The Prostate 75:1857–1867 (2015)

Effects of Hexanic Extract of Serenoa Repens (Permixon® 160 mg) on Inflammation Biomarkers in the Treatment of Lower Urinary Tract Symptoms Related to Benign Prostatic Hyperplasia

Alain Latil,[1]* Marie-Thérèse Pétrissans,[1] Jérôme Rouquet,[1] Grégoire Robert,[2] and Alexandre de la Taille[3†]

[1]*Institut de Recherche Pierre Fabre, Toulouse, France*
[2]*Department of Urology, Bordeaux Pellegrin University Hospital, Bordeaux, France*
[3]*Department of Urology and INSERM U955 Eq07, CHU Henri-Mondor, Créteil, France*

BACKGROUND. Chronic prostatic inflammation (CPI) could be a cause of symptomatic or complicated benign prostatic hyperplasia (BPH). In previous in vitro and in vivo studies, Hexanic Extract of Serenoa repens (HESr) namely Permixon® has demonstrated potent anti-inflammatory properties. With the aim to provide new insight onto HESr anti-inflammatory properties in human we explore its effect on CPI biomarkers in men with lower urinary tract symptoms (LUTS) related to BPH using a non-invasive method and investigate links between biomarkers and clinical symptoms.

METHODS. An international, randomized, double-blind, parallel-group, tamsulosin-controlled study was carried out in 206 men with BPH-related LUTS. Patients received oral daily HESr 320mg or tamsulosin 0.4 mg during 3 months. The first urine stream after digital rectal examination (DRE) was collected at Day 1 and Day 90 and mRNA was extracted from prostatic epithelial cells desquaming in the lumen of the glands and seminal plasma fluid after DRE. mRNA quantification of the 29 most significant published inflammation markers in BPH and protein detection in urine was performed.

RESULTS. At D90, a decrease in mean gene expression was observed for 65.4% of the markers detected in the HESr group versus 46.2% in the tamsulosin group. In the 15 most frequently expressed genes, this difference was higher (80% vs. 33% respectively). Three proteins (MCP-1/CCL2, IP-10/CXCL10, and MIF) were detected. At D90, a decrease in the number of patients who expressed MCP-1/CCL2 and IP-10/CXCL10 was observed only in the HESr group. Moreover, MIF expression was significantly reduced by HESr compared with tamsulosin ($P = 0.007$). Finally, in contrast to tamsulosin, the subgroup of patients treated by HESr and who over expressed MIF at baseline, had a higher response to the International Prostate Symptom Score (I-PSS) than those who did not over express this protein (mean I-PSS change: −6.4 vs. −4.5 respectively). As the study is exploratory, results should be confirmed in a powered clinical study.

[The copyright line was changed in October 2015 after original online publication.]

†International coordinator on behalf of study investigators.

Conflicts of interest: Including specific financial interests and relationships and affiliations relevant to the subject matter discussed in the manuscript are the following: Alain Latil, Marie-Thérèse Pétrissans and Jérôme Rouquet are employees of Pierre Fabre. Grégoire Robert is Investigator in the study and Scientific Advisor. Alexandre de la Taille is the International Study Coordinating Investigator.

*Correspondence to: Alain Latil, Institut de Recherche Pierre Fabre, Toulouse, France
E-mail: alain.latil@pierre-fabre.com
Received 15 June 2015; Accepted 22 July 2015
DOI 10.1002/pros.23059
Published online 26 August 2015 in Wiley Online Library (wileyonlinelibrary.com).

CONCLUSIONS. These results showed for the first time at clinical level the anti-inflammatory properties of HESr, already indicated in BPH-related LUTS. Thus, HESr could be of interest to prevent unfavourable evolution in patients with CPI. *Prostate 75:1857–1867, 2015.*

KEY WORDS: **benign prostatic hyperplasia; chronic prostatic inflammation; hexanic extract of serenoa repens; lower urinary tract symptoms; permixon®**

INTRODUCTION

A growing body of evidence suggests that chronic prostatic inflammation (CPI) leads to symptomatic or complicated benign prostatic hyperplasia (BPH) [1,2]. CPI is a very common condition in men over the age of 50 [3]: it has been observed in a large proportion of patients treated surgically for lower urinary tract symptoms (LUTS) due to BPH [4] and in the majority of histological BPH tissue obtained from autopsy series [5]. Moreover, CPI has been associated with higher prostate volume and a more severe International Prostate Symptom Score (I-PSS) [3,4].

Histologically, CPI is characterised by the presence of large confluent inflammatory nodules in prostatic tissue [3,4]. These nodules release multiple inflammatory mediators that have been shown to stimulate prostatic cell growth [6]. Nodules also damage the architecture of the gland, resulting in a chain reaction that further sustains the inflammatory response and promotes prostatic cell growth, prostatic enlargement, and bladder outlet obstruction [7].

CPI could be a target for medical treatment in patients with BPH-related LUTS. A recent review of randomised clinical trials suggested favourable effects of non-steroidal anti-inflammatory drugs (NSAIDs) [8], but their side effects related with long--term use mostly limit their prescription to acute worsening of urinary symptoms. Paubert-Braquet et al. [9] demonstrated in 1997 that Hexanic Extract of Serenoa repens (HESr), namely Permixon® and already indicated in BPH-related LUTS could antagonise 5-lipoxygenase metabolites, leading to an anti-inflammatory effect. This effect has been recently confirmed by in vitro and in vivo studies, indicating that HESr can modulate the expression of multiple inflammation-related genes [10–12].

The primary objective of the study was to assess the effect of HESr on CPI biomarkers in men suffering from BPH-related LUTS using a non-invasive method. The secondary objectives were to assess the clinical efficacy of HESr depending on the prostatic inflammation status of the patients.

MATERIALS AND METHODS

Study Participants

The study complied with the principles of the Declaration of Helsinki and was approved by the independent Ethics Committees of participating centres and countries.

Patients were required to understand and sign the informed consent form and understand and fill in questionnaires.

Inclusion criteria. To be included in the study, men were required to be between 45 and 85 years old with BPH- related LUTS for over 12 months, have an I-PSS score $\geq$12, prostatic volume $\geq$30 cm^3 determined by transrectal ultrasound, maximum flow rate (Qmax) 5–15 ml/s for a voided volume 150–500 ml, and total Prostate-specific antigen (PSA) $\leq$4 ng/ml or $\leq$10 ng/ml with ratio PSA (free)/PSA (total) $\geq$25% or negative prostate biopsy. Patients were required to be free of anti-androgens and LH-RH analog for at least 6 months, 5 alpha-reductase inhibitors and plant extracts for at least 3 months and alpha blockers and alpha/beta blockers for at least 1 month before screening. Patients taking the following oral medications at screening required a wash-out of 2 weeks: 5-PDE inhibitors for BPH treatment, NSAIDs corticosteroids, or antibiotics by systemic route, mepartricine, ACE inhibitors, calcium antagonists, beta blockers, diuretics, sympathomimetics, antihistamines, antidepressants (anticholinergic), atropine, antispasmodic drugs, antiparkinsonism drugs, pseudoephedrine, chlorpheniramine, or spironolactone (if unstable dose or initiated 6 weeks or less prior to selection). Moreover, these medications were prohibited for the duration of the study.

Non-inclusion criteria. Patients were excluded if they had a PVR > 200 ml (by suprapubic ultrasound), previous urological history including urethral stricture disease and/or bladder neck disease, active, or recent (<3 months) or recurrent urinary tract infection, urinary retention, indication of BPH surgery, stone in bladder, or urethra, acute, or chronic prostatitis, prostate, or bladder cancer, interstitial cystitis,

active upper tract stone disease causing symptoms, surgery of the prostate, bladder neck or pelvic region. In addition, any local and/or systemic inflammation disorders, orthostatic hypotension, any neurologic or psychiatric disease/disorder interfering with the detrusor or sphincter muscle, insulin-dependent diabetes mellitus and non-controlled non-insulin-dependent diabetes mellitus, chronic renal insufficiency, history of severe hepatic failure or other severe underlying disease excluded the participation of the patient in the study.

Study Design

This Phase IV trial (https://clinicaltrials.gov/ct2/show/NCT01604811) was conducted as an international, prospective, randomised, double-blind study in 2 parallel groups. After a 28–42 day wash-out period, men suffering from BPH-related LUTS were randomly assigned to receive daily HESr 320 mg (160 mg B.I.D hard capsule) or tamsulosin LP 0.4 mg capsule and were followed up over 90 days. Four visits were planned for each participant: selection visit, baseline visit (Day 1), first assessment visit (Day 30) and end-of-study visit (Day 90).

Methods

Urine sample collection. Urine samples were collected from the 203 patients treated with HESr (n = 102) or tamsulosin (n = 101). The first urine stream after digital rectal examination (DRE) was collected at D1, D30, and D90 in preservation tubes (Norgen Biotek Corp., Canada).

RNA isolation and PCR amplification. RNA extraction and PCR quantification were performed using standard methods. Briefly, 10 ml were centrifuged at room temperature for 15 min at 1000*g*. Two millilitre of the supernatant were removed and stored at −80°C for subsequent protein analyses.

Total RNA was isolated from the pellet by using the RNAble reagent and Qiagen RNeasy mini-preps according to the manufacturers' instructions (Eurobio and Qiagen, Courtaboeuf, France). The quantity and purity of extracted RNA were assessed with a NanoDrop ND 1000 spectrophotometer (Labtech International, Paris, France).

First-strand cDNA synthesis was performed with 100 ng of total RNA and SuperScript® VILO™ reverse transcriptase (Invitrogen) in a final volume of 20 µl.

Fourteen multiplex preamplification cycles of 2 pools of TaqMan Gene Expression Assays (n = 16 for each pool including KLK3 reference gene) was performed using the TaqMan PreAmp Master Mix Kit (Life Technologies).

The mRNA expression of the 29 most significant BPH inflammation markers was quantified [9–19]. Real-time qPCR was performed with TaqMan Gene Expression Assays (Life Technologie) using the following probes:

ALOX15 Hs00609608_m1, **ALOX15B** Hs00153988_m1, **ALOX5** Hs01095330_m1, **CAT** Hs00156308_m1, **CCL5** Hs00174575_m1, **HIF1A** Hs00153153_m1, **LTC4S** Hs00168529_m1 **MIF** Hs00236988_g1, **NFKB1** Hs00765730_m1, **PTGES2** Hs00228159_m1, **PTGES3** Hs04187821_g1, **PTGS2** Hs00153133_m1, **PTPRC** Hs04189704_m1, **SELP** Hs00927900_m1, **STAT3** Hs00374280_m1, **IL17A** Hs00174383_m1, **ICOS** Hs00359999_m1, **CCR7** Hs01013469_m1, **IL1B** Hs01555410_m1, **IL6** Hs00985639_m1, **IL8** Hs00174103_m1, **IL15** Hs01003716_m1, **PLA2G2A** Hs00179898_m1, **CXCL10** Hs01124251_g1, **CCL2** Hs00234140_m1, **CD40LG** Hs00163934_m1, **CTLA4** Hs03044418_m1, **FGF2** Hs00266645_m1, **CXCL6** Hs00605742_g1 and **KLK3** Hs02576345_m1

All qPCR reactions were performed with a QuantStudio™ 6 Flex System (Life Technologies, Foster City, CA, USA) and the TaqMan® Gene Expression Master Mix kit (Life Technologies). The thermal cycling conditions comprised an initial denaturation step at 95°C for 10 min, 40 cycles at 95°C for 15 sec and 60°C for 1 min.

Quantification of KLK3 (PSA gene), specific of prostatic cells, was also performed to confirm that the results of markers reflected only the expression of these markers in prostatic cells. The expression of each inflammation marker was therefore normalised to KLK3. In order to have an overview of inflammation markers expression in prostatic cells, we also quantified these markers in the prostate cell line LnCaP and used it as calibrator at baseline.

Enzyme-linked immunosorbent assays (ELISA). Protein levels were measured in urine supernatants with Quantikine ELISA kits according to the manufacturer's recommendations (R&D Systems Europe Ltd, Lille, France). Each experiment was repeated at least 3 times.

Efficacy and safety evaluation. The main efficacy criterion was the expression level on each mRNA gene at D90. The secondary efficacy criteria were the assessment of I-PSS and Quality of life (QoL) at all visits, and sexual function (MSF-4), Qmax (uroflowmetry), Post void residual urine volume (PVR) (suprapubic ultrasound) and prostate volume (transrectal ultrasound) at D1, D30, and D90.

A link between mRNA markers/proteins and BPH clinical symptoms on changes from baseline was investigated as well as the analysis of protein expression profile. Safety criteria included adverse events, physical examination and vital signs at each visit.

Statistical considerations. In the absence of previous information on the markers effect size, a sample size of around 200 patients seemed acceptable to reach the objectives of this exploratory analysis. For the primary criterion, downregulation and upregulation of gene expression were considered to occur when at least a two-fold change from baseline was observed. Wilcoxon rank-sum tests were used to compare both groups. Next, values were dichotomised as "expressed" (value>0) and "not detected". Values at D30 and D90 were fitted together using a method based on the generalised estimated equations (GEE). Finally, the changes from baseline to D90 were categorised into 4 classes and Cochran-Mantel-Haenszel tests based on the rank score were used to compare treatment groups. Protein expression profiles were analysed using the same description in classes and the same test.

To account for multiple testing on RNA markers, we applied a Bonferroni correction, which resulted in an adjusted α level of 0.0033. *P*-values larger than α but less than 0.05 were labelled as "nominally significant".

For the secondary efficacy criteria, I-PSS score, QoL, MSF-4 and Qmax, changes from baseline were fitted using a covariance analysis model adjusted for baseline value and treatment. These were also described with respect to the changes of markers.

RESULTS

Baseline Demographics, Disposition, and Disease Characteristics

A total of 206 men were randomised at 36 recruiting centres comprising 26 urologists in 4 countries (Spain, Portugal, Italy and France). The patient flow is illustrated in Figure 1.

Both treatment groups had similar demographics and other BPH baseline characteristics (Table I). According to the box plots, mRNA gene expression was globally similar in both groups despite the variability observed (data not shown).

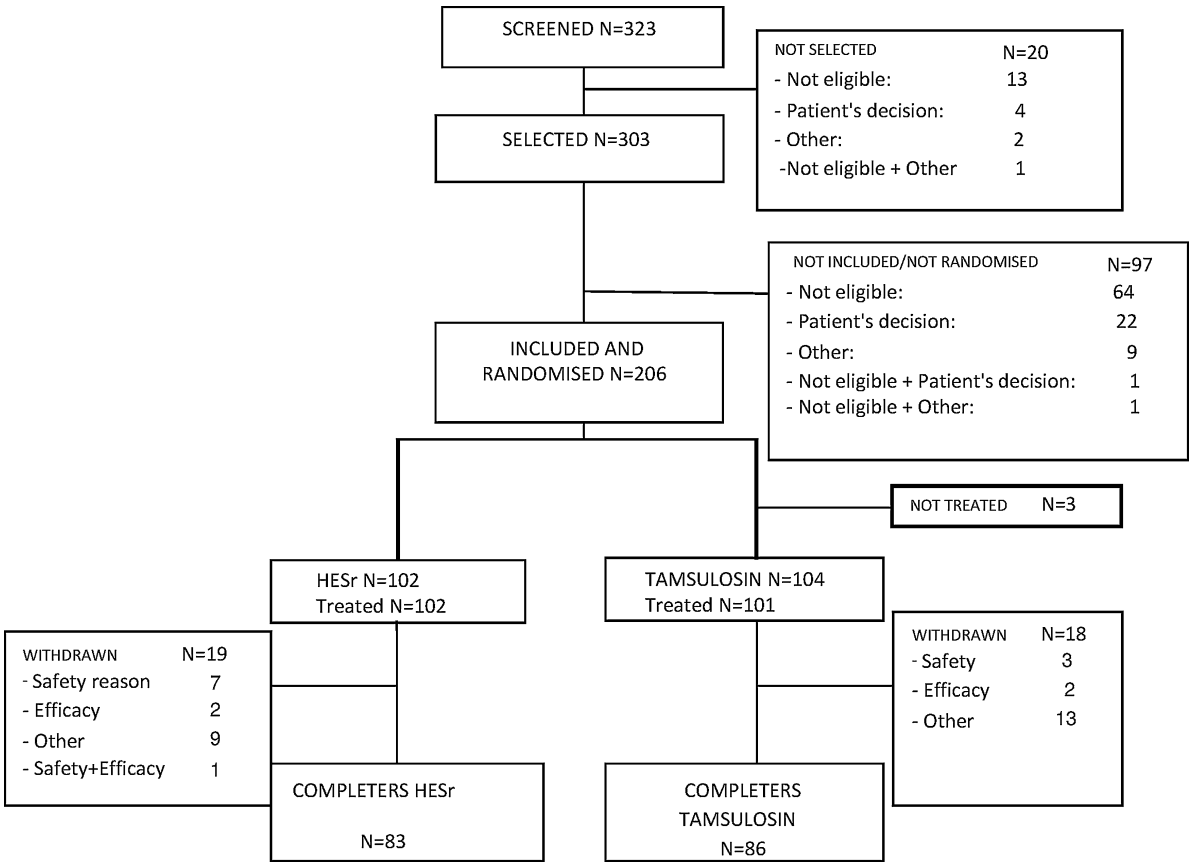

Fig. 1. Patient flow chart.

TABLE I. Evolution of Clinical Symptoms According to Treatment at D90

	HESr n = 102	Tamsulosin n = 101
I-PSS: Total score		
Baseline mean (SD)	17.7 (4.4)	16.8 (4.5)
Min/Median/Max	8/18.0/28	12/16.0/30
Value D90 mean (SD) LOCF**	13.2 (6.0)	10.3 (5.5)
Change D90-baseline LSMean*** (SE)	−4.28 (0.55)	−6.56 (0.55)
QoL score		
Baseline mean (SD)	3.9 (0.9)	3.8 (0.9)
Value D90 mean (SD) LOCF	3.0 (1.4)	2.5 (1.2)
Change D90-baseline LSMean*** (SE)	−0.87 (0.12)	−1.29 (0.12)
MSF4 score		
Baseline mean (SD)	7.4 (4.5)	6.9 (4.5)
Value D90 mean (SD) LOCF	7.7 (4.8)	7.7 (4.7)
Change D90-baseline LSMean*** (SE)	0.36 (0.35)	0.64 (0.35)
Qmax		
Baseline mean (SD)	10.88 (2.69)	10.60 (3.03)
Value D90 mean (SD) LOCF	12.53 (5.21)	12.73 (4.42)
Change D90-baseline LSMean*** (SE)	1.77 (0.46)	2.09 (0.45)
Transrectal prostate volume		
Baseline mean (SD)	48.82 (20.80)	46.29 (13.88)
Value D90 mean (SD) OC*	47.95 (20.05)	46.73 (16.83)
Change D90-baseline LSMean*** (SE)	−0.99 (1.08)	−0.53 (1.05)
Supra-pubic PVR volume (cm3)		
Baseline mean (SD)	53.82 (57.07)	42.04 (47.61)
Value D90 mean (SD) OC	64.11 (63.31)	47.41 (51.29)
Change D90-baseline LSMean*** (SE)	15.22 (5.80)	4.04 (5.84)

*Observed case method (OC).
**Last Observation Carried Forward method (LOCF).
***Adjusted means from the ANCOVA model: Change = Baseline + Treatment.

Primary Endpoints

Out of the 29 genes investigated at mRNA level, 26 were detected at baseline in at least 1 patient; 3 were not detected (CD40LG, CTLA4 and ICOS). At D90, a decrease in mean gene expression was observed for 17/26 (65.4%) markers in the HESr group *vs.* 12/26 (46.2%) in the tamsulosin group (it should be noted that for 7 of them, a decrease was observed in both groups).

We then focused on the most frequently expressed markers; 15 markers (ALOX5, ALOX15B, CAT, CCL2, HIF1A, IL1b, IL8, MIF, NFKB1, PLA2G2A, PTGES2, PTGES3, PTGS2, PTPRC, and STAT3) were expressed at baseline and D90 in at least 30 patients per group, a subgroup considered sufficient to be analysed. At D90, the decrease in the mean gene expression was observed in 80% of these markers in the HESr group versus 33% in the tamsulosin group (data not shown). Moreover, for 9/15 markers (60%) downregulation was observed more frequently in the HESr group compared with the tamsulosin group (5/15 markers —33.3%), No difference was observed between the groups for the fifteenth marker. In addition, for 11/15 markers (73.3%), upregulation was observed in fewer patients in HESr group compared with the tamsulosin group (4/15 markers—26.6%) (Fig. 2). Thus, combining higher decrease and lower increase of these markers resulted in a favourable effect of HESr in 73.3% of mRNA markers (including a nominally significant difference for HIF1A: $P = 0.008$ and PTGES3: $P = 0.0038$) compared with 26.6% for tamsulosin (Fig. 3).

Secondary Endpoints

Protein expression profile in urine. Based on these mRNA results, 10 proteins were selected, comprising 5 cellular proteins (ALOX5, ALOX15B, HIF1A, NFKB, and PTPRC) and 5 proteins potentially excreted in urine (MCP-1/CCL2, IL1B, IL8, MIF, and IP-10/CXCL10). Three proteins (MCP-1/CCL2, IP-10/CXCL10, and MIF) were detected.

At D90, a decrease was observed in the number of patients who expressed MCP-1/CCL2 and IP-10/

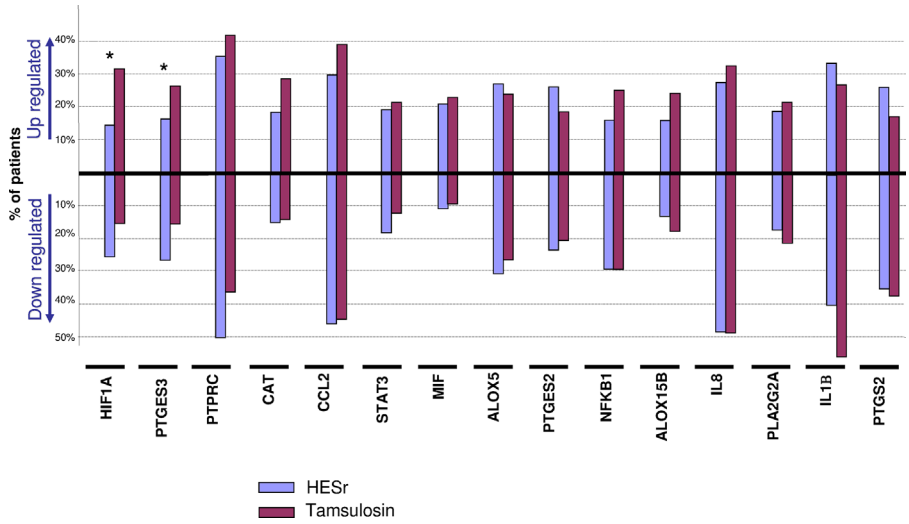

Fig. 2. Evolution of the 15 most frequently expressed inflammation genes (at least in 30 patients per group at baseline and D90). mRNA expression was quantified by qPCR. Percentage of patients for whom mRNA expression level was downregulated or upregulated between baseline and end of treament period (D90). Downregulation and upregulation were considered to occur when a change of at least twofold between baseline and D90 was observed (see Section 3.5: 'Statistical considerations'). Asterix * denoted nominally significant at $P < 0.05$.

CXCL10 in the HESr group (mean from 54.8% and 74.0% at baseline to 35.6% and 63.0% at D90 respectively) in contrast to the tamsulosin group (mean from 46.5% and 64.8% at baseline to 47.9% and 67.6% at D90 respectively). Moreover, in the HESr group, MCP-1/CCL2 and IP-10/CXCL10 were downregulated for a higher percentage of patients (37.0% and 39.7% respectively) and upregulated for a lower percentage of patients (20.5% and 34.3%) compared with the tamsulosin group (28.2% and 31% respectively for downregulation; 25.4% and 43.7% respectively for upregulation) (Fig. 4). It is interesting to note that in the HESr group, MCP-1/CCL2 and IP-10/CXCL10 were switched off for 27.4% and 20.5% of patients respectively compared with 15.5% and 12.7% of patients respectively in the tamsulosin group (data not shown).

MIF protein was expressed in all urine samples at D1 and D90 (Table II). A statistical significance was observed at D90 in favour of HESr with a higher percentage of patients for whom MIF expression was downregulated (42.5%) compared with the tamsulosin

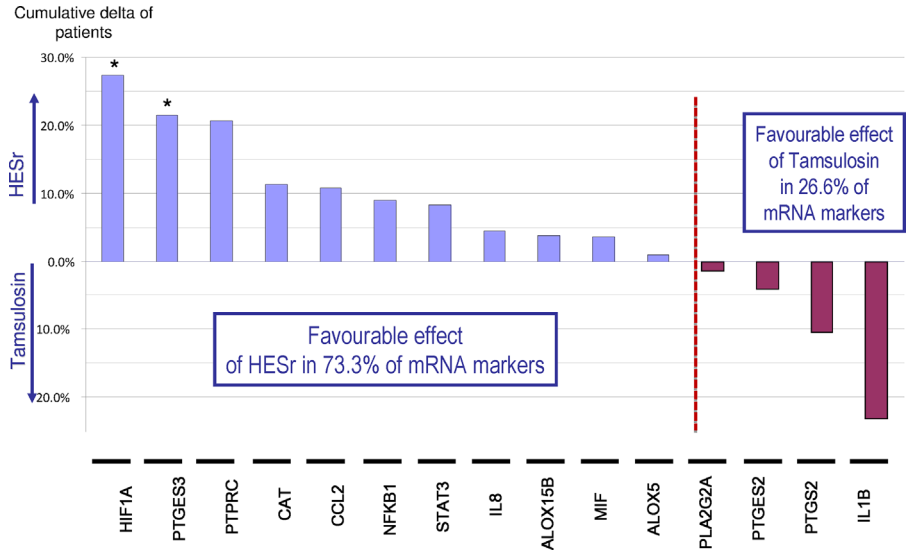

Fig. 3. Cumulative favourable effect by mRNA gene at the end of treatment (D90). For each mRNA gene, the global favourable effect corresponding to the sum of the delta of patients between treatment groups for downregulation and delta of patients for less upregulation was calculated. This was followed by a classification by group. Asterix * denoted nominally significant at $P < 0.05$.

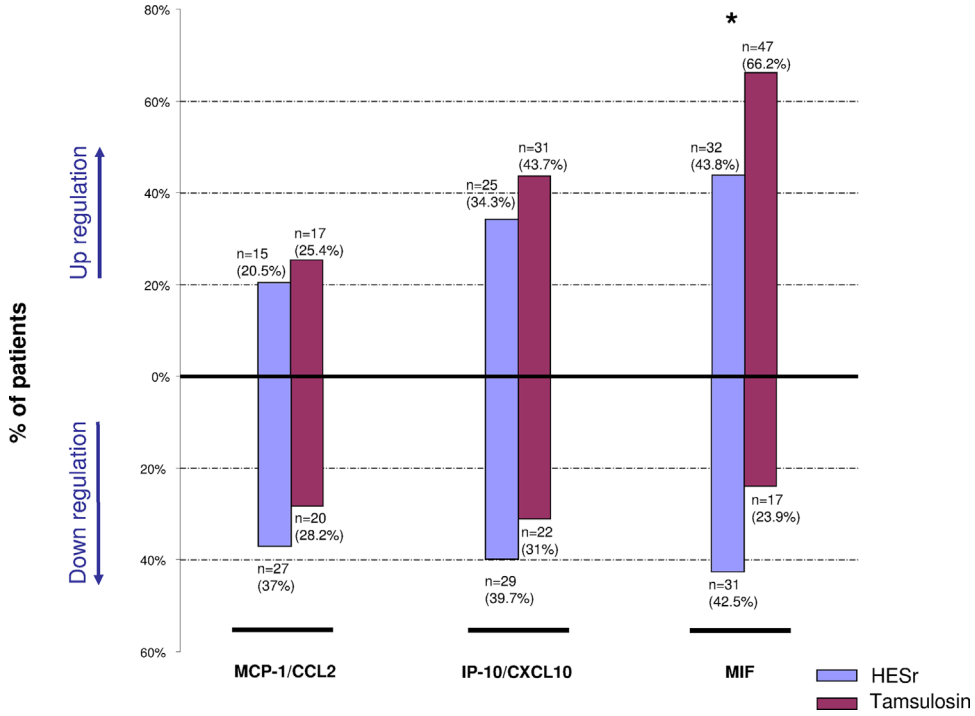

Fig. 4. Protein downregulation and upregulation at the end of treatment (D90). Downregulation and upregulation were considered to occur when a change of at least 25% was observed between D90 and baseline. Treatments were compared using a Cochran-Mantel-Haenszel test based on the rank score. Asterik * denoted $P < 0.05$.

group (23.9%), and a lower percentage of patients for whom the MIF expression was upregulated (43.8% vs. 66.2% respectively) ($P = 0.007$) (Fig. 4).

Clinical outcomes. Compliance to the study treatment was very good and similar in both groups (>95%). At D90, an improvement in I-PSS, QoL, Qmax and prostate volume was observed in both groups. Results are summarised in Table I. The curves for I-PSS over time in both groups are shown in Figure 5.

Link between mRNA expression level and clinical symptoms. No apparent relationship was identified between changes in clinical symptoms and changes in these markers, but, it should be noted the variability observed within groups and the small number of patients in subgroups).

TABLE II. Number of Patients who Expressed the Proteins at Baseline and D90

			HESr n=102		Tamsulosin n=101	
Protein detected	Number of available data		73		71	
MCP 1/CCL2	Baseline	Not detected	33	(45.2 %)	38	(53.5 %)
		Expressed	40	(54.8 %)	33	(46.5 %)
	Value at V4 (D90)	Not detected	47	(64.4 %)	37	(52.1 %)
		Expressed	26	(35.6 %)	34	(47.9 %)
IP 10/CXCL10	Baseline	Not detected	19	(26.0 %)	25	(35.2 %)
		Expressed	54	(74.0 %)	46	(64.8 %)
	Value at V4 (D90)	Not detected	27	(37.0 %)	23	(32.4 %)
		Expressed	46	(63.0 %)	48	(67.6 %)
MIF	Baseline	Not detected	—		—	
		Expressed	73	(100.0 %)	71	(100.0 %)
	Value at V4 (D90)	Not detected	—		—	
		Expressed	73	(100.0 %)	71	(100.0 %)

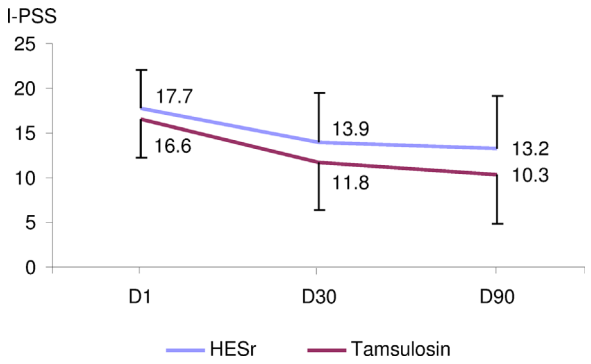

Fig. 5. I-PSS overtime in both treatment groups. I-PSS: International Prostate Symptom Score; D1: baseline, D30: follow-up visit Day 30; D90: end of treatment visit Day 90.

Protein expression profile and clinical outcomes. For the 3 proteins detected, we examined at the subgroup of patients who over expressed the protein at baseline (protein value >3rd quartile) compared with the other patients in the same group and analysed the I-PSS changes between baseline and D90 by treatment group.

No difference was observed between subgroups for MCP-1/CCL2 and IP-10/CXCL10.

In contrast, for patients treated by HESr and who over expressed MIF at baseline, a higher response to I-PSS was observed compared with the other patients in the same group (mean I-PSS change: −6.4 vs. −4.5 respectively). This improvement was not observed in the tamsulosin subgroup (mean I-PSS change: −6.5 vs. −6.3) (Fig. 6).

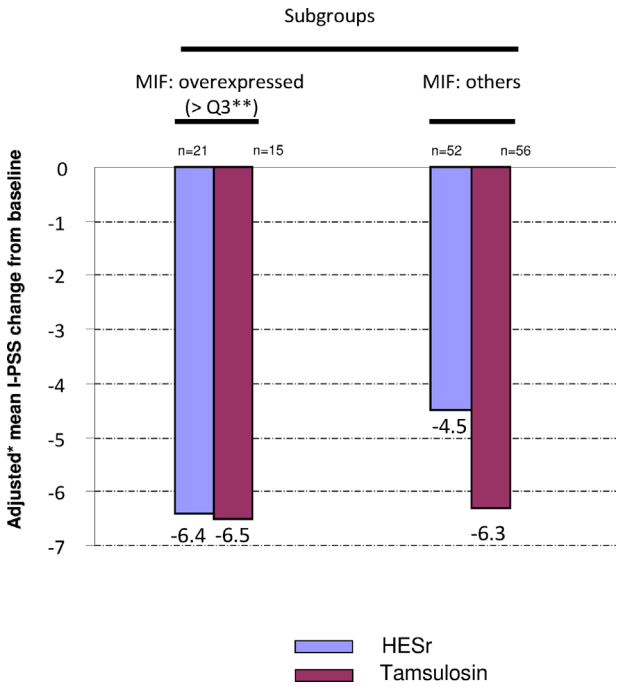

Fig. 6. I-PSS adjusted* mean change from baseline to end of treatment (D90). In HESr group, response to I-PSS at D90 was evaluated in patients who over expressed MIF protein at baseline (>3rd quartile) to those who did not over express this protein. *using Ancova model change=baseline+treatment. **>Q3 corresponds to the 25% of patients who expressed MIF at the highest level.

Safety Assessments

Eight serious adverse events (SAE) were reported during the study including 4 during treatment administration. One (a bilateral gynecomastia), declared in the tamsulosin group, was suspected by the investigator to be in relationship with the treatment. The percentage of patients with at least one adverse event (AE) was 29.4% in the HESr group (41 AEs reported) versus 30.7% in the tamsulosin group (50 AEs reported). A total of 10.8% patients in the HESr group *vs.* 8.9% in the tamsulosin group had at least one related treatment-emergent AE. The most frequent (>2% of patients) treatment-emergent AE (preferred term) were retrograde ejaculation (4% of patients), constipation (3%) and back pain (3%) with tamsulosin while no adverse event occurred at a frequency of more than 2% with HESr. No related treatment-emergent AE had a frequency over 1% in the HESr group compared with the tamsulosin group in which ejaculation failure (2%), retrograde ejaculation (2%) and asthenia (2%) were reported.

With respect to treatment discontinuation, 7.8% of patients in the HESr group *vs.* 3.0% in the tamsulosin group had at least one adverse event (AE) leading to study drug discontinuation (Table III).

DISCUSSION

To the best of our knowledge, this study is the largest randomised clinical trial specifically designed to investigate the anti-inflammatory effects of medical treatments in patients presenting BPH-related LUTS, and the only to use a non-invasive method. The study design was rigorous and followed the standards of quality clinical research: wash-out period, double--blind protocol and comparison between different active treatments. The baseline characteristics of the study population were in accordance with the required selection criteria. It should be noted the high mean I-PSS at baseline in both groups (mean I-PSS=17.7 in HESr group and 16.8 in tamsulosin group) which corresponds to moderate to severe LUTS.

With regard to the lack of placebo arm, given that the results showed the superior anti-inflammatory activity of HESr over tamsulosin, it was considered

TABLE III. List of Patients who Withdrew From the Study for Safety Reasons (Reported Terms)

Group	Subject-Sex-age	Adverse event reported term	Severity
HESr	M-57	Feeling stuffy nose	Mild
		Palpitation	Moderate
	M-69	Rash	Moderate
	M-74	Dizziness sensation	Moderate
		Persistant tiredness	Moderate
	M-73	Abdominal pain	Moderate
		Dry mouth	Moderate
		Insomnia	Moderate
		Nightmare	Moderate
	M-60	Erectile dysfunction	Mild
	M-68	Groin testicular. the patient suffered from pubic pain/ache	Mild
	M-67	Diarrhea	Moderate
		Joint swelling of both hands	Moderate
		Hypertension	Mild
	M-46	Epigastric pain	Mild
Tamsulosin	M-53	Bilateral gynecomastia	Moderate
	M-61	Anejaculation	Mild
	M-62	Weight loss (between v2-w3)	Moderate

that any potential effect of a placebo would have been even lower than that of tamsulosin. A placebo arm would therefore not have provided any additional value regarding the primary endpoint.

With regards clinical outcomes, the improvement in the I-PSS observed in both groups (−4.3 and −6.6) was in line with already published data on active LUTS/BPH treatments, thereby confirming the reliability of the clinical findings.

Although prostatic biopsies remain the reference method for investigating CPI [3], some urinary biomarkers were already successfully proposed to assess CPI in BPH with a reliable link between urine and tissue samples [14]. It was therefore decided that the study participants should not be exposed to any risk of complication related to an invasive procedure such as prostatitis. In this protocol, inflammatory status was monitored with a non-invasive method, which allowed for the collection of prostatic epithelial cells desquaming in the lumen of the glands and seminal plasma fluid after DRE.

Considering all detected markers, a higher decrease in mean mRNA expression was observed in HESr group compared to tamsulosin (65% vs. 46% respectively).

Regarding the 15 most frequently expressed genes at baseline, a nominally significant difference in favour of HESr was observed for HIF1A ($P = 0.008$) and PTGES3 ($P = 0.038$), though none remained significant after Bonferroni correction ($P < 0.0033$) (Fig. 2). Moreover, combining reduced, switched-off, and lower-increased genes, favourable anti-inflammatory activity of HESr was observed in 73.3% of the genes compared with 26.6% of the genes after tamsulosin treatment (Fig. 3). Thus, the trend clearly favoured the anti-inflammatory activity of HESr compared with tamsulosin.

The favourable effect of tamsulosin observed in some genes and/or patients could be explained by the obstruction relief associated with alpha-blocker therapy.

MCP-1/CCL2 and IP-10/CXCL10 mean protein expressions were clearly reduced after HESr treatment, whereas they were slightly raised after tamsulosin treatment (Table II). MIF protein expression was expressed in all samples and almost 50% of patients receiving HESr experienced a significant reduction in MIF (Fig. 6).

CCL2 encodes MCP-1 (monocyte chemoattractant protein-1), a chemotactic protein that plays a critical role in the recruitment and activation of monocytes and macrophages in inflammatory diseases [20]. MCP-1/CCL2 downregulation by HESr is in line with a previous in vitro study which demonstrated that HESr was able to reduce MCP-1/CCL2 expression in epithelial and stromal cell lines [10]; MCP-1/CCL2 was previously described as the most elevated protein secreted in the prostatic fluid of large prostatic glands [21]. The stimulation of prostatic epithelial cells by MCP-1/CCL2 resulted in increased cell proliferation, potentially leading to prostatic enlargement [21].

CXCL10 encodes IP-10 protein (interferon γ inducible protein 10), which plays an important role in the trafficking of monocytes and activated T cells. When activated $CD4^+$T cells, common prostate-infiltrating cells in BPH patients [7], were co-cultured with BPH cells, a significant increase in IP-10/CXCL10 was observed [22].

Human prostate stromal fibroblastic cells can secrete MCP-1/CCL2 and IP-10/CXCL10 cytokines [13,23] that are able to recruit and activate $CD4^+$T cells into the inflamed prostate, thereby generating an immune response leading to the development of chronic immune-mediated tissue destruction and fibromyomatosus growth, as observed in the pathogenesis of BPH [13]. Downregulation of MCP-1/CCL2 and IP-10/CXCL10 by HESr in patients with high CPI could therefore prevent LUTS/BPH progression.

MIF is a long-known T cell cytokine that has been recognised to be a key mediator of innate immunity and pleiotropic inflammatory cytokine [24]. MIF has a direct chemokine-like function, promotes "directed" cell migration (i.e. chemotaxis) and plays a prominent role in inflammatory and atherogenic leukocyte recruitment [25]. Another physiological function of MIF is to counter-regulate glucocorticoid suppression of immune cell responses [26].

MIF plays a pivotal role in the pathogenesis of acute and chronic inflammatory diseases by promoting and amplifying involved inflammatory reactions such as monocyte/macrophage survival or inflammatory cytokine release. Therefore, a direct action of HESr on MIF expression makes it an additional benefit in the management of BPH. However, MIF expression may locally result in higher inflammatory microenvironment [27], suggesting that the decrease in MIF expression could be the consequence of the overall anti-inflammatory effect of HESr treatment; in this way, we observed that HIF1A is downregulated under HESr treatment and it has been proved that HIF1A knockdown led to a reduction of MIF protein level in primary human $CD4^+$T cells [28].

Several clinical studies have indicated the usefulness of MIF as a biomarker for different diseases possessing an inflammatory component [29], it could be also considered as a biomarker of particular interest in LUTS/BPH treatment.

No link could be found between mRNA expression and the clinical outcomes. This was probably due largely to the low number of patients and the very high variability of gene expression at baseline.

This study was not designed to compare functional outcomes between groups, but significant improvement of I-PSS was observed in both groups at D90. However, a better response of −6.4 points I-PSS improvement was observed in the subgroup of patients under HESr with higher baseline MIF protein expression, compared with −4.5 points I-PSS observed in the other HESr subgroup.

These results suggest that HESr could be more effective in patients with higher MIF expression and higher CPI.

CONCLUSIONS

In this double-blind clinical study, HESr showed for the first time the anti-inflammatory activity in men with BPH-related LUTS. HESr, already well--known as a safe product indicated in the management of symptomatic BPH patients, could be particularly useful as an early treatment to prevent unfavourable evolution in patients with CPI.

ACKNOWLEDGMENTS

The authors thank all the investigators involved in the study: Arcangelo Pagliarulo, Rocco Damiano, Matthieu Durand, Emanuele Belgrano, Antonio Alcaraz, Hakim Fassi-Fehri, Bernard Malavaud, Philippe Igigabel, Xavier Durand, Raul Martos Calvo, Luis Campos Pinheiro, Cesare Selli, Riccardo Bartoletti, Henri Chaussade, Jean-Paul Boyes, Richard Yvon, Miguel Unda, Aurélien Descazeaud, Giorgio Carmignani, Régis Soulie, Jacques Tondut, Juan Morote, Alain Palomba, Massimo Porena, Philippe Remaud, Francesco Montorsi, Jean-François Foucault, Rafael Medina, Alfredo Rodriguez Antolin, Avelino Fraga, José Palma Dos Reis, Francisco Gomez Veiga, Benoît Daguzan, Gérard Tatareau, Bernard Boillot, Francisco Javier Burgos, Joaquin Carballido, José Manuel Cozar, Dario Garcia Rojo, Gilles Karsenty. The authors thank Pierre Bunouf for fruitfull collaboration in statistical analyses.

REFERENCES

1. Ficarra V,Rossanese M, Zazzara M, Giannarini G, Abbinante M, Bartoletti R, Mirone V, Scaglione F. The role of inflammation in lower urinary tract symptoms (LUTS) due to benign prostatic hyperplasia (BPH) and its potential impact on medical therapy. Curr Urol Rep 2014;15:463–469.
2. Gandaglia G, Briganti A, Gontero P, Mondeni N, Novara G, Salonia A, Sciarra A, Montorsi F. The role of chronic prostatic inflammation in the pathogenesis and progression of benign prostatic hyperplasia (BPH). BJU Int 2013;112:432–441.
3. Nickel JC, Roehrborn CG, O'Leary MP, Bostwick DG, Somerville MC, Rittmaster RS. The relationship between prostate inflammation and lower urinary tract symptoms: examination of baseline data from the REDUCE trial. Eur Urol 2008;54: 1379–1384.
4. Robert G, Descazeaud A, Nicolaiew N, Terry S, Sirab N, Vacherot F, Maillé P, Allory Y, de la Taille A. Inflammation in

benign prostatic hyperplasia: A 282 patients' immunohistochemical analysis. The Prostate 2009;69:1774–1780.

5. Zlotta AR, Egawa S, Pushkar D, Govorov A, Kimura T, Kido M, Takahashi H, Kuk C, Kovylina M, Aldaoud N, Fleshner N, Finelli A, Klotz L, Lockwood G, Sykes J, Kwast Tv.. Prevalence of inflammation and benign prostatic hyperplasia on autopsy in Asian and Caucasian men. Eur Urol 2014;66:619–622.
6. Begley LA, Kasina S, MacDonald J, Macoska JA. The inflammatory microenvironment of the aging prostate facilitates cellular proliferation and hypertrophy. Cytokine 2008;43:194–199.
7. Kramer G, Mitteregger D, Marberger M. Is benign prostatic hyperplasia (BPH) an immune inflammatory disease?. Eur Urol 2007;51:1202–1216.
8. Kahokehr A, Vather R, Nixon A, Hill AG. Non-steroidal anti-inflammatory drugs for lower urinary tract symptoms in benign prostatic hyperplasia: Systematic review and meta-analysis of randomized controlled trials. BJU Int 2013;111:304–311.
9. Paubert-Braquet M, Mencia Huerta JM, Cousse H, Braquet P. Effect of the lipidic lipidosterolic extract of Serenoa repens (Permixon) on the ionophore A23187-stimulated production of leukotriene B4 (LTB4) from human polymorphonuclear neutrophils. Prostaglandins Leukot Essent Fatty Acids 1997;57: 299–304.
10. Latil A, Libon C, Templier M, Junquero D, Lantoine-Adam F, Nguyen T. Hexanic lipidosterolic extract of Serenoa repens inhibits the expression of two key inflammatory mediators, MCP-1/CCL2 and VCAM-1, in vitro. BJU Int 2012;110:301–307.
11. Sirab N, Robert G, Fasolo V, Descazeaud A, Vacherot F, de la Taille A, Terry S. Lipidosterolic extract of serenoa repens modulates the expression of inflammation related-genes in benign prostatic hyperplasia epithelial and stromal cells. Int J Mol Sci 2013;14:14301–14320.
12. Bernichtein S, Pigat N, Camparo P, Latil A, Viltard M, Friedlander G, Goffin V. Anti-inflammatory properties of Lipidosterolic Extract of Serenoa Repens (Permixon®) in a mouse model of prostate hyperplasia. The Prostate 2015;75:706–722.
13. Penna G, Fibbi B, Amuchastegui S, Cossetti C, Aquilano F, Laverny G, Gacci M, Crescioli C, Maggi M, Adorini L. Human benign prostatic hyperplasia stromal cells as inducers and targets of chronic immuno-mediated inflammation. J Immunol 2009;182:4056–4064.
14. Robert G, Smit F, Hessels D, Jannink S, Karthaus HF, Aalders T, Jansen K, de la Taille A, Mulders PF, Schalken JA. Biomarkers for the diagnosis of prostatic inflammation in benign prostatic hyperplasia. The Prostate 2011;71:1701–1709.
15. Siejka A, Schally AV, Block NL, Barabutis N. Mechanisms of inhibition of human benign prostatic hyperplasia in vitro by the luteinizing hormone-releasing hormone antagonist cetrorelix. BJU Int 2010;106:1382–1388.
16. Stephan C, Xu C, Brown DA, Breit SN, Michael A, Nakamura T, Diamandis EP, Meyer H, Cammann H, Jung K. Three new serum markers for prostate cancer detection within a percent free PSA-based artificial neural network. The Prostate 2006;66: 651–659.
17. Tagaya M, Oka M, Ueda M, Takagaki K, Tanaka M, Ohgi T, Yano J. Eviprostat suppresses proinflammatory gene expression in the prostate of rats with partial bladder-outlet obstruction: A genome-wide DNA microarray analysis. Cytokine 2009;47: 185–193.
18. Theyer G, Kramer G, Assmann I, Sherwood E, Preinfalk W, Marberger M, Zechner O, Steiner GE. Phenotypic characterization of infiltrating leukocytes in benign prostatic hyperplasia. Lab Invest 1992;66:96–107.
19. Fan Y, Hu S, Liu J, Xiao F, Li X, Yu W, Cui Y, Sun M, Lv T, He Q, Jin J. Low intraprostatic DHT Promotes the Infiltration of CD8+ T Cells in BPH tissues via modulation of CCL5 secretion. Mediators Inflamm 2014;2014:397815. DOI: 10.1155/2014/397815.
20. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): An overview. J Interferon Cytokine Res 2009;29:313–326.
21. Fujita K, Ewing CM, Getzenberg RH, Parsons JK, Isaacs WB, Pavlovich CP. Monocyte chemotactic protein-1 (MCP-1/CCL2) is associated with prostatic growth dysregulation and benign prostatic hyperplasia. The Prostate 2010;70:473–481.
22. Vignozzi l, Cellai I, Santi R, Lombardelli L, Morelli A, Comeglio P, Filippi S, Logiodice F, Carini M, Nesi G, Gacci M, Piccinni MP, Adorini L, Maggi M. Antiinflammatory effect of androgen receptor activation in human benign prostatic hyperplasia cells. J Endocrinol 2012;214:31–43.
23. McDowell KL, Begley LA, Mor-Vaknin N, Markovitz DM, Macoska JA. Leukocytic promotion of prostate cellular proliferation. The Prostate 2010;70:377–389.
24. Greven D, Leng L, Bucala R. Autoimmune diseases: MIF as a therapeutic target. Expert Opin Ther Targets 2010;14:253–264.
25. Bernhagen J, Krohn R, Lue H, Gregory JL, Zernecke A, Koenen RR, Dewor M, Georgiev I, Schober A, Leng L, Kooistra T, Fingerle-Rowson G, Ghezzi P, Kleemann R, McColl SR, Bucala R, Hickey MJ, Weber C. MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. Nat Med 2007;13:587–596.
26. Calandra T, Bernhagen J, Metz CN, Spiegel LA, Bacher M, Donnelly T, Cerami A, Bucala R. MIF as a glucocorticoid-induced modulator of cytokine production. Nature 1995;377: 68–71.
27. Bucala R. MIF rediscovered: Cytokine, pituitary hormone, and glucocorticoid-induced regulator of the immune response. FASEB J 1996;10:1607–1613.
28. Gaber T, Schellmann S, Erekul KB, Fangradt M, Tykwinska K, Hahne M, Maschmeyer P, Wagegg M, Stahn C, Kolar P, Dziurla R, Löhning M, Burmester GR, Buttgereit F. Macrophage migration inhibitory factor counterregulates dexamethasone-mediated suppression of hypoxia-inducible factor-1 alpha function and differentially influences human CD4+ T cell proliferation under hypoxia. J Immunol 2011;186:764–774.
29. Grieb G, Merk M, Bernhagen J, Bucala R. Macrophage migration inhibitory factor (MIF): a promising biomarker. Drug News Perspect 2010;23:257–264.