Urgency Incontinence	Severity Scale: 0 – Omitted 1 – A little 2 – Moderately 3 – A lot	2.3 ± 0.6 1.4 ± 0.9 2.5 ± 0.5 2.1 ± 0.8	1.9 ± 0.8 * 0.3 ± 0.6 * 1.5 ± 0.6 * 0.8 ± 1.0 *	1.5 ± 0.7 * 0.5 ± 0.7 * 1.5 ± 0.7 * 1.0 ± 1.2 *
PPBC		Severity Scale: 1 – 6	3.6 ± 0.9	2.4 ± 1.0 *	2.4 ± 1.4 *

Data is expressed as the mean ± standard deviation

Higher KHQ subscale score indicates lower health-related quality of life

Higher PPBC score indicates more severe bladder problems

** a p<0.05 is statistically significant when compared to baseline*

Table 2. Mean urinary NGF/Cr and BDNF/Cr ratios, KHQ and PPBC scores in OAB patients at baseline, after 4 weeks and after 12 weeks of treatment with mirabegron 50mg, once daily. No significant changes

occurred in NGF/Cr and BDNF/Cr ratios during treatment, despite a statistical significant decrease in the mean KHQ and PPBC scores.

Figure 1

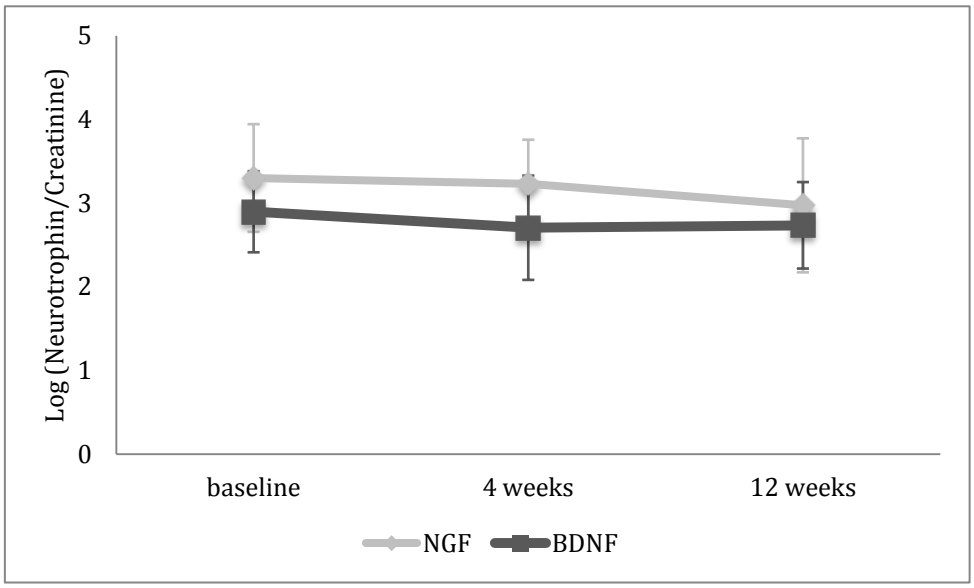

Figure 1. Mean urinary NGF/Cr and BDNF/Cr ratios (tick marks) in OAB patients at baseline (n=22), after 4 weeks (n=21) and after 12 weeks (n=20) of treatment with mirabegron 50mg, once daily. No significant changes occurred in NGF/Cr and BDNF/Cr ratios during treatment. Statistical significance was considered at $p<0.05$.

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Original and Research Articles should be arranged as follows: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, References, Tables, Legends. The title page should contain a concise, descriptive title, the names and affiliations of all authors, and a brief descriptive runninghead not to exceed 50 characters. One to five key words should be typed at the bottom of the title page. These words should be identical to the medical subject headings (MeSH) that appear in the Index Medicus of the National Library of Medicine. The abstract should not exceed 250 words (abbreviations are not to be substituted for whole words) and must conform to the following style: Purpose, Materials and Methods, Results and Conclusions.

References should not exceed 30 readily available citations for all articles (except Review Articles). Self-citations should be kept to a minimum. References should be cited by superscript numbers as they appear in the text, and they should not be alphabetized. References should include the names and initials of the first 3 authors, the complete title, the abbreviated journal name according to the Index Medicus of the National Library of Medicine, the volume, the beginning page number and the year. References to book chapters should include names and initials of the first 3 chapter authors, chapter title, book title and edition, names and initials of the first 3 book editors, city of publisher, publisher, volume number, chapter number, page range and year. In addition to the above, references to electronic publications should include type of medium, availability statement and date of accession. The statistical methods should be indicated and referenced. Enough information should be presented to allow an independent critical assessment of the data.

Digital illustrations and tables should be kept to a necessary minimum and their information should not be duplicated in the text. No more than 10 illustrations should accompany the manuscript for clinical articles. Magnifications for photomicrographs should be supplied and graphs should be labeled clearly. Reference to illustrations, numbered with Arabic numerals, must be provided in the text. Blurry or unrecognizable illustrations are not acceptable. Visit http://www.elsevier.com/author-schemas/artwork-and-media-instructions for detailed instructions for digital art. The use of color is encouraged at no charge to the authors.

Tables should be numbered and referred to in the text. In general, they should present summarized rather than individual raw data. Due to page constraints caused by the large number of high quality manuscripts being submitted to *The Journal of Urology,* the editors find it necessary to offer publishing alternatives. Therefore, authors may be requested to post tables and illustrations as supplementary material on The Journal website at no charge or print tables and illustrations in the article at a per page rate of $236.

Letters to the Editor should be useful to urological practitioners. The length should not exceed 500 words. Only Letters concerning articles published in the Journal within the last year are considered.

Review Articles (comprehensive only) should not be submitted without prior approval. Queries for these articles should be accompanied by a detailed outline of the proposed article, an abstract not to exceed 750 words and an estimate of the length of the manuscript to be submitted. The format is the same as that of an Original Article.

Systematic reviews do not require prior approval for submission, and are limited to 2500 words and 30 references.

Special Articles are scientific reports of original clinical research and state-of-the-art topics, and are designated as such by the Editors. The format is the same as that of an Original Article.

New Technology and Techniques feature high quality manuscripts that describe the innovative clinical application of new technology or techniques in all disciplines of urology, and are designated as such by the Editors. Addressing diagnosis or management of urological conditions, this feature covers the categories of 1) cutting-edge technology, 2) novel/modified techniques and 3) outcomes data derived from use of 1 and/or 2. The format is the same as that of an Original Article, although fewer words are preferred to allow more space for illustrations.

Opposing Views are submitted by invitation only.

Video Clips may be submitted for posting on The Journal web site. They are subject to peer review. Video files must be compressed to the smallest possible size that still allows for high resolution and quality presentation. The size of each clip should not exceed 10MB. File size limitation is intended to ensure that end-users are able to download and view files in a reasonable time frame. If files exceed the specified size limitation, they will not be posted to the web site and returned to the author for resubmission. For complete instructions e-mail: publications@auanet.org.

APÊNDICE(s)

Comprovativo de aprovação pela

Comissão de Ética para a Saúde

Ao CA

7.4.2014

3.4.2014

Tomei conhecimento. Nada a opor. Proponho que a autorização do projecto pelo CA sirva simultaneamente de autorização das despesas respectivas, a pagar com verbas do serviço de Urologia, num valor correspondente a 3000 (três mil) euros, para a terapêutica considerada (vale o dito pelo telefone, junho do Prof. Carlos Silva).

Exmo. Senhor

Presidente da Comissão de Ética para a Saúde do Hospital de S. João – EPE

Centro Hospitalar São João
Centro de Investigação
Prof. Dra. Ana Azevedo
Coordenadora CIC

Assunto: Pedido de apreciação e parecer para estudo/projecto de investigação; adenda a projecto de investigação já submetido e aprovado em 2011 – "O Papel dos Factores Neurotróficos nas Disfunções Miccionais – Implicações Fisiopatológicas e Clínicas".

Nome do Investigador Principal: Tiago Vieira da Conceição Antunes Lopes

AUTORIZADO

CONSELHO DE ADMINISTRAÇÃO REUNIÃO DE 17 ABR 2014

Presidente do Conselho de Administração (Prof. Doutor António Ferreira)

Directora Clínica (Dra. Margarida Tavares) | Enfermeira Directora (Enfermeira Eurídice Portela) | Vogal Executivo (Dr. João Oliveira) | Vogal Executivo (Dr. Arnaldo Ferreira)

Título do projecto de investigação: Adenda a projecto de investigação submetido previamente (2011): "O Papel dos Factores Neurotróficos nas Disfunções Miccionais – Implicações Fisiopatológicas e Clínicas".

O título da adenda ao projecto é: UNIOM – Urinary Neurotrophins in Idiopathic OAB Patients Treated with Mirabegrom

Pretendendo realizar no(s) Serviço(s) de Urologia do Hospital de S. João – EPE uma adenda a um projeto de investigação previamente submetido em 2011, solicito a V. Exa., na qualidade de Investigador/Promotor, a sua apreciação e a elaboração do respectivo parecer.

Para o efeito, anexo toda a documentação referida no dossier dessa Comissão respeitante a estudos/projectos de investigação.

Com os melhores cumprimentos.

Porto, 22 / Janeiro / 2014

O INVESTIGADOR/PROMOTOR

SÃO JOÃO
HOSPITAL

Comissão de Ética para a Saúde do HSJ

Parecer

Adenda intitulada "UNIOM – Urinary Neurotrophins in idiopathic OAB Patients treated with Mirabegrom" ao projecto de investigação submetido previamente (2011) intitulado "O papel dos fatores neurotróficos nas disfunções miccionais – implicações fisiopatológicas e clínicas".

O principal objectivo desta adenda consiste em investigar os níveis urinários de fatores neurotróficos (NGF; BDNF) em mulheres com síndroma de bexiga hiperativa antes e após o tratamento com o agonista seletivo dos receptores beta-3 Mirabegrom (fármaco recentemente aprovado pela FDA e pela EMEA como tratamento de primeira linha para o síndroma de bexiga hiperativa). O fármaco será fornecido às doentes pela farmácia do CHSJ, sendo financiado pelo Serviço de Urologia. *Solicita-se esclarecimento adicional sobre a questão do financiamento, designadamente por existir uma contradição, respetivamente nas páginas I e V do formulário da CES. Deverão ser melhor explicitados os pressupostos em que assenta o fornecimento de Mirabegrom pelo CHSJ, aos doentes com síndroma de bexiga hiperativa.*

Como benefícios potenciais do estudo pode considerar-se o fato das doentes terem acesso por seu intermédio a uma medicação de primeira linha para o tratamento sintomático do síndroma de bexiga hiperativa, durante a participação no estudo, não estando previstos riscos ou incómodos.

Está prevista a realização de questionários, dos quais *deverão ser facultadas as correspondentes cópias.* Está prevista a obtenção de consentimento informado, que é acompanhado de uma informação para as participantes que é esclarecedora sobre a natureza do estudo e que salvaguarda as questões éticas relevantes. Os exames a realizar são os que estão incluídos no padrão de acompanhamento dos doentes com esta patologia.

O investigador dispõe da competência científica para a realização do estudo, mas *deverá incluir-se autorização do diretor do serviço de Urologia.*

Em face da análise do protocolo proponho que a sua aprovação pela CES do HSJ fique a aguardar pela resposta do investigador às questões em itálico.

Porto, 25 de Fevereiro de 2014

Em face da resposta do investigador proponho a aprovação do protocolo pela CES do CIB

21.05.2014

relator

Prof. Manuel Pestana

8. TERMO DE RESPONSABILIDADE

Eu, abaixo-assinado, Tiago Vieira da Conceição Antunes Lopes, Interno de Formação Específica de Urologia, nº mecanográfico 1636, na qualidade de Investigador Principal, declaro por minha honra que as informações prestadas neste questionário são verdadeiras. Mais declaro que, durante o estudo, serão respeitadas as recomendações constantes da Declaração de Helsínquia (com as emendas de Tóquio 1975, Veneza 1983, Hong-Kong 1989, Somerset West 1996 e Edimburgo 2000) e da Organização Mundial da Saúde, no que se refere à experimentação que envolve seres humanos.

Porto, 21 / Janeiro / 2014

O Investigador Principal

PARECER DA COMISSÃO DE ÉTICA PARA A SAÚDE DO HOSPITAL DE S. JOÃO

emitido na reunião plenária da CES de 24 / Março / 2014

A Comissão de Ética para a Saúde APROVA por unanimidade o parecer do Relator, pelo que nada tem a opor à realização deste projecto de investigação.

Prof. Doutor Filipe Almeida
Presidente da Comissão de Ética