Role of p-ERK1/2 in Benign Prostatic Hyperplasia during Hyperinsulinemia

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Purpose: Using a rat model of hyperinsulinemia, the present study investigated the role of p-ERK1/2 in benign prostatic hyperplasia (BPH).

Materials and Methods: Forty male Sprague-Dawley rats were randomly selected and assigned to four groups: high fat diet (HFD)+BPH (n=10), HFD (n=10), BPH (n=10), and control (n=10) groups. Hyperinsulinemia was induced by HFD feeding, while BPH was induced using testosterone propionate. Plasma glucose, plasma insulin and bodyweight were examined weekly. Immunohistochemistry (IHC) and western blot analysis were used to analyze the expression of ERK1/2 and p-ERK1/2 in rat prostates.

Results: Plasma glucose and plasma insulin levels were significantly greater in the HFD+BPH and HFD groups, when compared to the other two groups ($P < 0.05$). Prostate weights were significantly greater in the HFD+BPH, HFD and BPH groups, than in the control group ($P < 0.05$). IHC and western blot analysis revealed that p-ERK1/2 expression was greater in the HFD+BPH group than in the other three groups ($P < 0.05$).

Conclusion: Androgens plus a hyperinsulinemic condition induced by HFD can result in prostatic cell hyperplasia, and this mechanism may be correlated to the upregulation of p-ERK1/2. Further investigations of this possibility are required.

Keywords: p-ERK1/2; Hyperinsulinemia; BPH; Androgen; ERK1/2

INTRODUCTION

Benign prostatic hyperplasia (BPH), which is caused by the nonmalignant-anomalous growth of the prostate gland, is the most common benign disease in elderly men and is characterized by augmented cell proliferation and/or contractility of the gland[1]. There are multiple causes for BPH, and its development and differentiation are affected by genetic, nutritional, and hormonal factors[2-4]. Recent studies have demonstrated that several other factors play a role in BPH development, including inflammatory mediators, oxidative stress, and ischemia. However, there is no consensus as to which is the most important[5-8]. A parallel increase in the incidence of type-2 diabetes mellitus and BPH has been reported[9]. The study conducted by Qu et al. revealed that prostate volume (PV) is correlated with diabetes in elderly BPH patients[10]. The study conducted by Ozden et al. demonstrated that the transition zone and PV have a positive relationship with serum insulin, suggesting that hyperinsulinemia is a general pathogenic factor for BPH[11]. Both experimental and clinical reports have shown an association between insulin resistance and BPH[12-14].

There are two major insulin signal transduction pathways: (1) the insulin receptor substrate/phosphatidylinositol 3-kinase (IRS/PI-3 kinase) pathway, which is correlated to the intake and metabolism of blood sugar; (2) the Raf/Mitogen-activated protein kinase (MEK)/extracellular-signal-regulated kinase (ERK) (Raf/MEK/ERK) pathway, which has profound effects on cellular proliferation. Both the Raf/MEK/ERK and IRS/PI-3 Kinase pathways control cellular proliferation and/or differentiation[13]. However, it is the IRS/PI-3 Kinase pathway that is inhibited during hyperinsulinemic conditions[15]. Thus, it was hypothesized that during hyperinsulinemic conditions, cellular proliferation and/or differentiation is a result of MEK/ERK activation. ERK, containing ERK1 and ERK2, is a member of the family of mitogen-activated protein kinases (MAPK), which has a wide distribution, and contributes to a number of physiological processes, including the regulation of cellular proliferation, differentiation and apoptosis. Phosphorylation by ERK (p-ERK) results in the translocation of several transcription factors to the nucleus (e.g. AP-1, ELK-1 and SAP), which promote cellular proliferation. It has now been established that hyperinsulinemia may be due to activation of the signaling pathway belonging to p-ERK1/2, which is a member of the family of MAPK[16]. The aim of the present study was to assess the role of ERK signaling during hyperinsulinemic conditions. Therefore, this trial was conducted to evaluate the role of p-ERK1/2 in the cause of prostatic hypertrophy.

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Received October 2019 & Accepted July 2020

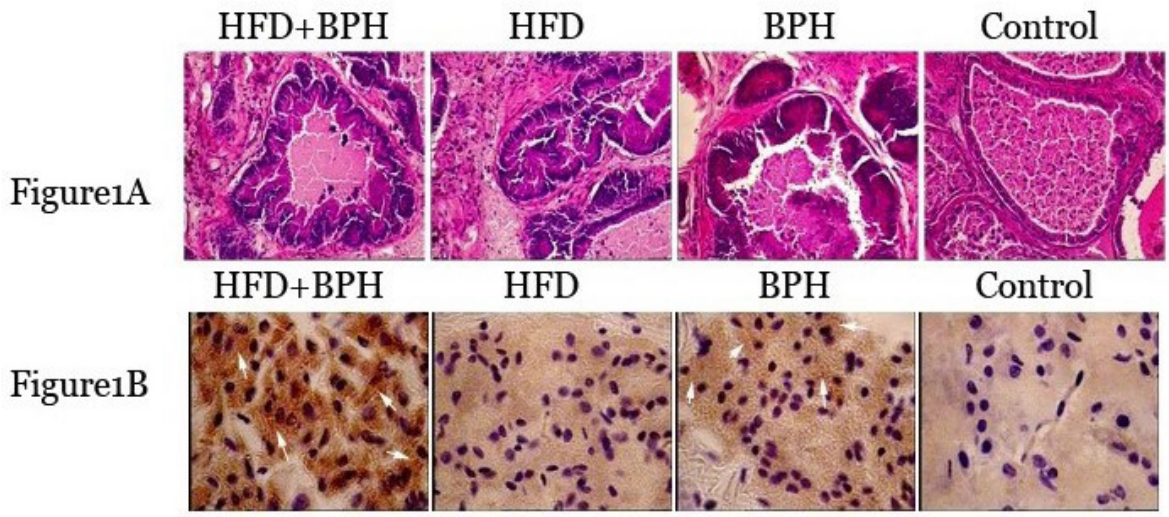

Figure 1. Showed the HE expression and p-ERK1/2 expression in the four groups.
Figure 1A shows the microscopic prostate gland cavity was bigger in the HFD+BPH and BPH groups than in the HFD and control groups. For these groups, adenomatosis was significant, stratified epithelium appeared and was disarranged, and interstitial fibrous tissue and smooth muscle tissue increased, when compared to the HFD and control groups. The microscopic prostate tissue in the HFD group had none of those characteristics, except for the irregular glandular cavity.
Figure 1B show the p-ERK1/2 expression in the cytoplasm of prostate tissues. A greater expression was identified in the HFD+BPH group, when compared to the other three groups. The HFD group and BPH group had detectable p-ERK1/2 expression levels, while the control group did not.

MATERIALS AND METHODS

Animals

All experiments were performed in accordance with the guiding principle of the Institutional Animal Ethics Committee, and approved by the Animal Care Committee of China Medical University. Male Specific pathogen-free (SPF) Sprague-Dawley (SD) rats (nine weeks, 190 ± 10 g) were purchased from the Experimental Animal Center of China Medical University. The animals were kept in our in-house colony with automatic day-night control (12/12 hours), and allowed free access to food and water. These animals were acclimated for one week prior to the start of the experiments.

Chemicals and dose administration

Testosterone propionate (TP, 25 mg/ml) was obtained from Shanghai General Pharmaceutical CO., Ltd. (batch 081004). Anti-ERK1/2, anti-p-ERK1/2 and IgG were purchased from Santa Cruz Biotechnology Inc. (CA, USA).

Experimental design

Hyperinsulinemia was induced by HFD feeding for 8 weeks, while BPH was induced by TP injection for 4 weeks. SD rats were randomly selected and assigned to four groups (each group contain 10 rats): high fat diet (HFD)+BPH, HFD, BPH, and control groups. Rats were tested for blood sugar and blood insulin levels each week. Four weeks after the start of the experiment, rats in the HFD+BPH and BPH groups were given subcutaneous injection of testosterone (20 mg/kg; Wako Chemicals, Tokyo, Japan) each week for four weeks. Rats in the HFD and control groups were given the same volume of olive oil (20 mg/kg). Eight weeks later, one rat, which was randomly chosen from each group, was sacrificed for confirmation of BPH. In total, 40 SD rats were used in the present study. All the animals were sacrificed by cervical dislocation.

Diet for the induction of experimental hyperinsulinemia

In the present study, high fat diet (HFD) is special feed for animals. HFD comprised of 5.3 kcal g−1, 17% carbohydrate, 25% protein and 58% kcal of fat for a period of eight weeks[17].

Immunohistochemistry

Immunohistochemistry was performed on 4-µm-thick sections after deparaffinization. Microwave antigen retrieval was performed in citrate buffer, pH 6.0, for 10 minute, prior to peroxide quenching with 3% H2O2 in phosphate buffered saline (PBS, pH 7.4) for 10 minutes. Then, the sections were washed in water and pre-blocked with normal goat or rabbit serum (Santa Cruz Biotechnology Inc.) for 10 minutes. For the primary antibody reaction, the slides were incubated with anti-p-ERK 1/2 HCV (Santa Cruz, CA, USA) (diluted at 1:100) overnight at 4°C. Then, the sections were incubated with biotinylated secondary antibodies (1:1,000) for one hour. After washing with PBS, streptavidin-horseradish peroxidase was applied. Finally, the sections were rinsed with PBS and developed using diaminobenzidine tetrahydrochloride substrate for 10 minutes. At least three random fields for each section were examined at 100× magnification. A Fiber Image Analysis Instrument (MetaMorph/DP10/BX51, Beijing, China) was used to determine the integrated optical density (IOD) of p-ERK1/2 and ERK1/2. Next, 10-40 fields per group were examined at 400× magnification. The analysis was performed by two individuals who were blinded to the analyzed groups. The results were presented as mean ± standard error of the mean (SEM) for two separate observations[18].

Western blot analysis

Western blot was performed, as previously described

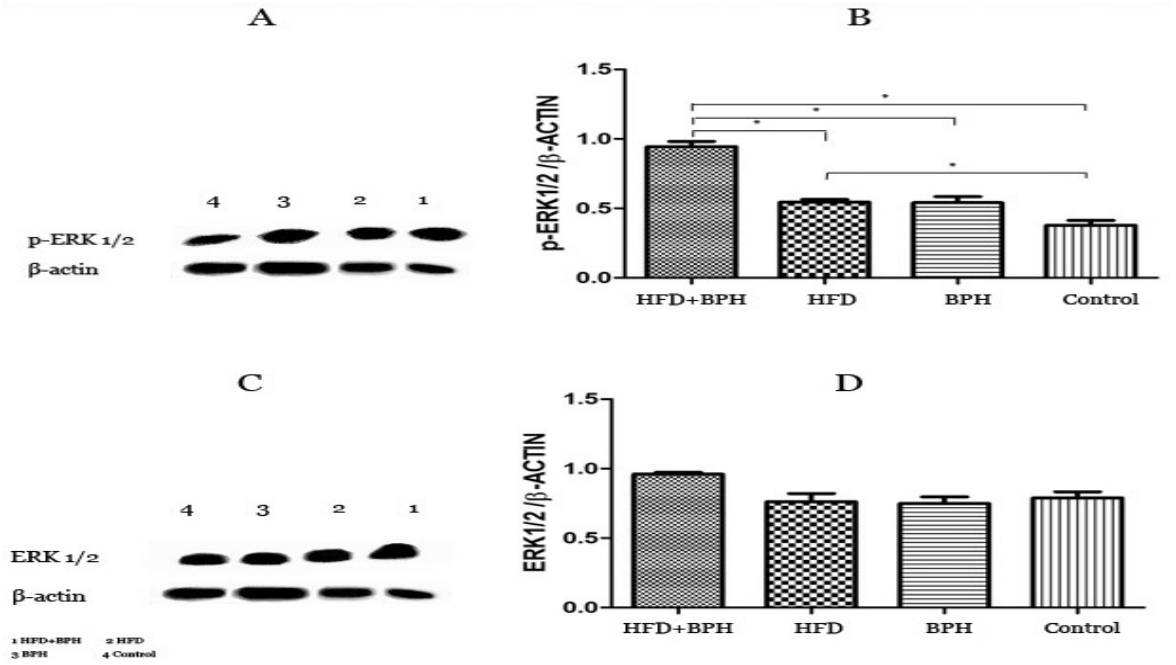

Figure 2. Western-blot expression of **(C, D)** ERK1/2 and **(A, B)** p-ERK1/2 in the four groups.
As shown in Figure 2 **(C, D)**, the expression of ERK1/2 was strongest in the HFD+BPH group. However, the differences among the four groups were not statistically significant. As shown in **Figure 2 (A, B)**, the p-ERK1/2 expression differed among groups, with the strongest expression observed for the HFD+BPH group, which was significantly different from the other three groups (all, $P < 0.01$). The expression of p-ERK in the HFD group was statistically greater than in the Control group ($P < 0.01$).

[16]. Briefly, the membranes were incubated overnight at 4°C with the primary antibody against either MEK1/2 (1:400, Cell Signaling Technology, Beverly, MA, USA), or p-MEK1/2 (1:400, Cell Signaling). The signals were detected using an enhanced chemiluminescence kit (Amersham, Buckinghamshire, UK). β-actin (1:1,000, Santa Cruz Biotechnology) was used as the loading control. Three independent experiments were performed for each animal.

Statistical methods

Data are expressed as the mean ± standard deviation (SD). The significance of differences among groups for the quantitative index was determined using one-way ANOVA, followed by a post hoc LSD test. The hepatic histopathologic evaluation was performed using the Mann-Whitney U test. The statistical analysis was conducted using SPSS 19.0 software, and a $p < 0.05$ was considered statistically significant.

RESULTS

General results

As shown in Table 1, the HFD+BPH and HFD groups had greater plasma glucose and plasma insulin levels, suggesting that hyperinsulinemia has been established ($P < 0.05$). Bodyweight in the HFD+BPH and HFD groups were significantly greater than that in the BPH and control groups (all $P < 0.05$). Prostate weights were greater in the HFD+BPH, HFD and BPH groups than those in the control group ($P < 0.05$). Prostate weights were greater in the BPH group than those in the control group, but the difference was not statistically significant. However, as for prostatic index, HFD does not increase prostate mass with (0.705 versus 0.778) or without BPH (0.638 versus 0.657). Hence, under this experimental model HFD influence was not so direct.

Immunohistochemistry

Figure 1A demonstrates that microscopic prostate gland cavities were bigger in the HFD+BPH and BPH groups than in the HFD and control groups. For these groups, adenomatosis was significant, stratified epithelium appeared and was disarranged, and interstitial fibrous tissue and smooth muscle tissue increased, when compared to the HFD and control groups. The microscopic prostate tissues in the HFD group had none of these characteristics, except for irregular glandular cavities. Figure 1B shows the p-ERK1/2 expression in the cytoplasm of prostate tissues. A greater expression was identified in the HFD+BPH group than in the other three groups. The HFD and BPH groups had detectable p-ERK1/2 expression, while the control group did not.

Western blot analysis

As shown in **Figures 2C and 2D**, the expression of ERK1/2 was strongest in the HFD+BPH group. However, the difference among the four groups was not statistically significant. Furthermore, p-ERK1/2 expression differed among groups, with the strongest expression observed in the HFD+BPH group, which was significantly different from the other three groups (all, $P < 0.01$; Figures 2A and 2B). Furthermore, the expression of p-ERK1/2 was statistically greater in the HFD group than in the control group ($P < 0.01$).

DISCUSSION

Benign prostatic hyperplasia is a very complex phenomenon that includes structural and functional development, and this phenomenon is mainly administered by androgens, estrogen and mesenchymal-epithelial

Table 1. Effect of high-fat diet-feeding and testosterone on the body weight and biochemical parameters

Groups	Body weight (g)	Prostatic wet weight (mg)	Prostatic index (mg/g)	Plasma glucose (mg/dl)	Plasma insulin (ng/ml)
Control	312.34 ± 3.5	205.32 ± 5.4	0.657 ± 0.02	96.60 ± 2.7	0.70 ± 0.3
HFD	369.51 ± 4.8* *P* = 0.023	235.82 ± 6.5& *P* = 0.024	0.638 ± 0.02* *P* = 0.014	127.63 ± 3.5*& *P* = 0.021, *P* = 0.032	2.41 ± 0.3*& *P* = 0.024, *P* = 0.030
BPH	324.81 ± 4.2	252.60 ± 7.8& *P* = 0.035	0.778 ± 0.03& *P* = 0.016	97.40 ± 2.6	0.84 ± 0.2
HFD+BPH	376.53 ± 5.6* *P* = 0.031	265.42 ± 8.5*& *P* = 0.026, *P* = 0.023	0.705 ± 0.03* *P* = 0.042	125.33 ± 3.2*& *P* = 0.015, *P* = 0.031	2.30 ± 0.2*& *P* = 0.024, *P* = 0.035

*$P < 0.05$, compared with BPH; & $P < 0.05$, compared with control group

interactions[19]. It has been reported that hyperinsulinemia, secondary to insulin resistance (IR), is an independent risk factor for BPH development[20,21]. Prostatic vascular lesions can also be induced by atherosclerosis, which could lead to heavier prostate tissue due to ischemia, and contribute to the development of BPH[22]. Ozden et al.[11] found that both transition zone and prostate volumes were positively correlated to serum insulin levels, which suggests that hyperinsulinemia may be a general pathogenic factor for BPH. In the present study, a model of rat hyperinsulinemia was successfully established, and it was found that HFD feeding could significantly increased body weight. Moreover, it was found that testosterone produced greater prostate weight gain, when compared with HFD feeding. This result demonstrates the importance of androgens in BPH. However, combination of testosterone injections with HFD did not increase prostatic index compared with BPH group, while HFD without testosterone injections did not cause prostate enlargement versus control group. Hence HFD alone does not influence prostatic index.

It has been considered that MEK1 binds ERK1/2, and phosphorylates either a tyrosine or threonine residue of ERK1/2, and subsequently, MEK1 dissociates. Monophosphorylated ERK1/2 is again bound by activated MEK-1 and double phosphorylated. Activated in this fashion, ERK phosphorylates p90 RSK, which translocates to the nucleus and phosphorylates transcription factor Elk-1. The regulation of the Raf/MEK/ERK signaling cascade is central to the control of cellular proliferation and differentiation[23-27]. In the present study, it was found that the expression of p-ERK1/2 significantly increased in the HFD+BPH group, when compared with the control group. Vikram et al.[12-14] found significantly increased levels of p-ERK in the prostate of hyperinsulinemic rats, suggesting the involvement of MEK/ERK.

It was established that the HFD and BPH groups do not have significant differences between themselves in the expression of p-ERK1/2 and ERK1/2, but with a combination of fat diet and testosterone, the expression of these factors significantly increases in HFD+BPH group, especially pERK1/2. Therefore, we can assume that the effect of testosterone on the prostate mass is significantly enhanced in the presence of a fat diet and the resulting hyperinsulinemia. In the absence of testosterone, hyperinsulinemia does not significantly affect the mass of BPH in this model.

Diabetes mellitus (DM) patients typically have heavier prostates than non-DM patients. Srinivasan et al. reported in 2004 that patients with DM had more prostate volume and greater International Prognostic Scoring System (IPSS) scores[28]. In the present study, it was found that prostate weight and expression of p-ERK1/2 was statistically higher in the HFD+BPH group, when compared to that in the other three groups. On the base of these data, we can assume that the hyperinsulinemia rises p-ERK1/2 expression in the BPH model induced with testosterone. This result suggests the role for HFD and androgens in BPH. Further investigation of this possibility is required.

The current study suffers from the following limitations: First, this study lacked specific data on ventral and dorsolateral prostate. Second, the quality of the western blot data was limited.

CONCLUSIONS

In conclusion, androgens plus a hyperinsulinemic condition induced by HFD can result in prostatic cell hyperplasia, and the mechanism may be correlated to the upregulation of p-ERK1/2. Further investigations of this possibility are required.

ACKNOWLEDGEMENT

This study was funded by Department of Education in Liaoning Province (201202251). We are particularly grateful to all the people who have given us help on our article.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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