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Thesis autoreferate

**NOVEL TREATMENT MODALITIES FOR PELVIC FLOOR
DYSFUNCTION**

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SUMMARY IN CZECH

Naším cílem bylo testování vybraných nových léčebných modalit pro dysfunkci pánevního dna a genitourinární syndrom menopauzy. Oba stavy jsou u žen běžné a negativně ovlivňují kvalitu jejich života. Současné možnosti léčby však nejsou optimální.

Náš výzkum jsme zahájili rozsáhlým studiem literatury. Nejprve jsme shrnuli informace o zvířecích modelech a jejich vlastnostech pro zkoumání patofyziologie POP a nových terapií. Ze všech zkoumaných zvířat se spontánně vyvine POP pouze u primátů, jejich použití je však kontroverzní. Došli jsme k závěru, že mnoho studií má metodologické nedostatky a postrádá standardizaci, ale že existuje několik zvířat, která lze použít jako chirurgický model pro POP, každé z nich je vhodně pro jiné účely. Pro náš pozdější výzkum jsme vybrali kysu pro opravu POP syntetickou sítkou.

Systematicky jsme také prozkoumali literaturu o objektivních účincích neablativního Er: YAG LASER na kůži a vaginální stěnu. Energie Er: YAG LASERU indukuje měřitelné změny v hlubších vrstvách kůže nebo vaginální stěny procesem buněčné aktivace, produkce extracelulární matrix a remodelace tkáně, nicméně úroveň důkazů byla nízká s vážným rizikem bias ve většině článků a širokým spektrem měřených parametrů. Tato review nám pomohla navrhnout design pozdější studie na zvířatech. Prozkoumali jsme také literaturu o LASER terapii pro POP a UI. Všechny studie uváděly zlepšení POP nebo UI po použití LASERU, ale kvalita studií byla většinou špatná a riziko bias vážné. Bohužel i zde byla široká heterogenita nastavení LASERU, aplikačních protokolů a zkoumaných parametrů a vysoká míra bias. Tato review pomohla naší skupině navrhnout tři RCT, jednu pro GSM, jednu pro POP a jednu pro inkontinenci moči; Těmito studii se nyní zabývají mí mladší kolegové.

V experimentální části své práce jsem provedla dvě translační studie na zvířatech. V první jsme provedli randomizovanou kontrolovanou studii zaměřenou na měření účinků neablativního Er: YAG LASER na vaginální atrofii v ovčím menopauzálním modelu. Er:YAG LASER jsme srovnali s vaginální manipulací a systémovým podáním estrogenů. Ukázali jsme, že jak vaginální epiteliální tloušťka, tak vaginální

poddajnost byly modifikovány aplikací LASERU a vaginální manipulace v podobném rozsahu. Tato modifikace byla menší, než bylo pozorováno u zvířat po systémové náhradě estrogeneru.

V druhém experimentu jsme preklinicky testovali síťku z polyvinylidenfluoridu (PVDF), používanou pro chirurgickou opravu POP. Materiál jsme implantovali do incizionálního krysího modelu břišní kýly. Porovnali jsme výsledky s těmi, které byly získány po implantaci strukturálně identické sítky vyrobené z polypropylenu (PP). Hlavním výstupním měřením bylo biomechanické chování explantátů, dále jsme zkoumali zánětlivou reakci hostitele a integraci sítky do tkáně hostitele. Biomechanické testování neprokázalo žádný rozdíl mezi těmito dvěma materiály. Také reakce hostitele a integrace byly téměř totožné. Vedlejším pozorováním je, že oba implantáty způsobily určitý stupeň svalové atrofie v pozdějších časových bodech. Závěrem lze říci, že léčba GSM, POP a UI Er: YAG laserem není dostatečně podložena kvalitními důkazy. Za druhé, v ovčím modelu menopauzy nemá laserová terapie jiný účinek než vaginální manipulace a obě terapie mají menší účinek než systémové estrogenery. Za třetí, u krysího modelu implantáty, které mají stejnou textilní strukturu, ale jsou vyrobeny z jiného polymeru (PVDF nebo PP), a proto mají jinou hmotnost, vytvářejí stejné biomechanické vlastnosti, odezvu hostitele a integraci tkáně. Oba ve střednědobém horizontu vyvolávají svalovou atrofii.

SUMMARY IN ENGLISH

We aimed to test selected novel treatment modalities for pelvic floor dysfunctions and genitourinary syndrome of menopause. Both conditions are common in female, they negatively affect their quality of life, and current treatment options are not optimal.

We started our research with an extensive literature search. First, we summarized the information on animal models for, and the utility they may have in the investigation of the pathophysiology of POP and novel therapies. From all screened animals, only the non-human primate develops POP spontaneously, however their use is controversial. We concluded that many studies have methodological shortcomings and lack standardization in reporting outcomes. Also,

several other animals can be used as a model of surgery for POP, each of them with different purposes. For our later research we chose the rat model to simulate POP repair with synthetic mesh.

We also systematically reviewed the literature on the objective effects of non-ablative Er:YAG LASER on the skin and vaginal wall. Er:YAG LASER energy induces measurable changes in the deeper skin or vaginal wall by a process of cell activation, production of extracellular matrix and tissue remodelling, however the evidence level was low with serious risk of bias in most articles, and a wide spectrum of outcome measures. This review helped us to build the design of an animal study. We also reviewed the literature on LASER therapy for POP and UI. We included 31 studies on 1530 adult women. All studies reported an improvement of POP or UI after LASER use, but the quality of studies was mostly poor and risk of bias serious. Unfortunately, there was a wide heterogeneity of LASER settings, application protocols and outcome measures. That review helped our group designing three RCT, one in GSM, one in POP and one for urinary incontinence; work that will be reported by one of my successors.

In the experimental part of my thesis, I conducted two animal translational studies. In the first we did a randomised controlled trial aiming to measure effects of non-ablative Er:YAG LASER on vaginal atrophy in the ewe menopausal model, as compared to sham and oestrogen application. We demonstrated that both the vaginal epithelial thickness as well as the vaginal compliance were modified by LASER and SHAM manipulation to a similar extent, but less than what was observed following systemic oestrogen replacement.

In the second experiment we preclinically tested a polyvinylidene fluoride (PVDF) mesh, used for POP surgical repair. We implanted the material in the rat incisional abdominal hernia model. We compared outcomes to those obtained after implantation of a structurally identical mesh but made from polypropylene (PP). Main outcome measure was biomechanical behaviour of explants, next to host inflammatory response and tissue integration. Biomechanical testing showed no difference between the two materials. Also, the host response and tissue integration were almost identical, and both implants caused ultimately some degree of muscle atrophy in later

time points. In conclusion, we first demonstrate there is no difference in host response to implants either made from PP or PVDF when they have the same textile properties.

In conclusion, treatment of GSM, POP, and UI with Er: YAG laser is not supported by good quality evidence. Second, in the ovine menopause model laser therapy has an effect that is no different from that of sham manipulation, and both have less effects than systemic oestrogens. Third, in the rat model, implants that have an identical textile structure but that are made from a different polymer (PVDF or PP), hence have a different weight, generate the same biomechanical properties, host response and tissue integration. Both induce muscle atrophy on the medium term.

INTRODUCTION

In the last century, life expectancy has increased rapidly in the developed world. In Belgium resp. in the Czech Republic the average life expectancy is 83.5 resp. 81.7 year for women.¹ However, longevity is not necessarily paralleled with a sustained quality of life, which is decreasing with age.² Herein we will focus on selected age-related health changes in aging women, more specifically on genitourinary syndrome of menopause (GSM) and selected forms of Pelvic floor disorders (PFD).

PFD comprise a wide spectrum of interrelated conditions including pelvic organ prolapse (POP) and urinary and anal incontinence (UI, AI). The underlying early cause of most PFD are nerve, levator muscle and anal sphincter injuries in the context of pregnancy and vaginal delivery.³ Most injuries often remain undiagnosed as they are not immediately bothering or suspected, yet predispose for symptoms later in life⁴ as well as recurrence after treatment.⁵ In POP patients, native tissue repair is the standard surgical treatment yet it has a 30% recurrence and reintervention rate.^{6,7} For that purpose mesh is used, yet graft related complications became a concern, in particular for vaginal mesh insertion.⁸ This seems to be mainly driven by surgical, patient yet also mesh related factors. In essence, the host response is dependent on the nature of the graft material.⁸ Therefore new polymers and textile structures are being considered, which one can and should test preclinically.⁹ The polymer PolyVinylideneFluoride

(PVPDF) is new to pelvic floor meshes, and is claimed to induce a different and more moderate host response.¹⁰ This claim has however not been substantiated, in particular not at the gene expression level.

Genitourinary syndrome of menopause (GSM) is caused by a lack of oestrogens. In menopausal women, vaginal epithelial thickness decreases as well as the amount of collagen and elastin fibers¹¹ leading to frailty of the vagina. Also the vaginal microbiome is changing, a distinct bacterial state with relative low abundance of Lactobacillus is associated with GSM.¹² This results in complaints such as dryness, irritation, dyspareunia and contact bleeding.¹¹ GSM occurs in up to 70% of women after the menopause¹³ and has considerable influence on quality of life and sexual satisfaction.¹⁴ When occurring earlier, e.g. following ovariectomy, the symptoms are usually worse.¹⁵ The gold standard for treatment of GSM is local or systemic application of estrogens.¹¹ This treatment is efficient but not widely accepted a.o. because of its perceived and true risks. Higher levels of oestrogens in the menopause increase the risk of breast and endometrial cancer, which is known to the lay public.¹⁶ Moreover, in women treated for oestrogen-dependent malignancies systemic hormonal treatment is often discouraged. Recently, LASER therapy of GSM was introduced, based on previous experience with skin rejuvenation proven by years of in-vitro and preclinical research¹⁷. Vaginal rejuvenation with LASER is claimed to reverse GSM symptoms without additional risks¹⁸, but in contrast to skin rejuvenation, robust evidence is missing¹⁹. Few clinical studies have been published, describing the histological changes in the vaginal wall following LASER application²⁰⁻²². However, no translational research underpins this claim so far.

SPECIFIC STUDY AIMS:

The overall aim of my research was to experimentally assess novel treatment modalities for PFD and GSM.

Literature search:

- Hypothesis: animal models used in urogynecological research are not well characterized and probably misused
- Aim was to summarize current knowledge on animal models in the study of in the pathogenesis and treatment of selected PFD, and on the use of LASER therapy for GSM and PFD.

Experimental studies:

- Hypothesis: Vaginal Er:YAG laser effect is not different from vaginal manipulation
- Aim: Preclinically the effects of vaginal LASER therapy, using the sheep menopausal model.

- Hypothesis: host response and biomechanical behaviour to PVDF mesh is comparable to PP mesh of the same textile structure.
- Aim: to test preclinically a novel mesh material for pelvic floor surgery, i.e. a PVDF mesh, which is implanted in the rat abdominal hernia model and compare it to standard polypropylene mesh.

MATERIAL AND METHODS

In the **theoretical part** of this thesis, we have summarized the current knowledge on animal models for, and the utility they may have in the investigation of the pathophysiology of POP and novel therapies in 4 review articles.

In the first article - systematic review²³, we searched MEDLINE, Embase, Cochrane and the Web of Science to establish what animal models are used in the study of suggested risk factors for the development of POP, including pregnancy, labour, delivery, parity, aging and menopause. Lack of methodological uniformity precluded meta-analysis hence results are presented as a narrative review.

In the second article - literature review²⁴, we summarize the recent literature on the use of different animal models for testing existing and new materials for treatment of pelvic organ prolapse.

In the third article - systematic review²⁵, we searched MEDLINE, Embase, Cochrane and the Web of Science. Included were studies investigating objectively measured effects of non-ablative Er:YAG laser on the skin or vaginal wall. Included were studies of any designs. Due to the lack of methodological uniformity, no meta-analysis could be performed and therefore results are presented as a narrative review.

In the fourth article – systematic review²⁶, we searched Pubmed, Web Of Science and Embase were searched for relevant articles, using a three concept (POP, UI, LASER therapy) search engine composed as (concept 1 OR concept 2) AND concept 3. Only full text clinical studies in English. Data on patient characteristics, LASER setting, treatment outcome and adverse events were independently collected by two researchers. Due to the lack of methodological uniformity meta-analysis was not possible and results are presented narratively.

In the experimental part of my thesis, I conducted two animal translational studies.

In the first experimental study²⁷, menopause was surgically induced, after which the ewes were randomised to three groups receiving either (1) vaginal Er:YAG LASER application three times, with a one-month interval; (2) three sham manipulations with a month interval; or (3) oestrogen replacement and sham manipulations. At given intervals, ewes were clinically examined, and vaginal wall biopsies taken. Vaginal compliance was determined by passive biomechanical testing from explants taken at obduction.

In the second experimental study^(manuscript in preparation), we designed an experiment in a rat incisional hernia model, wherein two implants with identical textile structure were compared, and that only differed

by the polymer they were made off. Tested polymers were polypropylene (PP), which is most widely used, and polyvinylidene fluoride (PVDF), proposed for its milder host response and higher compliance. Passive biomechanical testing of explants revealed no differences between the two polymers. Only at one time-point (90d), explant with PP was stiffer compared to SHAM operated animals.

RESULTS

In the first article - systematic review²³, 7,426 studies were identified of which 51 were included in the analysis. Pregnancy has a measurable and consistent effect across species. In rats, simulated vaginal delivery induces structural changes in the pelvic floor, without complete recovery of the vaginal muscular layer and its microvasculature, though it does not induce POP. In sheep, first vaginal delivery has a measurable effect on vaginal compliance; measured effects of additional deliveries are inconsistent. Squirrel monkeys can develop POP. Denervation of their levator ani muscle facilitates this process in animals that delivered vaginally. The models used do not develop spontaneous menopause, so it is induced by ovariectomy. Effects of menopause depend on the age at ovariectomy and the interval to measurement. In several species menopause is associated with an increase in collagen content on the longer term. In rodents there were no measurable effects of age apart of elastin changes. We found no usable data for other species.

In the second article – literature review²⁴, we have found that though animal experimentation with novel candidate implants is advocated, there is a lack of standardization in reporting. The concept of resorbable construct is being revived, as durable materials have caused clinical graft related complications. Large animal experiments seem to provide interesting and more comprehensive information, yet their use may be contested.

In the third article - systematic review²⁵, we identified *in vitro* or *ex vivo* studies on human cells or tissues, studies in rats and clinical studies. Most studies were on the skin (n=11); the rest were on the

vagina (n=4). The quality of studies is limited and setting of the laser was very diverse. Though the methods used were not comparable, there were demonstrable effects in all studies. Immediately after application the increase of superficial temperature, partial preservation of epithelium and subepithelial extracellular matrix coagulation were documented. Later, an increase of epithelial thickness, inflammatory response, fibroblast proliferation, an increase of collagen amount and vascularization were described.

In the third article - systematic review²⁶, thirty one studies recruiting 1530 adult women met the inclusion criteria. All studies showed significant improvement either on UI, POP or both, however the heterogeneity of LASER settings, application and outcome measures was huge. Only one study was a randomized controlled trial, two studies were controlled cohort studies. All three were on UI and used standardized validated tools. The risk of bias in the RCT was low on all seven domains; the controlled studies had a serious risk of bias. No major adverse events were reported, mild pain and burning sensation were the most common described adverse events.

In the fifth article - experimental study²⁷, we have found out that animals exposed to Er:YAG LASER application and sham manipulation, but not to oestrogens, displayed a significant and comparable increase in vaginal epithelial thickness between baseline and seven days after the third application (69% and 67% respectively, both $p < .0008$). In LASER treated ewes, temporary vaginal discharge and limited thermal injury were observed. Oestrogen substituted ewes displayed a more prominent increase in epithelial thickness (202%; $p < .0001$) and higher vaginal compliance ($p < .05$). None of the interventions induced changes in the lamina propria.

In the sixth article - experimental study^(manuscript in preparation), the rate of clinical complications was very low for both implants. Differences in gene expressions, histology and immunohistochemistry were small. In rats, implants with an identical textile structure, though based on a different polymer with a different weight, induce a

comparable host response and tissue integration, without measurable difference in passive biomechanical properties.

DISCUSSION AND CONCLUSIONS

In conclusion, treatment of GSM, POP, and UI with Er: YAG laser is not supported by good quality evidence.

Second, in the ovine menopause model laser therapy has an effect that is no different from that of sham manipulation, and both have less effects than systemic oestrogens.

Third, in the rat model, implants that have an identical textile structure but that are made from a different polymer (PVDF or PP), hence have a different weight, generate the same biomechanical properties, host response and tissue integration. Both induce muscle atrophy on the medium term.

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PUBLICATION ACTIVITY:

Publications used in the thesis manuscript (*all with impact factor*):

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