Comparison of Reproductive Damages in Male Rats Induced with Valproate for 7 and 10 days

การเปรียบเทียบความเสียหายของระบบสืบพันธุ์ในหนูแรทเพศผู้ที่ถูกชักนำด้วยวาลโปรเอท เป็นเวลา 7 และ 10 วัน

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ABSTRACT

Valproate (VPA) is widely used in treating for epileptic patients. However, adverse effects of VPA on male reproductive system especially testicular damages were documented. It is unclear whether VPA induction for 7 days can damage the rat testicular histology as reported from a previous study. Therefore, the present study was aimed to reinvestigate it and attempt to observe the critical days that can effectively induce rat testicular damages compared between 7 and 10 days. The Wistar rats were injected (i.p.) with normal saline or VPA (500 mg/kg BW) for 7 and 10 consecutive days and observed reproductive organ weights, testicular histology, seminiferous tubular diameter, and sperm concentration, respectively. Significantly, the results showed that VPA induction for 10 days can obviously damage testicular histology and decrease reproductive organ weights, seminiferous tubular diameter, and sperm concentration as compared with 7 days induction which is similar to that of the control. In conclusion, 10 days of VPA induction are optimum days to induce damages of reproductive organs in Wistar rats for using them as animal model.

บทคัดย่อ

วาลโปรเอท (VPA) ถูกนำมาใช้อย่างแพร่หลายในการรักษาผู้ป่วยลมชัก อย่างไรก็ตามผลของ VPA ต่อระบบสืบพันธุ์เพศชาย โดยเฉพาะอย่างยิ่งความเสียหายของอัณฑะที่มีการรายงานมาก่อน ยังขาดความชัดเจนในการชักนำด้วย VPA เป็นเวลา 7 วัน สามารถทำลายจุลกายวิภาคอัณฑะของหนูแรท โดยมีวัตถุประสงค์เพื่อศึกษาและตรวจสอบช่วงเวลาที่มีประสิทธิภาพสามารถทำให้เกิดความเสียหายของอัณฑะหนูแรทเปรียบเทียบระหว่าง 7 และ 10 วัน หนูแรทถูกฉีดด้วยน้ำเกลือหรือ VPA (500 มก./ กก. นน.ตัว) เป็นเวลา 7 และ 10 วันตามลำดับ และตรวจสอบน้ำหนักอวัยวะสืบพันธุ์ จุลกายวิภาคของอัณฑะ เส้นผ่าศูนย์กลางหลอดเก็บอสุจิ และความเข้มข้นของอสุจิ ผลการศึกษาแสดงให้เห็นว่า การชักนำด้วย VPA เป็นเวลา 10 วัน พบการทำลายจุลกายวิภาคของอัณฑะ และมีการลดน้ำหนักของอวัยวะสืบพันธุ์ เส้นผ่าศูนย์กลางหลอดเก็บอสุจิและความเข้มข้นอสุจิเมื่อเปรียบเทียบกับ 7 วัน ซึ่งคล้ายกับกลุ่มควบคุม สรุปผลการศึกษา 10 วันของการชักนำด้วย VPA เป็นระยะเวลาที่เหมาะสมที่จะใช้เป็นตัวชักนำให้เกิดความเสียหายของอวัยวะสืบพันธุ์ของหนูแรทในงานวิจัย

Key Words: Valproate, Reproductive damages, Male rats

คำสำคัญ: วาลโปรเอท ความเสียหายของระบบสืบพันธุ์ หนูแรทเพศผู้

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Introduction

Valproate (VPA) is widely used as an anti-convulsant drug for treatment of epilepsy, anorexia nervosa, panic attacks, and bipolar disorders (Spina and Perugi, 2004). In addition, VPA has been reported to have anticancer effects against various tumor cells (Ouyang et al., 2011; Travaglini et al., 2009). However, many adverse effects of VPA on human and animal reproductive organs have been documented. In epileptic patients, VPA induced polycystic ovaries and endocrine disorders in women and decreased sperm number, motility, normal sperm, and serum testosterone levels in men (Isojarvi et al., 1993; Krogenase et al., 2008; Roste et al., 2001). Similar to male animals induced with VPA in various days (7, 10, 15, and 30 days) showed histopathology of testis with decreasing of male reproductive organ weights and sperm concentration (Soliman et al., 1999; Vijay et al., 2010). Although, Hamza and Amin (2007) demonstrated the histological severe damages in testis of VPA treated rats, the results gained from our laboratory for testing of VPA negative control are totally different from the control. To clarify these damages with same does of VPA and rats used in Hamza and Amin (2007), this study attempted to investigate and compare the degrees of reproductive damages in male rats treated with valproate for 7 and 10 days. The reproductive organ weights, testicular histology, seminiferous tubular diameter, and epididymal sperm concentration were the parameters used to reveal concentration for such damages.

Objective of the study

The present study was aimed to investigate and compare the reproductive damages in male rats induced VPA between 7 and 10 days.

Methodology

Animals

Male Wistar rats (180-200 g), were purchased from the National Laboratory Animal Center, Salaya, Nakhon Pathom, Thailand. Animals were provided on standard pellet and water ad libitum. All rats were kept in cages under controlled environmental conditions at room temperature (25.1±1ºC) and controlled cycle of 12 h and 12 dark. Then, rats were acclimatized to the environmental conditions for 5 days prior to the commencement of the experiments. This study was approved by the Animals Ethics Committee of Khon Kaen University, based on the Ethic of Animals Experimentation of National Research Council of Thailand (AEKK 50/2556).

Experimental design

Twenty four male rats were randomly divided into four groups. Each group (N=6) was treated as follows:

Group 1: Control group (7 days), rats were injected (i.p.) with 0.5 ml normal saline for 7 days .

Group 2: Control group (10 days), rats were injected (i.p.) with 0.5 ml normal saline for 10 days .

Group 3: VPA 7 days, rats were injected (i.p.) with VPA (500 mg/kg BW) for 7 days.

Group 4: VPA 10 days, rats were injected (i.p.) with VPA (500 mg/kg BW) for 10 days.

This VPA does (500 mg/kg BW) was reported to damage the testis of Wistar rats testes. (Hamza and Amin, 2007)

Histology examination of the testis and seminiferous tubular diameter (STD) assessment

After 7 and 10 days of VPA administration, all rats were euthanized by cervical dislocation and

sacrificed to collected male reproductive organs (testes, epididymes plus vas deferens, and seminal vesicles). Then these organs were weighted. The percentage of organ weight per body weight was calculated and expressed as the relative organ weight. To examine histology of testes, the testes were fixed with 10% formalin in PBS (pH 7.4), dehydrated and embedded in paraffin. The testicular paraffin blocks were sectioned (5µm) and stained with hematoxylin and eosin. The slide sections were observed under light microscope (Iamsaard et al., 2003). The photographs were captured by digital camera (Nikon ECLIPSE E200 microscope, Tokyo, Japan). To determine the seminiferous tubular diameter, the imageJ program was used to its diameters (using round 100 seminiferous tubules on sections of an animal).

Epididymal sperm concentration assay

Left epididymis and vas deferens were operated to collect mature sperm. Epididymal sperm fluid were dipped in 1 ml of phosphate buffer saline (PBS, 37 ºC, pH 7.4) and centrifuged (500 x g, 37 ºC, 5 min) to wash and separate mature sperm pellet from its fluid. To analyze the epididymal sperm concentration, the sperm pellets were resuspension with 1 ml PBS, 37 ºC, pH 7.40. In triplicate preparations, the sperm solution (1:10 dilution) were counted by using a Neubauer's counting chamber and calculated for its concentration (Iamsaard et al., 2003).

Statistical analysis

The independent t–test was used to examine the significant differences between two data set using Sigma Stat (version 3.1.1.). All quantitative results were represented as the mean ± SD.

Results

1. Effect of VPA on male reproductive organ weights between 7 and 10 days

Only rats treated with VPA (10 days) were significantly decreased ($P < 0.05$) the relative weights of testes and seminal vesicles as compared to that of control group and 7 days group (Table 1). In contrast, relative weight of epididymes plus vas deferens in both VPA groups was not significantly different from control group (Table 1).

2. Effect of VPA on testicular damages

Histology of testes showed atrophic tubules in both VPA treated groups as compared to control group (Fig. 1). More atrophic tubules and germ cell degeneration were observed in 10 days VPA treated group than 7 days (Fig. 1). This finding was confirmed by the reductions of seminiferous epithelial

Table 1 Effect of VPA on male reproductive organ weights for 7 and 10 days

Parameters (relative weight)	Control (7 and 10 days)	VPA	
		7 days	10 days
- *Testes* (g/100g)	0.57 ± 0.02	0.53 ± 0.03	0.40 ± 0.05*
- *Epididymes plus vas deferens* (g/100g)	0.17 ± 0.03	0.17 ± 0.01	0.16 ± 0.01
- *Seminal vesicles* (g/100g)	0.29 ± 0.04	0.16 ± 0.01*	0.19 ± 0.05*

* significant differences ($P < 0.05$) as compared to control group

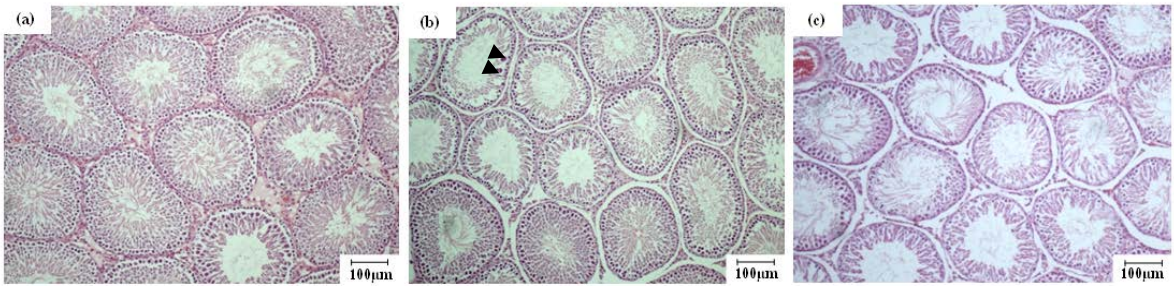

Fig.1 Photograph of the testicular histology (H&E) focusing on seminiferous tubules of control group (a) VPA treated group 7 days [Arrowheads represent giant cells] (b) and VPA treated group 10 days (c).

height (SEH) and seminiferous tubular diameter (Fig. 1b and 2b). In contrast, seminiferous tubular lumen (STL) and tubular space were more increased in 10 days VPA group (Fig 1c). In addition, few giant cells in seminiferous tubules were only observed within 7 days VPA treated group.

3. Effects of VPA on sperm concentration and seminiferous tubular diameter.

Sperm concentration and seminiferous tubular diameter were reduced in both VPA treated groups as compared to that of control group (Fig. 2a and 2b).

Discussion and Conclusions

The results demonstrated that treatment with VPA 7 days has less affect on reduction of reproductive organ weights, except for seminal vesicles as compare to those of 10 days group (Table 1). For histological examination of testes, the degree of testicular damages was clearly observed in 10 days group (Fig 1c). This histophatology was as similar to the results on 7 days VPA treatment demonstrated by Hamza and Amin, (2007). Therefore, induction by VPA for 10 days to demonstrate testicular damages in Wistar rats is appropriate for our laboratory. Moreover, 10 days VPA treated group also affected the decrease relative weights of testes and seminal vesicles (Table 1), sperm concentration, and seminiferous tubular diameter as compared to the control group (Fig. 2), which are similar to VPA effects shown by previous reports (Hamza and Amin, 2007; Nishimura et al., 2000; Yashiwanth et al., 2110). The reduction of seminiferous tubular diameter

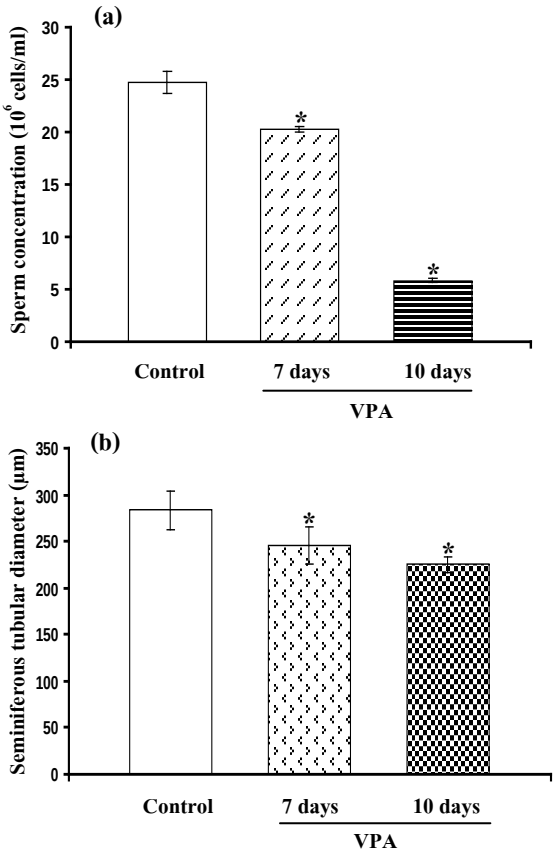

Fig.2 Effects of VPA on sperm concentration (a) and seminiferous tubular diameter (b) in rats. All the values are expressed as mean ± SD, $^{*}P < 0.05$ *vs.* control.

corresponded to their testicular histology. It was observed that atrophic tubules, seminiferous epithelial height, and germ cell degeneration were severe in male rats treated with valproate for 10 days than 7 days.

In conclusion, this study suggested that 10 days of VPA treatment to significantly induce male reproductive organ damages especially testis in Wistar rats.

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