

Efficacy and safety of oral porcine relaxin (pRLX) in adjunct to physical exercise in the treatment of peripheral arterial disease (PAD)

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Summary

Introduction PAD medical therapy has a number of limitations. RLX showed promises in experimental model mainly through NO release. Our study is the first to evaluate the efficacy and safety of RLX in PAD.

Materials-Methods eligible PAD La fontaine IIA-IIb patients were randomized in 2 groups. Group A was treated with physical therapy plus oral pRLX, 20 ug b.i.d for 12 weeks, group B received physical therapy alone. Pain Free Walking Distance (PFWD) and Maximum Walking Distance (MWD) at 3 and 12 wks and at follow up 3 months after treatment interruption were performed.

Results The percentage increases of PFWD in group B were 23±9, 65±17, and 35±4 respectively at 3 and at 12 weeks. and 3 months after termination. In Group A showed significantly higher percentage increases: 74±16 $p<0.01$, 168±28 $p<0.001$, and 122±15 $p<0.001$ at the corresponding time points. The percentage increases of MWD in the B group were 29±7, 55±10 and 54±8 at the above time points, while in the A group were 55±10 $p<0.001$, and 99±12 $p<0.001$. The RLX patients referred a better physical and mental status. No adverse events during or after the treatment were recorded.

Comment-RLX resulted very effective in PAD. Our results may suggest that the observed functional benefits should come not only from hemodynamic improvement but also from positive vascular remodeling.

Introduction

Peripheral arterial disease (PAD) is caused by chronic arterial occlusive disease of the lower extremities caused by atherosclerosis. PAD may cause intermittent claudication which is pain or weakness with walking that is relieved with rest. Muscle pain or weakness after exercise occurs distal to the arterial obstruction. Since the superficial femoral and popliteal arteries are most commonly affected by atherosclerosis, the pain of intermittent claudication is most commonly localized to the calf. Measurement of ankle and brachial artery systolic blood pressures using a Doppler stethoscope and blood pressure cuffs allows calculation of the ankle-brachial index (ABI) which is normally 0.9 to 1.2. An ABI of less than 0.90 is 95% sensitive and 99% specific for the diagnosis of PAD. The lower the ABI, the more severe the restriction of arterial blood flow, and the more serious the ischemia. The prevalence of PAD increases with age. Schroll and Munck reported that the prevalence of PAD was 16% in men and 13% in women aged 60 years [1]. Treatment of chronic PAD is mainly based on

cholesterol and blood pressure control, antiaggregants such as acetylsalicylic acid or Clopidogrel. Cilostazol has been documented in numerous trials to improve exercise capacity in patients with intermittent claudication and in a dose of 100 mg twice daily, was shown to be superior to both placebo and pentoxifylline [2]. Another option is exercise for intermittent claudication. Exercise rehabilitation programs have been shown to increase walking distance in persons with intermittent claudication through improvements in peripheral circulation, walking economy, and cardiopulmonary function [3-4]. The optimal exercise program for improving claudication pain distance in persons with PAD uses intermittent walking to near-maximal pain during a program of at least 6 months [5]. Supervised exercise training is recommended for a minimum of 30-45 min in sessions performed at least 3 times per week for a minimum of 12 weeks and preferably for 6 months or longer.

Since there is room for better treatment option we investigated the role of Relaxin (RLX) an hormone that belongs to the relaxin hormone super-family, which in humans includes 3 RLX molecules and 4 insulin-like peptides. Of these, luteal RLX is the main circulating form in animals and man. RLX, formerly known for its effects on reproduction and pregnancy, has been demonstrated to act on numerous other targets, including the cardiovascular, central and peripheral nervous, respiratory, tegument, excretory and digestive systems. Most of these effects have been studied in animal models, but there is compelling evidence that RLX also acts in the human. RLX may be a novel therapy in cardiovascular disease because it induces microvessel dilation, blood flow increase, platelet inhibition and neo-angiogenesis, mainly through nitric oxide release. This article report the first clinical experience with purified preparation of oral porcine RLX (pRLX) that is now available and fits well for chronic use.

AIM OF THE STUDY

The aim of the study was to evaluate the safety and the efficacy of oral porcine relaxin in adjunct to physical exercise in patients with chronic peripheral artery disease la fontaine stage IIA-IIB in comparison to physical exercise alone. We investigated the effect on the two main parameters of walking capability: the pain free walking distance and the maximum walking distance.

DESIGN OF THE STUDY

This is a randomized, open label, single center, controlled trial comparing the efficacy and safety of oral porcine relaxin in adjunct to physical exercise versus physical exercise alone.

POPULATION OF THE STUDY

Consecutive patients both male and female affected by PAD stage IIA-IIB who gave informed consent to the participation to the study and that fulfill all the following inclusion criteria with no exclusion criteria:

INCLUSION CRITERIA

- 1) Presence of claudicatio intermittens that relieves few minutes after stopping activity.
- 2) Patients of age ranging from 18 to 85 years.
- 3) Stable symptoms of intermittens claudicatio for at least one month before enrollment
- 4) Documented PAD by echo-color doppler or conventional angiography
- 5) Subjects judged affordable that accept all the appointments for control and protocol visit
- 6) The subject or its legal tutor have to sign the informed consent
- 7) Female can participate only if potentially not fertile :
 - a) women potentially not fertile (menopause from more than one year or surgically sterile
 - b) women potentially fertile for whom pregnancy is excluded by an urinary test in the screening visit and that ensure an adequate contraception during the study period

Exclusion criteria

- 1) Patients with PAD causing ischemic pain at rest or trophic lesions (patients stage III e IV) according to la Fontaine classification.
- 2) Patients in whom endovascular procedure is the treatment of choice (in example single stenosis of the common iliac artery or of the external iliac artery < 3cm)
- 3) one of the following contraindications to physical exercise (severe coronary disease, severe kidney failure, patients hemodynamically unstable, need of continuous oxygen therapy for chronic respiratory insufficiency, orthopedic diseases that limits walking capability, recent myocardial infarction, hemiplegy, uncontrolled hypertension)
- 4) Patients with a medical history for allergy to drugs
- 5) Patients with an history of alcohol abuse or suspect alcohol or drug addiction in the former two years
- 6) Subject with low compliance to therapy assumption or that at judgment of the investigator should be at risk of low compliance
- 7) Sever gastrointestinal disease that could interfere with relaxin absorption
- 8) women in pregnancy or lactation.
- 9) Women potentially fertile with no adequate contraception
- 10) significant alteration of the laboratory test
- 11) Subjects that participated to another trial in the month before the screening visit or that plan to be enrolled to a protocol that use an experimental drug or devices

Material and methods

A total of 18 Consecutive eligible patients attending to Prosperius Institute from March 2010 to 2011 were evaluated, 2 were excluded 1 for an history of allergy to medication and another for low compliance. Sixteen patients 14 males and 2 females (mean age 67,8) were randomised in two groups. 8 patients in Group A received

physical exercise alone while 8 patients of group B received oral porcine relaxin at 20 mcg every 12 hours in adjunct to physical exercise for 12 weeks.

Physical exercise was conducted on tapis roulant at 3,2 km/hr speed at zero degree incline.

Exercise lasted from 30 to 45 minutes for session and was repeated three times a week

All Patients underwent EKG and echocolor Doppler of the artery of the lower limbs and ankle brachial index evaluation at baseline. Patients had also blood taken for sampling for complete blood count, renal function, hepatic function coagulation electrolyte the echodoppler of the artery of the lower limbs was repeated at 12 and 24 weeks. Blood samples were also taken to determine the same parameters of baseline at Visit 1 (after 3 weeks) and at visit 2 (after 12 weeks).

Endpoints:

Primary endpoints:

Effect of treatment on pain free walking distance and on maximum walking distance. Treadmill test for evaluation of PFWD and of MWD was executed at 2.5km/hr speed, starting from 6% inclination and increasing 2 % inclination every 2 minutes. This test was repeated at 3-12 and 24 weeks

Pain free walking distance was determined as the distance where the pain began. Maximum walking distance was calculated as the maximum distance that the patients could walk. Stop was determined by pain at maximum entity (of a scale from 1 to 5) or by dyspnea, or tachycardia or intense fatigue

Secondary endpoints

Variation of ankle brachial index

Valuation of treatment satisfaction questionnaire modified (TSQM) for patients treated with relaxin variation in renal and hepatic function or glycosilated hemoglobin.

Statistical analysis

Differences were calculated as percent variation of individual initial values. was done by Student's test and two-way ANOVA,

The results were calculated as percentage variation of individual initial values. Statistical analysis was done by Student's *t* test and two-way ANOVA followed by Bonferroni's multiple comparison test.

OUTCOME MEASURES

The primary objective of our study was to evaluate the improvement induced by oral porcine RLX treatment on Pain-Free Walking Distance (PFWD) and of Maximum Walking Distance (MWD) registered at a follow-up of 3 and 12 weeks and 3 months.

RESULTS PFWD

Both groups improved during and after the trial. The percentage increases of PFWD in patients assigned to physical exercise were $23 \pm 9\%$, $65 \pm 17\%$, and $35 \pm 4\%$ respectively at 3 and a 12 weeks and 3 months after placebo/physical exercise termination. In comparison, patients treated with oral porcine relaxin group showed significantly higher percentage increases 74 ± 16 $p < 0.01$, 168 ± 28 $p < 0.001$, and 122 ± 15 $p < 0.001$ at the corresponding time points.

RESULTS MWD

Similarly, the percentage increases of MWD in the group assigned to physical exercise were 29 ± 7 , 55 ± 10 and 54 ± 8 at 3-12 and 24 weeks, while in the group receiving oral porcine relaxin were 55 ± 10 $p < 0.001$, and 99 ± 12 $p < 0.001$. The therapeutical action of pRLX appeared very soon and lasted up to 3 months after withdrawal. The RLX patients referred a better physical and mental status. No adverse events during or after the treatment were recorded. Of interest 6 of the 8 patients assigned to oral relaxin stopped their exercise for dispnea or tachycardia and not for maximum tolerated pain

ADDITIONAL RESULTS

Variations on ankle-brachial index (Winsor Index) were also evaluated.

Possible side effects of the treatment were carefully monitored.

Treatment satisfaction questionnaire for medication (TSqM) was done at the end of treatment by all patients. Blood assays were carried out to monitor routine blood parameters, kidney and liver functions, and glycated hemoglobin.

Treatment satisfaction to oral porcine RLX was very high, 81,25% regarding efficacy, 100% considering tolerability, 78,65% on the easiness of use and 95,25% as a whole.

No changes were observed in ankle-brachial index between the groups at any test time (before treatment: physical exercise, 0,81; physical exercise + oral porcine relaxin, 0,82. The same parameters evaluated after treatment: physical treatment, 0,68; physical treatment + oral porcine relaxin, 0,67. No effects were observed in glycemic status: before and after the treatment, glycated hemoglobin was 6,27% and 6,36% in the physical exercise group, 6,70 and 6,77 in the physical exercise plus oral porcine relaxin group. No changes related to the treatment were observed in routine blood tests as well as on the markers of liver and kidney function. No adverse events were reported during treatment and during the follow-up period

Discussion

PAD is a chronic disease carrying an higher risk of cardiovascular complications and death than healthy population. On the other hand PAD at stage II is a disease

with a slow progression and somewhat stable, therefore patients often are not worried by the disease itself. Symptoms are characterized by pain during walking that can be localized also in the buttocks, hips, thighs, or the inferior back muscles. Atherosclerotic obstruction of the distal aorta and its bifurcation into the two iliac arteries. Criqui *et al.* showed that the prevalence of PAD was 5.6% in persons aged 38 to 59 years, 15.9% in persons aged 60 to 69 years old, and 33.8% in persons aged 70 to 82 years old [6]. In the Cardiovascular Health Study, PAD was present in 13.9% of 2,214 men aged ≥ 65 years and in 11.4% of 2,870 women aged ≥ 65 years without cardiovascular disease [7]. The ACCP recently published guidelines for antithrombotic drug therapies for primary and secondary prevention of cardiovascular disease as well as for the relief of lower-extremity symptoms in persons with chronic peripheral arterial disease (PAD) stage IIa-IIb. [8]. For secondary prevention of cardiovascular disease in patients with symptomatic PAD (including patients before and after peripheral arterial bypass surgery or percutaneous transluminal angioplasty), the usual therapy is long-term aspirin (75-100 mg/d) or clopidogrel (75 mg/d) (Grade 1A). Warfarin plus aspirin in patients with symptomatic PAD was not recommended (Grade 1B). For patients with refractory claudication despite exercise therapy and smoking cessation, the addition of cilostazol (100 mg bid) to aspirin (75-100 mg/d) or clopidogrel (75 mg/d) (Grade 2C) was suggested. Two drugs, pentoxifylline and cilostazol, have been approved by the United States Food and Drug Administration for symptomatic treatment of intermittent claudication.

Scientific awareness of the existence of relaxin (RLX) dates back to 1926, when Frederick Hisaw found that the serum from pregnant guinea pigs or rabbits into virgin guinea pigs contained a hormone-like substance which induced relaxation of the interpubic ligament [9], has been long considered pertinent to reproduction, based on early studies demonstrating its ability to promote elongation of the interpubic ligament in mammals [10]. Four years later, the peptide substance held responsible for this effect was purified and identified from pregnant sow luteal extracts and designated *relaxin*. RLX has a molecular weight of about 6,000 Dalton and consists of 2 chains, termed A and B, which are covalently linked by 2 interchain disulfide bonds with a further intra-disulfide bond in the A chain. Extra-luteal sources of H2 peptide H1 RLX have been detected in a few reproductive tissues including the decidua, placenta and prostate. Exhaustive information on this issue is available on previously published reviews [11-12]. The notion that RLX is a cardiovascular hormone dates back to the late 1950s, when an impure porcine RLX preparation, injected to patients suffering for peripheral vascular diseases and Raynaud's syndrome, was shown to cause a dramatic, albeit transient, amelioration of symptoms and signs of ischemia [13]. In some patients who also suffered for ischemic heart disease, RLX treatment led to the reduction of the daily glyceryl trinitrate requirements. This observation clearly suggests that RLX has a direct, dilatatory effect on peripheral and coronary vasculature. Recognition of the blood vessels as specific RLX targets came from the identification of RLX binding sites/receptors on cells of the vascular wall [14-16]. RLX promotes vasodilatation in reproductive organs, such as the uterus [17, 18] and the mammary gland [19], as well as in non-reproductive targets, including mesocaecum [20], kidney [21], liver [22], lung [23] and heart [24,25]. Vasodilatation appears as a physiological effect of RLX since it is fully manifested at hormone concentrations which are in the nanomolar range, similar to the RLX blood levels of normal human

pregnancy. RLX is extremely potent as a vasorelaxant: in the isolated, perfused rat and guinea pig heart, the dose-dependent increase in coronary flow induced by RLX is significantly higher than that obtained with similar doses of typical vasodilatory agents such as acetylcholine or sodium nitroprusside. This latter concept indirectly suggested that these effects of RLX would be mediated by mechanisms involving a potent vascular effector and, once again, NO appeared as the perfect candidate. This was the first clinical aiming to evidence the efficacy and safety of oral porcine RLX in patients with chronic ischemic PAD stage Ia Fontaine IIa-IIb. Results indicate a significant additive effect in adjunct to exercise therapy. These results were obtained without the presence of any adverse event related to the treatment. Of note the amelioration was present from the first three weeks and was sustained over 12 weeks after stopping treatment. Although preliminary for the limited number of treated patients, indeed, the results suggest that oral porcine RLX given to patients with chronic PAD at the described administration regimen is capable of inducing a prompt and significant amelioration of the primary outcome parameters of walking capability, associated with an improvement of the patients' general conditions. This increase in walking capability was not due to the reopening of the major artery, in fact the ankle brachial index was not modified. Of interest, the treatment resulted safe and well tolerated by the patients, who subjectively reported high satisfaction and reported an ease of administration and a lack of negative effect. In our opinion these results strongly encourage further clinical research on the effect of relaxin on cardiovascular disease

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