ARTICOLI ORIGINALI

Muscle stimulation in elderly patients with CKD and sarcopenia

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Abstract

Objectives: Aim of our study was to assess the potential effects of high-tone external muscle stimulation (HTEMS) on improvement of endothelial dysfunction (ED) and kidney damage in elderly patients with chronic kidney disease (CKD), sarcopenia and/or serious physical disability with a high Multidisciplinary Prognostic Index (MPI).

Methods: We enrolled 12 consecutive CKD patients with MPI > 0,66 from January 1st, 2008 to December 31st, 2014. Six patients underwent a 2-hours HTEMS during the first day (group A) and the other six patients (group B) underwent a sham experiment with HTEMS without power supply. After 24 hours, patients of group A were shifted to group B and viceversa. Nitrite/nitrate (NOx), endotheline-1 (ET-1) and urine creatinine concentration were measured in all patients.

Results: During HTEMS urine amount increased by 22% (p=0.049), so did urine creatinine that increased by 40%, (p=0.034) and creatinine clearance that increased by 26% (p=0.041). There was no statistical difference in urine nitrogen (that raised by 11%, p=0.526), urine sodium (that reduced by 42%, p=0.121) and urine potassium levels (p=0,491). At the same time, NOx changed from 44.1±5.1 to 38.4±5.3 µM/L after 1 hour, to 36.4±4.8 µM/L after 2 hours, to 41.1±5.7 µM/L after 3 hours and to 46,9±5.0 µM/L after 4 hours (p=0.008) during HTEMS, while it did not vary during the sham section of the experiment, respectively 43.6±6.1 µM/L, 43±6.4 µM/L, 42.8±5.5 µM/L, 43±4.7 µM/L, and 42.8±5.8 µM/L (p=0.992).

Conclusion: Our study showed that HTEMS may improve microcirculation and, through this mechanism, may reduce kidney damage in elderly patients with CKD and severe muscle atrophy.

Key words: sarcopenia, electrical muscle stimulation, nitric oxide

Introduction

Muscle weakness, defined as inability to generate strength, is common in patients with chronic kidney disease (CKD) [1] (full text), and is progressive during the course of CKD [3]. Reduction of muscle mass leads to both a reduction of muscle fiber's size (atrophy) and of muscle fiber's number (hypoplasia). Several factors present in CKD condition may be responsible of muscle mass reduction, such as hormonal or immunological changes, inflammation, metabolic acidosis, reduced protein-energy intake and physical activity, increased levels of angiotensin II, anomalies of insulin or insulin-like growth factor I (IGF-I), and myostatin with reduced cellular function [4].

On the other side, the number of elderly patients and their age increases exponentially in CKD population, so that elderly patients represent themselves a category susceptible to develop a uremic muscle wasting, also due to physical inactivity with increasing age. Older patients with serious disability are at high risk of sarcopenia [4].

Recently, the importance of endothelial dysfunction and nitric oxide (NOx) in muscle weakness has been described [5]. Also, the importance of physical activity has been studied in preventing muscle degradation, and increasing insulin sensitivity and NOx formation [6] (full text) [7] (full text). Moreover, it is possible to prevent muscle atrophy and preserve muscle protein synthesis during prolonged periods of immobilization [8]; in a published study high tone external muscle stimulation (HTEMS) was effective inducing extremely high frequencies ranging from 4,100 to 33,000Hz which were only associated with vibrations and micro-contractions of muscles' fibers and were well tolerated [9].

Based on this knowledge, the aim of our study was to study 12 consecutive elderly patients (with age>75 years) with CKD and sarcopenia and/or serious physical disability (Multidimensional Prognostic Index [MPI]>0,66 [10]) to understand whether HTEMS can improve renal function.

Methods

Study population

We enrolled 12 consecutive patients from January 1st 2008 to December 31st 2014. Inclusion criteria were: CKD (stage 3b-4), age $\geq$ 75 years, MPI>0,66, renal function stable for at least 3 months; exclusion criteria were: acute illnesses, immunosuppressive drugs, immunological diseases. The cause of disability was *ictus cerebri* in 8 out of 12 patients and spinal medulla trauma in 4 out of 12 patients.

Experimental design

A sham section and an experimental section were conducted in 2 different and consecutive days. Six patients underwent a 2-hours HTEMS during the first day (group A) and the other six patients (group B) underwent a sham experiment with HTEMS without power supply. All patients received 500ml of 5% glucose solution per *endovena* before the experimental and the sham section. After 24 hours, patients of group A were shifted to group B and *viceversa*. In both study sections all patients collected urine during the 4 hours following the experiment.

Intervention by HTEMS application during experimental time.

As previously described, HTEMS was performed with a HiToP 184 appliance (gbo Medizintechnik, Rimbach, Germany), which is a 230 V power supply device [11]. The frequency varied in short intervals (3 seconds) between 4100 and 33000Hz and the amplitude and electrical frequency were modulated simultaneously. During the 2-hours HTEMS therapy, the electrodes were placed at femoral muscles' level and in some cases, on the calves. Electrical stimulation intensity was adapted to reach a comfort level for each patient without causing discomfort or pain.

Multidimensional Prognostic Index

Multidimensional Prognostic Index (MPI) is a prognostic instrument to evaluate 1-year mortality risk in the elderly using a Multidimensional Evaluation [10]. MPI is formed by 8 domains with 63 items coming from the following instruments of VMD: Activities of Daily

Living (ADL), Instrumental Activities of Daily Living (IADL), Short Portable Mental Status Questionnaire (SPMSQ), Mini Nutritional Assessment (MNA), Exton-Smith Scale, Comorbidity Index Rating Scale (CIRS), number of drugs and social-housing state [10].

MPI is able to identify 3 groups of subjects with a different mortality risk: low (0-0.33), moderate (0.34-0.66), and severe (>0.66). MPI has a prognostic mortality risk higher than the other domains of the index taken individually. On the other hand, the knowledge of prognosis and outcome in elderly patients is important to lead diagnostic and therapeutic management [12] [13] [14] (full text) [15] [16].

Measurement of nitrite/nitrate concentrations

As previously described by our group [11], nitrite/nitrate (NOx) concentration, a surrogate marker of NO generation, was measured in serum samples [17]. Plasma samples were incubated with nitrate reductase (0.1U/ml) and NADPH (1 mm), and FAD (50μm) at 37°C. After 15 minutes samples were incubated with LDH (100U/ml) and sodium pyruvate (10mm) for 5 minutes. The total NOx concentration in the samples was measured by Griess reaction, by adding 100μl of Griess reagent (0.1% naphthylethylenediamide dihydrochloride in H2O and 1% sulfanilamide in 5% conc. H2PO4; vol.1:1) to 100μl of samples, each in triplicate. The optical density at 550nm (OD550) was measured at 540nm in a microplate reader Titertek (Dasit, Cornaredo, Milan, Italy). Total NOx concentrations (μm) were calculated from a standard curve of sodium nitrate. Results were expressed as μM/L.

Tabella 1. Data of patients

	Before HTEMS	After HTEMS	p
Number	12		
Males	6		
Age, years	79.32±3.08		
BW, kg	55±7		
MPI	0.69±0.03		
Creatinine	2.61±0.34	2.49±0.35	0.403
Urea	96±21	92±21	0.645
Emoglobin	11.3±2.2	10.9±2.5	0.681
Albumin	3.7±0.2	3.6±0.2	0.234
Na	140.5±1.9	140.7±1.7	0.788
K	4.86±0.68	5.13±0.54	0.293
Ca	9.24±0.66	9.18±0.61	0.819
P	4.24±0.48	4.24±0.42	0.995
Bicarbonate	21.33±1.5	21.5±1.31	0.77
Cl	103±5	105±5	0.338
LDH	314±66	304±72	0.726
CPK	111±66	112±21	0.961
GOT	14.1±3.2	13.8±3.6	0.94
GPT	9.0±2.4	8.6±2.7	0.705
Troponin	66±38	64±41	0.902
Mioglobin	107±14	106±16	0.872

Measurement of ET-1 concentrations

ET-1 serum protein concentration was assessed by Enzyme-linked immunosorbent assay (ELISA) method using a commercial kit for human ET-1 according to manufacturer's instruction (R&D Systems, Minneapolis, MN, USA). Results were expressed as pg/mL [11].

Statistical analysis

All values were reported as means ±SD unless otherwise specified. Analysis of variance (ANOVA) was used to compare the two groups. Variable statistical significance was defined as $p<0.05$.

Results

Table 1 shows basal data of study's participants. Mean age was 79.32±3.08 years; 50% of them were male; mean eGFR was 22±6ml/min mean, and MPI was 0.69±0.02 (range 0.65-0.76).There was no statistical difference between the 2 groups in biochemical parameters despite a reduction of creatinine levels of 4.6% during HTEMS (p=NS).

Table 2 shows changes of urine amount that increased of 22% during the experimental section of the study (p=0.049), and of urine creatinine that increased of 40% (p=0.034), with a following improvement of creatinine clearance that increased of 26% (p=0.041). There was no difference in the 2 groups between urine nitrogen that increased of 11% (p=0.526), urine sodium levels that reduced of 42% (p=0.121), and urine potassium levels (p=0,491).

As shown in Figure 1, NOx varied from 44.1±5.1 to 38.4±5.3μM/L after 1 hour, to 36.4±4.8μM/L after 2 hours, then returned to 41.1±5.7μM/L after 3 hours and to 46,9±5.0μM/L after 4 hours (p=0.008) during HTEMS, but it remained stable during the sham section (p=0.992), 43.6±6.1μM/L , 43±6.4μM/L, 42.8±5.5μM/L, 43±4.7μM/L, and 42.8±5.8μM/L respectively.

ET-1 did not change compared to basal value (2.26±0.61pg/L) neither during HTEMS (p=NS) nor during the sham section of the study (basal value: 2.33±0.65pg/L).

Figure 2 shows variations of urine creatinine (mg/min) in the 2 phases of the study: it increased to 85% during HTEMS after 1 hour and to 48% after 4 hours; in the sham section urine creatinine did not change at all. On the other hand, as shown in Figure 3, urine volume increased to 31% after 1 hour and to 21% after 4 hours.

Discussion

Uremic myopathy has been described for more than 100 years and it has been defined as muscle strength reduction following an altered ATP-dependent proteolysis of muscles' pro-

Tabella 2. Urinary data of patients

	Before HTEMS	After HTEMS	p
Urinary flow, ml/min	0.71±0.22	0.87±0.15	**0.049**
Urinary Creatinine, mg/min	0.32±0.19	0.53±0.26	**0.034**
Urinary Urea, mg/min	6.79±2.19	7.55±3.45	0.526
Urinary Na, mmol/min	0.059±0.026	0.034±0.047	0.121
Urinary K, mmol/min	0.020±0.007	0.018±0.007	0.491
GFR, ml/min	21.8±5.8	27.4±4.5	**0.039**

teins, metabolic acidosis, micro-inflammation, hormonal changes, immune mechanisms, Angiotensin II levels, and other clinical conditions associated to CKD [4]. Disability and reduced physical activity induce a worsening of uremic myopathy, especially in elderly patients with reduced cognitive and physical ability. Furthermore, it is already well known that endothelium influences blood flow regulation, barrier permeability, blood clotting, and inflammation [17]. Also NOx conditions renal blood flow and other factors such as glomerular medullary hemodinamics, tubulo-glomerular feedback, renin secretion and extracellular fluid volume homeostasis [18] [19] (full text) [20] (full text) [21] (full text) [22] (full text).

Our group has already used HTEMS in patients with Acute Kidney Insufficiency (AKI) and showed that HTEMS reduced duration of anuria and induced an earlier recovery of renal function; NOx was involved in these effects [11] [12] [13] [14] (full text) [15] [16] [17] [18] [19] (full text) [20] (full text) [21] (full text) [22] (full text) [23].

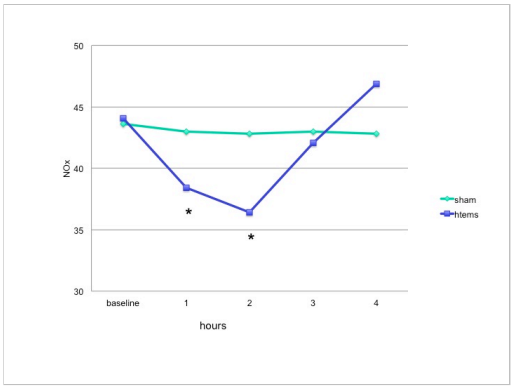

Figura 1.
Andamento dei valori di NOx durante HTEMS (p<0.05)*

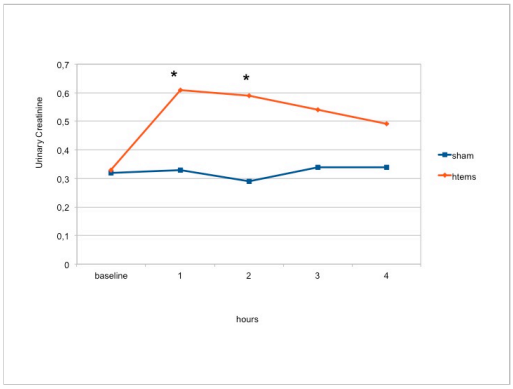

Figura 2.
Andamento della creatininuria durante HTEMS (p<0.05)*

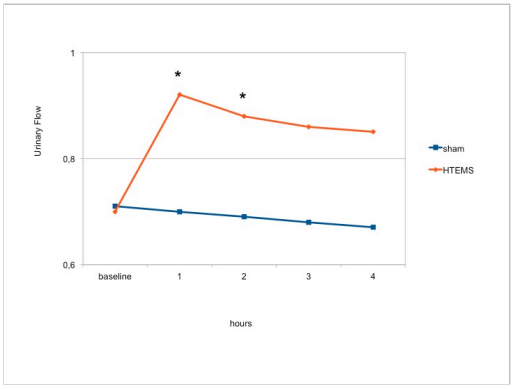

Figura 3.
Variazioni del volume urinario durante HTEMS (p<0.05)*

We hypothesized that muscle stimulation induced by HTEMS stimulated microcirculation of peripheral tissues [24] [25]. Karavidas et al showed that HTEMS stimulates blood flow locally and also at systemic level. In critical care patients, its application to lower legs improved microcirculation of the thenar muscle [26]. There are also observations that an acute application of HTEMS to the thighs may increase GFR [27]. HTEMS induced circulatory effects through an enhanced bioavailability of NO. In vitro and in vivo studies showed that shear stress is a key activator of eNOS and NOx generation [28] (full text) [29] (full text).

Our data evidenced beneficial effects of HTEMS on renal function with increment of urine output and of urinary creatinine excretion in subjects with severe muscle atrophy following absence of physical activity.

Analyzing NOx levels, it is possible that muscle stimulation may produce an increased NOx consumption (then an increased production as consequence), indicating a role of NOx in the kidney.

Our experiment is a pilot study, then there is no scientific evidence on this topic, that is relatively unexplored. Hence, it has several limitations: in fact, the study enrolled a small number of patients observed in a follow-up period, but it has not statistical power neither to study long-term effects nor to understand therapeutic consequences. Further studies are needed in this field that appears very interesting due to HTEMS's possible therapeutic application and low cost. In fact, it has been recently evidenced that a low-frequency electrical stimulation may have beneficial effects in preventing muscle atrophy in 5/6 nephrectomized mice [30]. It has been showed that electrical stimulation inhibits IGF-1 reduction induced by uremia and induces an increase of protein synthesis and myogenesis with a consequent improvement of protein metabolism [30]. This study is an animal experiment but its results are similar to ours.

In conclusion, our data show that HTEMS improves microcirculation; then it may reduce kidney damage through this mechanism in elderly patients with CKD and severe muscle atrophy.

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