



### Effects of pollen extract EA-10, P<sub>5</sub> on chronic prostatitis or infertility with chronic prostatitis

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**KEY WORDS:** prostatitis; infertility; free radicals; pollen

#### ABSTRACT

**AIM:** To determine the drug action mechanism of pollen extract EA-10, P<sub>5</sub> on the treatment of chronic prostatitis (CP) or infertility with CP. **METHODS:** Malondialdehyde (MDA), super oxide dismutase (SOD), and nitrogen monoxide (NO) were measured by biochemical assay, and zinc content was assayed by atomical spectrophotography in the pre-treatment and post-treatment of CP or infertility with CP. **RESULTS:** Compared with control group, leukocytes in expressed prostatic secretion (LEPS), MDA, and NO were increased, and zinc content and SOD were decreased significantly in the pre-treatment of CP. After the treatment, LEPS was improved, and MDA and NO were reduced, while zinc content were increased apparently and the alteration of SOD was not evident ( $P>0.05$ ). In the pre-treatment of infertility with CP, LEPS, MDA, NO, sperm viability, and seminal leukocytes were obviously higher and seminal plasma SOD, zinc content, and sperm motility were obviously lower than those in control group. After the treatment, LEPS, sperm motility, and sperm viability were improved, MDA, NO, and seminal leukocytes were decreased, SOD and zinc content were increased markedly. **CONCLUSION:** There was inter-correlation between oxygen free radicals (OFR) and occurrence, development, and recovery of CP; Change of OFR may be involved in the drug action mechanism of EA-10, P<sub>5</sub> in the treatment of CP or infertility with CP.

#### Introduction

Chronic prostatitis (CP) is one of the most common diseases in andrology. Its therapeutic efficacy is not very satisfactory. Recent studies showed that CP might defect semen quality. Thus, it is significant to make an investigation of pathogenesis and medication of CP.

Oxygen free radicals (OFR) which causes tissue damage by lipid peroxidation (LPO)<sup>[1]</sup>, includes mainly super oxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), hydroxyl free radical ( $\cdot OH$ ), and nitrogen monoxide (NO). LPO has yielded several types of secondary free radicals and a large number of reactive compounds (including MDA), resulting in the destruction of cellular portion. Of course, cells are equipped with

various antioxidant, such as vitamin E, vitamin C, glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and so on. These can scavenge supernumerary OFR and protect organism from cytotoxic effect of OFR<sup>[2,3]</sup>. In addition, there was apparent negative correlation between semen OFR level and semen quality, but with the increasing of semen OFR level and prolonging of contact time between OFR and sperm, sperm vital force would obviously decrease<sup>[4,5]</sup>. Studies also showed seminal MDA might be increased apparently in chronic bacterial prostatitis, resulting in the influence of sperm vitality and sperm motility<sup>[6,7]</sup>. These data indicated that OFR played an important role in pathogenesis of CP and infertility.

EA-10, P<sub>5</sub> is regarded as a satisfactory drug in the treatment of CP. At present, it is still unknown that whether OFR, antioxidase, and zinc content in semen will be regulated in the treatment of CP or infertility with CP by EA-10, P<sub>5</sub>. Therefore, we investigated whether EA-10, P<sub>5</sub> could inhibit LPO, and thus to obtain the primary conclusion about drug action mechanism of EA-10, P<sub>5</sub> in our treatment.

## MATERIALS AND METHODS

**Population.** All 68 cases of CP (group I) and 63 cases of infertility with CP (group II) were divided into two groups, which were then subdivided into three treatment subgroups respectively (group A: EA-10, P<sub>5</sub> + Roxithromycin, group B: EA-10, P<sub>5</sub> alone, and group C: Roxithromycin alone). Twenty cases who were normal healthy donors of proven fertility were used as control group. The treatment period was four weeks. Group A received EA-10, P<sub>5</sub> (product from Sweden Pharmacia Allergon AB, 375 mg/pill) and roxithromycin (150 mg/pill) twice daily. Group B-C received respectively EA-10, P<sub>5</sub> and Roxithromycin twice daily. During the treatment, all 131 cases were treated with sitting bath in hot water and controlled diet (wine and pungent diet prohibited).

**Semen samples and treatment.** Semen samples were obtained from all cases by masturbation after 3 d of abstinence. Samples were incubated for 20 min in 37 °C warm bath box. Firstly, regular semen analysis and seminal MDA content were analyzed after semen has been liquefied completely; Secondly, liquefied semen was centrifuged at 1000×g for 10 min, and seminal plasma was used to determine the content of NO and SOD. Finally, surplus seminal plasma was frozen at -20 °C until further use for zinc content assay.

**Determination of seminal MDA content and SOD activity.** Seminal MDA content was determined by thiobarbituric acid (TBA) method<sup>[8]</sup>. SOD activity was measured as the inhibition of nitroblue tetrazolium reduction due to superoxide anion generation by xanthine plus xanthine oxidase<sup>[9]</sup>.

**Zinc and NO content in seminal plasma assay.** Zinc content was assayed by a method based on atomical spectrophotography<sup>[10]</sup>. The NO concentration was estimated by a method based on nitrite salt response with sulfanilamide to form diazole, which could appear purplish red color reacting with naphthalene ethylenediamine in the acid conditions. The absorbance of 530 nm was measured<sup>[11]</sup>.

**Semen parameters.** All semen analysis adopt with color quality analysis system of WLJY-9000, which was devised by skill-trade Company Weili Peking. All parameters were settled down to refer to standard of World Health Organization (WHO)<sup>[12]</sup>.

**Statistical.** Data were expressed as mean ±SD and analyzed with *t*-test. Value of *P*<0.05 was considered to be statistically significant.

## RESULTS

**Changes in symptom and LEPS in CP or infertility with CP.** After the treatment by EA-10, P<sub>5</sub> +Roxithromycin, EA-10, P<sub>5</sub> alone, and roxithromycin alone in CP or infertility with CP, remissive rate of symptom was 92 %, 66.67 %, 68.17 %, and 90 %, 61.91 %, 63.64 %, while effective rate of LEPS was 88 %, 57.14 %, 59.09 %, and 85 %, 52.38 %, 54.55 %, respectively. Therapeutic efficacy in group A was significantly higher than that in group B or C (*P*<0.01) (Tab 1, 2).

**Tab 1. Changes in symptom and LEPS in different treated groups of CP. <sup>b</sup>*P*<0.05 vs EA-10, P<sub>5</sub>+Roxithromycin groups.**

Treatment	Case	Symptom improved		LEPS improved	
		Efficiency	Percent/%	Efficiency	Percent/ %
EA-10, P <sub>5</sub> +Roxithromycin	25	23	92	22	88
EA-10,P <sub>5</sub>	21	14	66.67 <sup>b</sup>	12	57.14 <sup>b</sup>
Roxithromycin	22	15	68.17 <sup>b</sup>	13	59.09 <sup>b</sup>

**Tab 2. Changes in the symptom and LEPS in different treated groups of infertility with CP. <sup>b</sup>P<0.05 vs EA-10, P<sub>5</sub>+roxithromycin groups.**

Treatment	Case	Symptom improved		LEPS improved	
		Efficiency	Percent%	Efficiency	Percent%
EA-10, P <sub>5</sub> +roxithromycin	20	18	90	17	85
EA-10, P <sub>5</sub>	21	13	61.91 <sup>b</sup>	11	52.38 <sup>b</sup>
Roxithromycin	22	14	63.64 <sup>b</sup>	12	54.55 <sup>b</sup>

**Changes in LEPS, MDA, SOD, Zinc content, and NO in CP.** Compared with control group, LEPS, MDA, and NO were increased, while zinc content and SOD were decreased significantly in the pre-treatment (P<0.01). After the treatment, LEPS and zinc content were improved, while MDA and NO were decreased apparently vs pre-treatment (P<0.01), but there was no obvious alteration of SOD (P>0.05) (Tab 3).

**Changes in LEPS, MDA, SOD, Zinc content, NO, and semen parameters in infertility with CP.** In the pre-treatment, LEPS, MDA, NO, sperm viability, and seminal leukocytes were obviously higher and SOD, zinc content, and sperm motility were obviously lower than those in controlled group (P<0.01). After the treatment, LEPS, SOD, zinc content, sperm motility, and sperm viability were improved and MDA, NO, and seminal leukocytes were decreased significantly (P<0.01). Compared with the pre-treatment, MDA levels and seminal leukocytes were reduced significantly in group A than these in group B or C in the post-treatment (P<0.01) (Tab 4).

## DISCUSSION

In this test, we have used EA-10, P<sub>5</sub> and roxithromycin to treat CP and infertility with CP.

Roxithromycin has a good effect to chlamydia besides much of Gram-negative bacteria [13].

Therapeutic efficacy was lower in our works than that in literature. But our therapeutic efficacy was still satisfactory. We considered that the reason may be as follows: (1) Chronic bacterial prostatitis may be selected in all the chosen cases, which might influence therapeutic efficacy of EA-10, P<sub>5</sub>. (2) The treatment period was shorter compared with that illustrated in literature. In addition, we have found that therapeutic efficacy in group A was better than in group B or C. This indicated that EA-10, P<sub>5</sub> should be used together with effective antibiotic in the treatment of CP.

Some studies have proved that OFR was related to occurrence and development of CP [3-4,14]. In our studies, MDA was higher and SOD was lower significantly in the pre-treatment of CP than those in the control group, which suggested that there be an increase of OFR, a decrease of antioxidation, and reinforce a of LPO. But MDA was decreased after the treatment, indicated that OFR was scavenged massively and LPO was obviously inhibited.

Similarly, MDA was higher and SOD was lower significantly in pre-treatment of infertility with CP than those in the control group, which suggested that oxidation be increased and antioxidation be decreased in semen. At the same time, we

**Tab 3. Changes in LEPS, MDA, SOD, Zn<sup>2+</sup> content, and NO in different treated groups of CP. Mean±SD. <sup>b</sup>P<0.05, <sup>c</sup>P<0.01 vs control. <sup>d</sup>P>0.05, <sup>e</sup>P<0.01 vs pre-treatment at the same group. <sup>f</sup>P<0.05 vs EA-10, P<sub>5</sub>+Roxithromycin group.**

	Control (n=20)	EA-10, P <sub>5</sub> +Roxithromycin (n=25)		EA-10, P <sub>5</sub> (n=21)		Roxithromycin (n=22)	
		pre-treat	post-treat	pre-treat	post-treat	pre-treat	post-treat
LEPS /Hp	3.4±2.1	25±16 <sup>b</sup>	5.0±2.8 <sup>f</sup>	23±13 <sup>b</sup>	7±4 <sup>f</sup>	25±14 <sup>b</sup>	7±4 <sup>f</sup>
MDA/μmol·L <sup>-1</sup>	4.1±1.1	8.3±1.9 <sup>c</sup>	4.3±1.4 <sup>f</sup>	8.3±1.7 <sup>c</sup>	5.4±1.6 <sup>bfb</sup>	8.4±1.8 <sup>c</sup>	5.2±1.2 <sup>bfb</sup>
Zn <sup>2+</sup> /mmol·L <sup>-1</sup>	2.3±0.6	1.2±0.4 <sup>b</sup>	1.8±0.5 <sup>f</sup>	1.2±0.5 <sup>b</sup>	1.6±0.5 <sup>f</sup>	1.2±0.4 <sup>b</sup>	1.6±0.5 <sup>f</sup>
SOD/kU·L <sup>-1</sup>	920±119	850±118 <sup>b</sup>	851±122 <sup>d</sup>	838±110 <sup>b</sup>	840±113 <sup>d</sup>	829±120 <sup>b</sup>	831±123 <sup>d</sup>
NO/μmol·L <sup>-1</sup>	4.6±1.6	63±20 <sup>c</sup>	39±16 <sup>bf</sup>	63±20 <sup>c</sup>	45±18 <sup>bf</sup>	63±21 <sup>c</sup>	47±18 <sup>bf</sup>

**Tab 4. Changes in LEPS, MDA, SOD, Zinc content, NO, and Semen parameters in different treated groups of infertility with CP. Mean±SD. <sup>a</sup>P>0.05, <sup>b</sup>P<0.05, <sup>c</sup>P<0.01 vs control. <sup>d</sup>P>0.05, <sup>e</sup>P<0.05, <sup>f</sup>P<0.01 vs pre-treatment at the same group. <sup>h</sup>P<0.05 vs EA-10, P<sub>5</sub>+Roxithromycin groups.**

	Control (n=20)	EA-10,P <sub>5</sub> +Roxithromycin (n=25)		EA-10,P <sub>5</sub> (n=21)		Roxithromycin (n=22)	
		pre-treat	post-treat	pre-treat	post-treat	pre-treat	post-treat
LEPS/Hp	3.4±2.1	23±1.3 <sup>c</sup>	6±4 <sup>f</sup>	23±1.2 <sup>c</sup>	7±5 <sup>f</sup>	23±1.2 <sup>c</sup>	6±4 <sup>f</sup>
MDA/μmol·L <sup>-1</sup>	4.1±1.1	9.2±1.6 <sup>c</sup>	5.5±2.1 <sup>f</sup>	9.1±1.9 <sup>c</sup>	7.5±2.4 <sup>bef</sup>	9.1±1.7 <sup>c</sup>	7.2±2.5 <sup>bef</sup>
Zn <sup>2+</sup> /mmol·L <sup>-1</sup>	2.3±0.6	1.1±0.4 <sup>c</sup>	1.6±0.4 <sup>bef</sup>	1.1±0.4 <sup>c</sup>	1.5±0.4 <sup>bef</sup>	1.1±0.3 <sup>c</sup>	1.4±0.4 <sup>bef</sup>
SOD/kU·L <sup>-1</sup>	920±119	653±115 <sup>c</sup>	736±125 <sup>bef</sup>	663±91 <sup>c</sup>	727±104 <sup>bef</sup>	660±97 <sup>c</sup>	722±109 <sup>bef</sup>
NO/μmol·L <sup>-1</sup>	4.6±1.6	78±2.0 <sup>c</sup>	55±18 <sup>bef</sup>	76±2.7 <sup>c</sup>	63±27 <sup>bef</sup>	77±2.5 <sup>c</sup>	61±21 <sup>bef</sup>
10 <sup>9</sup> ×Sperm density/L <sup>-1</sup>	76±24	82±49 <sup>a</sup>	79±46 <sup>ad</sup>	79±4.2 <sup>a</sup>	77±41 <sup>ad</sup>	80±41 <sup>a</sup>	79±40 <sup>ad</sup>
Sperm motility/%	75±12	37±14 <sup>c</sup>	46±14 <sup>bef</sup>	38±17 <sup>c</sup>	43±19 <sup>bef</sup>	37±16 <sup>c</sup>	43±18 <sup>bef</sup>
Sperm viability/%	14±8	36±14 <sup>c</sup>	24±10 <sup>bef</sup>	34±14 <sup>c</sup>	28±11 <sup>bef</sup>	34±13 <sup>c</sup>	28±11 <sup>bef</sup>
10 <sup>9</sup> ×Seminal leukocytes/L <sup>-1</sup>	0.5±0.3	1.6±0.9 <sup>c</sup>	0.7±0.4 <sup>ef</sup>	1.6±0.8 <sup>c</sup>	0.9±0.4 <sup>bef</sup>	1.6±0.8 <sup>c</sup>	0.9±0.5 <sup>bef</sup>

discovered that sperm motility was declined and sperm viability was raised significantly.

But after the treatment, MDA was decreased and SOD was increased significantly than those in the pre-treatment (P<0.01), accompanying with improvement of sperm motility and sperm viability apparently. This indicated that LPO was inhibited and antioxidation was reinforced. From the result above, we believed that EA-10, P<sub>5</sub> could reduce LPO and enhance antioxidation in the treatment of CP or infertility with CP.

In our treatment, antibiotic and EA-10, P<sub>5</sub> were used not only to cure CP but also to improve semen quality. We found that EA-10, P<sub>5</sub> had an effect on weakening oxidative stress and increasing antioxidation in prostatic secretion and semen. This suggested that change of OFR may be involved in the drug action mechanism of EA-10, P<sub>5</sub> in the treatment of CP or infertility with CP. At present, it is known that ferulic acid was an antioxidant containing phenolic hydroxyl<sup>[15]</sup>; and P<sub>5</sub>, one of valid portion in pollen extract EA-10, P<sub>5</sub>, may have anti-oxidative effect owing to providing phenolic hydroxy too. Nevertheless this view still needs to be confirmed by more investigation.

It was reported that zinc content in prostatic secretion and semen was higher than in other organ and body fluid, which showed that zinc played an important role in keeping function of prostate and other accessory sex glands. Our studies showed that zinc content was increased accompanying with improvement of an illness state. EA-10, P<sub>5</sub> can enhance zinc content in

seminal plasma, which may be related to improve local circumstance.

In summary, all these results could provide us with a possible therapeutics approach to treat infertility with CP. In order to improve therapeutic efficacy, anti-infection and anti-oxidation should be adopted in the treatment of CP or infertility with CP.

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