Kasetsart J. (Nat. Sci.) 45 : 59 - 69 (2011)

Effect of a Pyrimethamine-Sulfanilamide Combination on Induced Temporal Infertility in Male Wistar Rats

Panas Tumkiratiwong[1]* and Kittiya Lerkchundhakriat[2]

ABSTRACT

The interaction of a pyrimethamine-sulfanilamide combination on the fertility of reversible infertile effects in male Wistar rats was investigated. Sample groups of rats were administered orally for 49 d based on kilograms of body weight (kgbw) as group I: 1.5 mL corn oil/d; group II: 25 mg/kgbw/d of pyrimethamine; group III: 170 mg/kgbw/d of sulfanilamide; and group IV: 25 and 170 mg/kgbw/d of combined pyrimethamine and sulfanilamide. All groups then had 35 d of drug withdrawal. Males were cohabited with untreated females to diagnose infertility on days 7, 21, 35, and 49 and reversible infertility on days 56, 70, and 84. Males were sacrificed on days 49 and 84, when spermatogram (numbers of spermatids and epididymal spermatozoa, sperm motility, viability of epididymal spermatozoa) and testicular and epididymal histopathology were examined. Male rats showed significant reductions in fertility on days 21, 35 and particularly 49 from the combined treatment, corresponding to significant impairments of spermatogram. Desquamation and multinucleated giant cells in seminiferous tubules as well as hyperplasia, degenerative and cloudy chief cells were observed in the epididymis of rats treated with the combined drugs. Infertility, spermatogram, testicular and epididymal histopathology reversibly became normal by day 35 after drug withdrawal. It was concluded that the combined administration of pyrimethamine and sulfanilamide potentiated temporal infertility effect in male Wistar rats.

Keywords: interaction, male contraception, pyrimethamine-sulfanilamide, reversible infertility, rat

INTRODUCTION

With the human population of the world currently around 6.76 billion (U.S. Census Bureau, 2009) and growing at an explosive rate, the need for additional forms of readily available male contraception appears paramount. To date, contraception techniques in males have been very limited (Cosentino and Matlin, 1997). Certain sulfa drugs containing the sulphonamide functional group, as well as pyrimethamine have been documented recently as being associated with male infertility. Sulphonamides are original synthetic antimicrobial agents that contain the sulphonamide functional group and are the basis of several groups of sulfa drug, namely, sulfasalazine and sulfanilamide. However, sulfasalazine (2-hydroxy-5-[[4-[(2-pyridinylamino) sulphonyl] phenyl] azo] benzoic acid; SASP) is commonly used to treat inflammatory bowel diseases (IBDs), including ulcerative colitis and Crohn's disease. It is also effective in several types of arthritis, particularly

1 Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.
2 Department of Animal Science, Faculty of Agriculture, Silpakorn University, Phetchaburi 76120, Thailand.
* Corresponding author, e-mail: fscipnt@ku.ac.th

Received date : 16/06/10 Accepted date : 17/09/10

rheumatoid arthritis. SASP consists of sulfapyridine (SP) linked to 5-aminosalicylic acid (5-ASA) by an azo bond. 5-ASA is the active therapeutic moiety of SASP (Horimoto *et al*., 2000), but SP, the sulphonamide carrier molecule for 5-ASA, has been linked with adverse reproductive effects (Riley *et al*., 1987) when SASP was described first as a male infertility drug by Levi *et al*. (1979). SASP did not disturb the kinetics of spermatogenesis, but decreased sperm concentrations and altered motility (Hoyt *et al*., 1995). Concurrently, Marmor (1995) reviewed that fertility disturbances of SASP were due to either SASP therapy, severe inflammatory bowel diseases or to their association, but fertility alterations had never been reported in SASP-treated rheumatologic patients. Diverse mechanisms by which sulfa drugs, especially SASP, caused adverse effects on male fertility have been proposed by many authors (Collen, 1980; O'Moran *et al*., 1984; Cosentino *et al*., 1984; Wu *et al*., 1989; Fukushima *et al*., 2007 and Alonso *et al*., 2009). Pyrimethamine (2,4 diamino-5-p-chlorophenyl-6-ethylpyrimidine) is commonly used for both treatment and prevention of malaria, while it was reported first as a cause of male infertility by Bialy and Alexander (1992). Pyrimethamine decreased the sperm motility as well as the sperm count and the fertility rate reduced to zero and testicular histology was altered. However, following 45 d of drug withdrawal, all the parameters mentioned above were returned to normal (Kalla *et al*., 1997).

There is widespread use of the resistance of sulphonamides or pyrimethamine to microbes. Therefore, pyrimethamine has been combined typically with sulphonamides. They work synergistically, with the sulphonamides blocking dihydropteroate synthetase to inhibit folic acid synthesis and then pyrimethamine blocks dihydrofolate reductase to inhibit ribonucleic acid and deoxyribonucleic acid production from the folic acid in microbes. Sulfanilamide (4-aminobenzenesulphonamide) is a sulphonamide antibacterial drug which has had little evaluation for its effects on male reproductive toxicity. Moreover, a combination of pyrimethamine and sulfanilamide with regard to male reproductive toxicity has still to be investigated. It was expected that a combination of pyrimethamine and sulfanilamide would provide a good model to investigate the male reproductive toxicity. Therefore, it was hypothesized that the combination of pyrimethamine and sulfanilamide could potentiate male infertility and the reversible effect of infertility following drug withdrawal.

MATERIALS AND METHODS

Animals and treatments

Male Wistar rats, aged 9 wk and weighing 300 g ± 10 g, were obtained from the National Laboratory Animal Center (NLAC), Mahidol University, and were acclimatized to the laboratory for 7 d prior to treatment. All rats were housed individually in stainless steel metabolic cages (203 × 305 × 127 mm). The controlled temperature ranged between 22 and 24°C, with relative humidity between 55 and 60% and a daily light-dark period of 12 h. All rats were provided with a commercial diet and water *ad libitum*. Male rats were selected for fertility testing prior to the experiment.

A suspension of drugs was prepared in 1.5 mL corn oil prior to daily administration, via the esophagus, for 49 d (day 49). Fertile-proven male rats were allocated according to their kilograms of body weight (kgbw) into four groups of 14 animals in each group. In group I, each animal was given corn oil 1.5 mL as a control. In group II, 25 mg/kgbw/d of pyrimethamine (Sigma Chemical Co., St. Louis, MO) suspended in 1.5 ml corn oil was provided to each animal (50 mg/kgbw/d, based on Kallal *et al*., 1997 was found to be toxic to some rats in a preliminary study). In group III, 170 mg/kgbw/d of sulfanilamide (Sigma

Chemical Co., St. Louis, MO) suspended in 1.5 ml corn oil was given to each animal, with 170 mg/kgbw/d modified appropriately in a preliminary study from the amount of 150 mg/kgbw/d of sulphonamide used in the experiment of Pholpramool *et al*. (1990). Finally, in group IV, each animal was given 25 mg/kgbw/d of pyrimethamine and 170 mg/kgbw/d of sulfanilamide as a combination suspended in 1.5 mL corn oil. One half of the animals in each group (seven animals) remained 35 d (day 84) without treatment as a recovery period. On days 49 (7 animals/group) and 84 (7 animals/group), all male Wistar rats were sacrificed under light ether and testicles were collected to monitor the spermatozoal characteristics and conduct testicular and epididymal histopathology.

Fertility testing

Female rats in the proestrous phase were introduced to males in a ratio of 2:1. Females that had sperm plugs in the vagina were separated, and pregnancy was maintained for 10 d. Mated females were then sacrificed with light ether and the male fertility index was tested by counting the number of foetuses divided by the number of corpora lutea (WHO, 1983b). Each male with fertility greater than or equal to 85% was chosen for random allocation into one of the four groups. Fertility was also evaluated on days 7, 21, 35, 49 (49 d of drug treatment) and days 56, 70 and 84 (after 35 d of drug withdrawal).

Spermatozoal characteristics

Spermatozoal characteristics were monitored, including numbers of spermatids (Amann and Howards, 1980) and epididymal spermatozoa, sperm motility and viability of epididymal spermatozoa (WHO, 1983).

Histopathology

One of the two testes, caput, corpus and cauda of epididymis was fixed in Bouin's solution, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax. Sections were cut to a thickness of 5 mm, stained with Harris hematoxylin and eosin, and then observed under a light microscope. The tubules were evaluated for the existence of complete spermatogenesis and for desquamation and multinucleated giant cells. Alterations of epididymal cells were also studied (for example, hyperplasia, degeneration and cloudy swelling) (Linares *et al*., 2005).

Body weight measurement

Body weights were measured each week to adjust the drug dosage according to body weight and to monitor the changes in body weight throughout the experiment.

Statistical analysis

Data were expressed as mean ± standard error ($\bar{x}$ ± SE). Normal distribution and homogeneity of variances were analyzed employing the Kolmogorov-Smirnov test and Levene test, respectively. If variances were homogenous, one-way analysis of variance (ANOVA) was used and the mean differences among groups were analyzed by Duncan's new multiple range test. The Kruskal-Wallis test was used whenever variances were not homogenous. Significant differences between the medians of two independent groups were tested by the Mann-Whitney U-test. The significant level was set at $P<0.05$ and highly significant testing was at $P<0.01$.

RESULTS

Male fertility and sperm characteristics during drug treatment

No significant differences ($P>0.05$) in fertility were found among the four groups on day 7, but there was significant ($P<0.05$) infertility on days 21, 35 and 49 in group II (Figure 1), and highly significant ($P<0.01$) infertility on days 21,

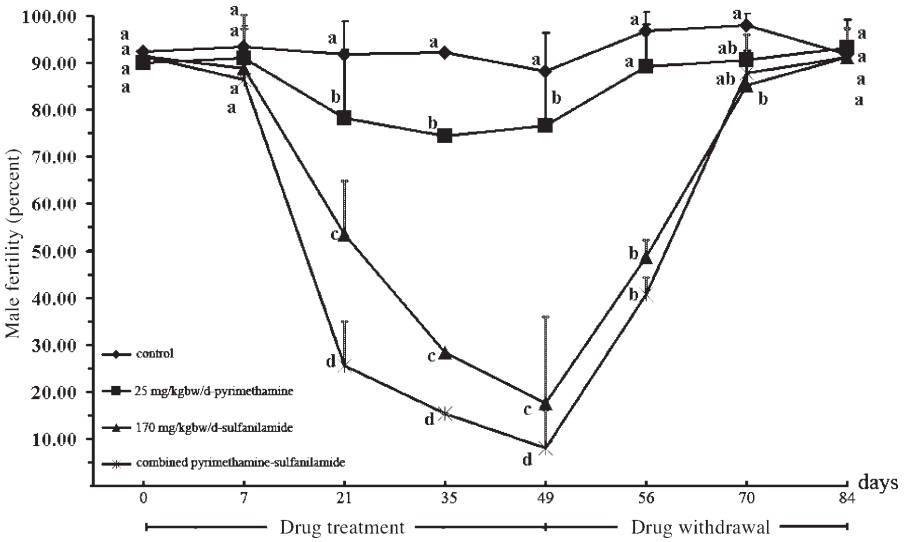

Figure 1 Male fertility percentage of Wistar rats at days 0, 7, 21, 35, 49, 56, 70 and 84 of the experiment. Each point represents $\bar{x}$ ± standard error of 7 Wistar rats per group. One-way ANOVA and Duncan's new multiple range test were employed, values with different letters (a, b) were significantly different at P<0.05 and (c,d) highly significantly different at P<0.01.

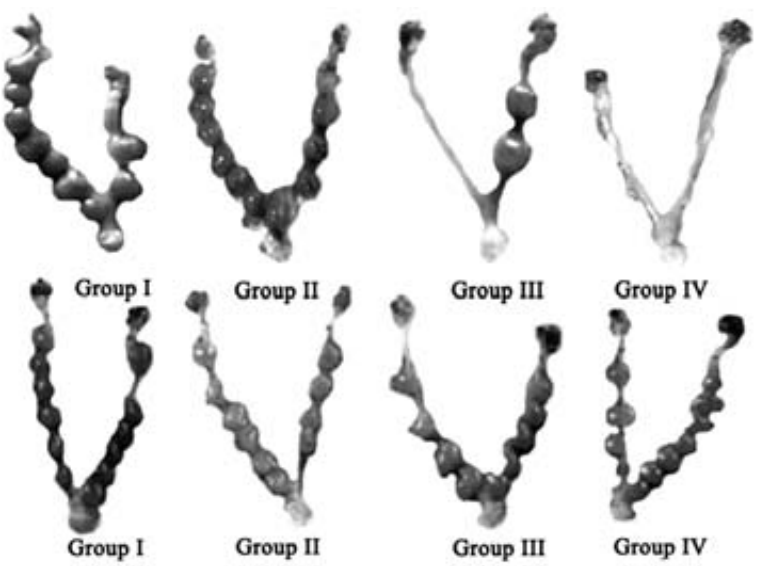

Figure 2 Foetus implants in left and right uterine horns of female rats. Top row (left to right): Conceived uteri of female rats that were mated on day 49 of drug treatment with fertility percentage ($\bar{x}$ ± standard error); group I, 88.28 ± 8.15; group II, 76.69 ± 19.64; group III, 17.55 ± 18.45; group IV, 7.94 ± 8.25. Bottom row (left to right): Conceived uteri of female rats that were mated on day 84 (after 35 d of drug withdrawal); Group I, 91.80 ± 2.72; group II, 93.17 ± 5.98; group III, 91.25 ± 5.95; group IV, 91.27 ± 6.08. (Fertility percentage = number of foetuses divided by number of corpora lutea, multiplied by 100).

35 (Figure 1) and 49 (P<0.01) (Figures 1 and 2) in groups III and IV compared with the control group. Numbers of spermatids and epididymal spermatozoa, sperm motility and viability of epididymal spermatozoa were also significantly (P<0.05) lower in groups II, III and IV on day 49 (Table 1). Figure 2 shows typical images of implanted uteri of females that were mated with treated males, on days 49 and day 84 (35 d after withdrawal of drug treatments).

Histopathology during drug treatment

The seminiferous tubules of group I showed normal features with successive stages of transformation of spermatogonia into spermatozoa (Figure 1A). However, pyrimethamine caused atrophy of the seminiferous tubules, less spermatozoa in some tubules and germinal cell desquamation (De; Figure 1B). Histological defects in sulfanilamide were not prominent (Figure 1C), but combined pyrimethamine-sulfanilamide produced less desquamation (De) and fewer spermatozoa and multinucleated giant germinal cells (G; Figure 1D). The caput of the epididymis of rats in the control group showed well organized chief cells (Ch; Figure 2A). However, pyrimethamine caused cloudy swelling of chief cells (Figure 2B). Sulfanilamide caused chief cell degeneration and hyperplasia (Dc and Hy; Figure 2C). Combined pyrimethamine-sulfanilamide affected chief cell hyperplasia and cloudy swelling (Hy and Cs; Figure 2D). The corpus of the epididymis of control rats demonstrated a normal arrangement of chief cells (Figure 3A). Pyrimethamine was responsible for some cloudy swelling of chief cells (Cs; Figure 3B). Sulfanilamide caused chief cell hyperplasia (Hy; Figure 3C), while combined pyrimethamine-sulfanilamide caused some chief cell degeneration and hyperplasia (Dc and Hy; Figure 3D). The cauda of the epididymis of control rats showed well organized chief cells (Figure 4A). Either pyrimethamine or sulfanilamide caused chief cell

Table 1 Effects of administration of pyrimethamine and sulfanilamide on sperm characteristics of male Wistar rats.

Sperm characteristic	Day 49 (N=7 rats/group)				Day 84 (N=7 rats/group)			
	Control	Pyrimethamine	Sulfanilamide	Sulfanilamide+ pyrimethamine	Control	Pyrimethamine	Sulfanilamide	Sulfanilamide+ pyrimethamine
No. of spermatids ($x10^6$cells/g-testis)	18.81±1.72^a	13.42±3.13^b	10.20±1.31^c	7.92±1.01^d	17.55±0.86^a	17.36±0.93^a	17.10±1.63^a	16.58±1.41^a
No. epididymal spermatozoa ($x10^6$ cells/g-epididymis)	439.40±44.64^a	225.25±55.77^b	211.88±34.83^b	162.74±50.77^c	457.82±70.57^a	494.29±48.81^a	497.89±22.02^a	447.60±66.18^a
Spermatozoal motility (%)	61.63±5.99^a	35.82±4.91^b	37.15±5.35^b	22.3±4.39^c	55.02±2.18^a	46.18±4.58^a	46.32±10.14^a	53.26±1.86^a
Live spermatozoa (%)	77.78±3.40^a	61.26±3.21^b	52.68±2.78^c	22.84±2.82^d	80.48±3.03^a	77.15±3.11^a	77.56±2.89^a	79.87±3.72^a

Note: Results are expressed as ($\bar{x}$ ± standard error). One-way ANOVA and Duncan's new multiple range test were used. Values in the same row not sharing a common letter (a, b, c and d) are significantly different at P<0.05.

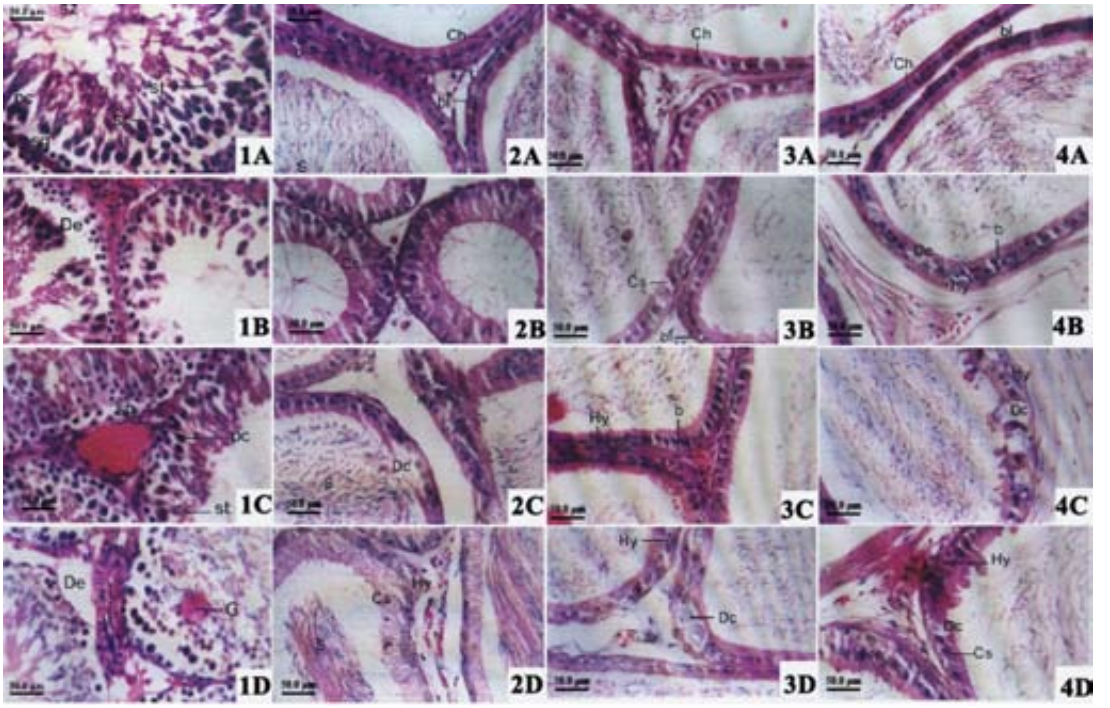

Figure 3 Photomicrograph of seminiferous tubules (1A, 1B, 1C, 1D); caput of epididymis (2A, 2B, 2C, 2D); corpus of epididymis (3A, 3B, 3C, 3D) and cauda of epididymis (4A, 4B, 4C, 4D) on day 49 of control (group I) and drug-treated (groups II-IV) Wistar rats. (Figure letter A = group I; B = group II; C = group III and D = group IV. Magnification = x 400. G = multinucleated giant cell; De = desquamation; pc = primary spermatocyte; sc = secondary spermatocyte; sg = spermatogonia; st = spermatid; sz = spermatozoa; b = basal cell; bl = basal lamina; Ch = chief cell or principal cell; Cs = cloudy swelling; Dc = degenerative cell; Hy = hyperplasia; S = sperm in lumen).

degeneration and hyperplasia (Dc and Hy; Figure 4B and 4C, respectively). Combined pyrimethamine-sulfanilamide caused severe degeneration, hyperplasia and cloudy swelling of chief cells (Dc, Hy and Cs; Figure 4D).

Infertility reversion and histopathology during drug withdrawal

After 35 d (day 84) of drug withdrawal, fertility gradually returned to normal on days 56 and 70 (Figure 1) and was fully reversed on day 84 ($P>0.05$) (Figures 1 and 2) in all four groups, with no significant ($P>0.05$) differences among numbers of spermatids and epididymal spermatozoa, sperm motility, and viability of epididymal spermatozoa on day 84 (Table 1). Testicular, caput, corpus and cauda of epididymis architectures were restored to normal when compared with the control group (Figure 4).

Body weight

There was no significant ($P>0.05$) difference in body weight throughout the experiment (Figure 5).

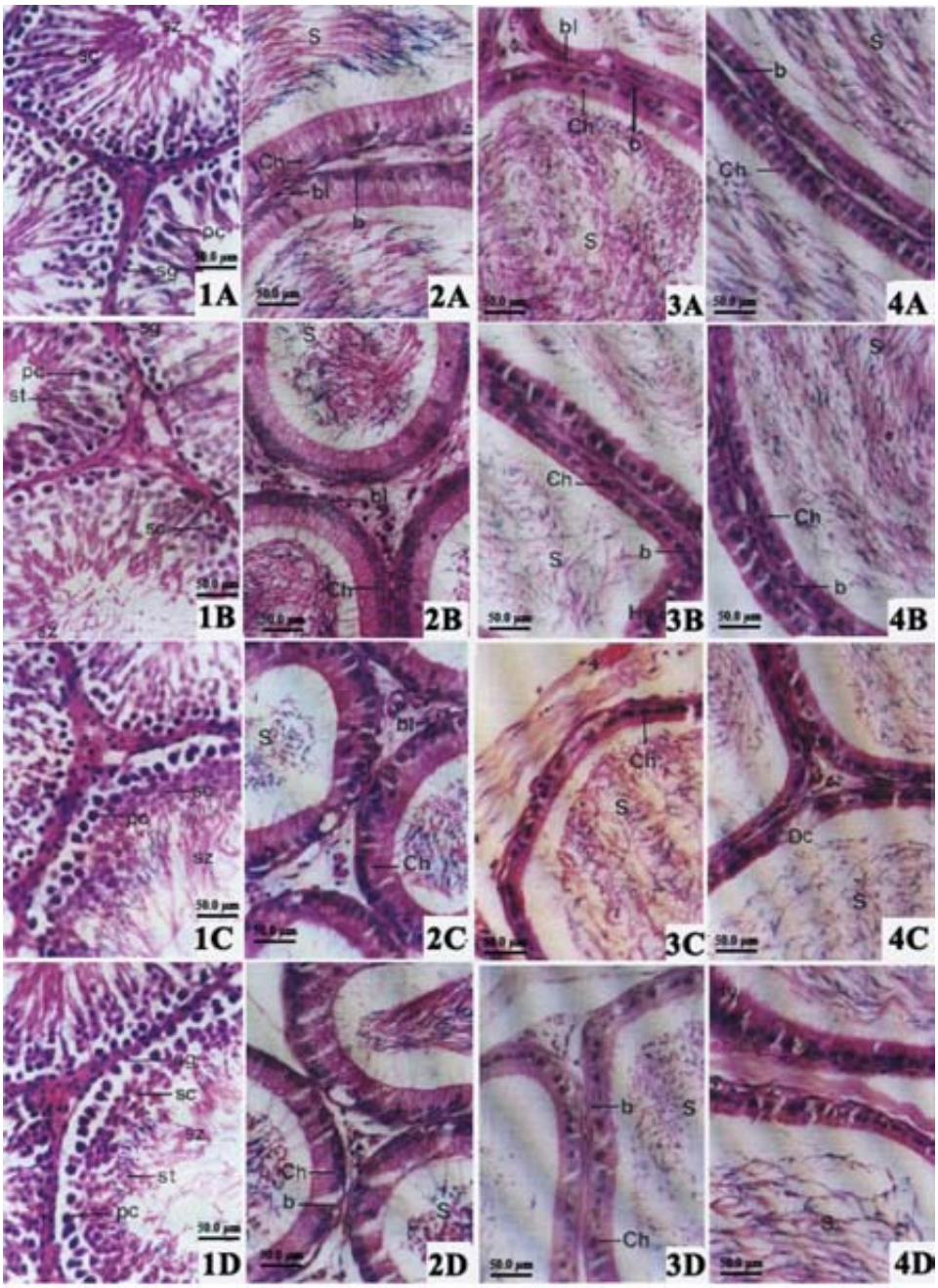

Figure 4 Photomicrograph of seminiferous tubules (1A, 1B, 1C, 1D); caput of epididymis (2A, 2B, 2C, 2D); corpus of epididymis (3A, 3B, 3C, 3D) and cauda of epididymis (4A, 4B, 4C, 4D) on day 84 of control (group I) and drug-treated (groups II-IV) Wistar rats. (Figure letter A = group I; B = group II; C = group III and D = group IV. Magnification = x 400. pc = primary spermatocyte; sc = secondary spermatocyte; sg = spermatogonia; st = spermatid; sz = spermatozoa; b = basal cell; bl = basal lamina; Ch = chief cell or principal cell; Dc = degenerative cell; S = sperm in lumen).

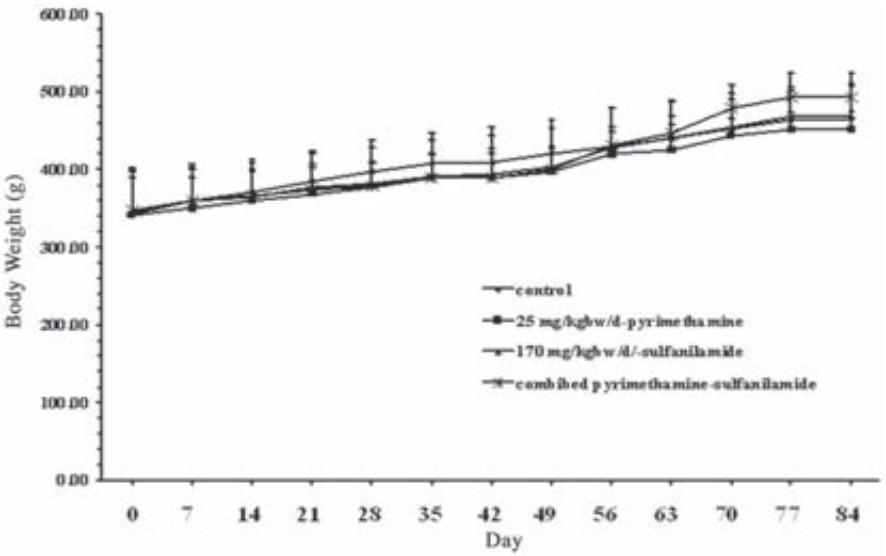

Figure 5 Mean weekly body weight (g) of male Wistar rats among four groups from prior to (day 0) until the end of the experiment (day 84). Positive standard error ranges are shown by the vertical bars. One-way ANOVA and Duncan's new multiple range test were used. Values with no letter were not significantly different at P>0.05.

DISCUSSION

This study demonstrated that 25 mg/kgbw/d of pyrimethamine, 170 mg/kgbw/d of sulfanilamide or a combined amount of 25 mg/kgbw/d of pyrimethamine and 170 mg/kgbw/d of sulfanilamide administered to male Wistar rats for 49 d caused a significant severe decrease in fecundity which was attributed to the male infertility property of these drugs. Awoniyi *et al.* (1993) reported that the consecutive administration of pyrimethamine at a rate of 400 mg/kgbw/d for 56 d to male rats reduced their fertility from 100 to 20%, and that the reduction of fecundity recovered to normal after 56 d of drug withdrawal. Kalla *et al.* (1997) reported that the oral administration of only 50 mg/kgbw/d of pyrimethamine consecutively for 30 d could induce the arrest of spermatogenesis, reduction in the numbers of testicular spermatids and epididymal spermatozoa, and a decrease in epididymal sperm motility, and was restored to normal levels 45 d after drug withdrawal. The provision of 25 mg/kgbw/d of pyrimethamine consecutively for 49 days demonstrated significantly less male fertility, numbers of spermatids, viability of epididymal spermatozoa ($P<0.05$) and numbers of epididymal spermatozoa ($P<0.05$) than those of 170 mg/kgbw/d-sulfanilamide. But there was no significant ($P>0.05$) difference between levels of sperm motility and morphological damage in the seminiferous tubules, caput, corpus and cauda of the epididymis. It was speculated that more than 25 mg/kgbw/d of pyrimethamine preferably demonstrated a more severe level of male infertility. However, all the features of reduced fertility that were observed after the administration of 25 mg/kgbw/d of pyrimethamine or 170 mg/kgbw/d of sulfanilamide could be restored to normal levels after 35 d of drug withdrawal. Pholpramool *et al.* (1990) reported that the administration of 150 mg/kgbw/d of sulphonamide given subcutaneously for 42 d caused reduced

fertility in male rats. Surprisingly, they also reported that the reduction could be reversed only 1 d after drug withdrawal. However, SASP rather than sulfanilamide was used in that experiment and mostly, it has been reported to reduce male fertility, but its action is still unknown (Marmor, 1995). With regard to drug combinations, Uche-Nwachi and Caxton-Martin (1998) found that a therapeutic dose of antimalarial pyrimethamine and sulfadoxine combination (0.72 mg/kgbw/d) administered early in gestation resulted in complete embryo resorption in Wistar rats. Based on this experiment, the present study demonstrated that combined pyrimethamine-sulfanilamide administered consecutively for 49 d to male Wistar rats reduced fertility markedly, as evidenced by significant reduction in the numbers of spermatids and epididymal spermatozoa, and in spermatozoal motility and sperm viability in an additive manner comparable with the administration of individual amounts of pyrimethamine or sulfanilamide. However, some histopathological features showed different effects, with rather adverse alterations in the seminiferous tubules and throughout the epididymis. Moreover, it was shown that the infertility induced by the combined administration of these drugs was reversible in male Wistar rats, because they showed a complete recovery from infertility 35 d after withdrawal of administering the drug. Conclusively, the combined administration of pyrimethamine and sulfanilamide showed an additive function on the induction and reversibility of severe infertility in male Wistar rats.

Possible modes of action of either pyrimethamine or SASP (excluding sulfanilamide) on male infertility have been proposed by many authors. Pyrimethamine did not cause indirect inhibition of serum and plasma testosterone, luteinizing hormone (LH) and follicle stimulating-hormone (FSH) (Awoniyi *et al*., 1993), while Cosentino *et al*. (1990) suggested that the action of pyrimethamine was attributable to its antifolate action. The mode of action of SASP on male fertility has not been elucidated and SASP has been proposed as being an inhibitor of digestive absorption of the folate (Marmor, 1995). Men with high folate intake had lower overall frequencies of several types of aneuploid sperm (Young *et al*., 2008). Jolanda *et al*. (2009) suggested that low folate concentrations in the microenvironment of spermatozoa may have detrimental effects on sperm DNA stability. High concentrations of sperm DNA damage are associated with poor sperm cell motility and morphology. Additionally, SASP was found to inhibit seminal prostaglandins secretion via prostaglandin synthase blockage (Collen, 1980), but no correlation was found between spermatogram results and the level of seminal prostaglandins (Cosentino *et al*., 1984). In addition, Wu *et al*. (1989) found no correlation between reactive oxygen species (ROS) production and sperm density or motility during SASP therapy of inflammatory bowel diseases. Fukushima *et al*. (2007) hypothesized that 600 mg/kgbw/d of SASP suppressed sperm motility and acrosome reaction after 7 d of treatment. Moreover, epididymal, not testicular, expression of *CD 59* and *DAF* genes decreased as early as day 1 of the treatment, thereby SASP suppressed sperm maturation in the epididymis. Alonso *et al*. (2009) reported that 600 mg/kgbw/d of SASP induced a significant decrease of superoxide dismutase (SOD) and glutathione reductase (GR), but a significant increase in catalase (CAT) and thiobarbituric acid-reactive substances (TBARS) in both the testis and epididymis. They concluded that there was SASP-induced variation of oxidative stress markers in the testis and epididymis, which, in turn, might act as a possible mechanism of male-induced infertility. Based on this study, a combination of pyrimethamine and sulfanilamide might cause more severe defects of desquamation and multinucleated giant germinal cells in the seminiferous tubules and hyperplasia, degenerative and cloudy swelling of chief cells in

the epididymis of rats than did either pyrimethamine or sulfanilamide.

In the present study, it was proposed that the combined administration of pyrimethamine and sulfanilamide is one of the possible candidates for the induction of reversible infertility in male rats, although the mechanisms are still unclear.

ACKNOWLEDGEMENTS

This research was funded by the Kasetsart University Research and Development Institute (KURDI). In addition, appreciation is sincerely expressed to all staff members of the Department of Zoology.

LITERATURE CITED

Alonso, V., V. Linares, M. Belles, M.L. Albina, J.J. Sirvent, J.L. Domingo and D. Sanchez. 2009. Sulfasalazine induced oxidative stress: A possible mechanism of male infertility. **Reprod. Toxicol.** 27: 35-40.

Amann, R.P. and S.S. Howards 1980. Daily spermatozoal production and epididymal spermatozoal reserves of the human male. **J. Urol.** 24: 211-215.

Awoniyi, C.A., V. Chandrashekar, B.S. Hurst, W.K. Kim and W.D. Schlaff. 1993. The effects of chronic administration of pyrimethamine on spermatogenesis and fertility in male rats. **J. Androl.** 14: 174-179.

Bialy, G. and N.J. Alexander. 1992. Developments in male contraception. **Health Sexual** 3: 10-11.

Collen, M.J. 1980. Azulfidine induced oligospermia. **Amer. J. Gastroenterol.** 74: 441.

Cosentino, M.J. and S.A. Matlin. 1997. Pharmacological developments in male contraception. **Expert Opin. Inv. Drug.** 6: 635-653.

Cosentino, M.J., R.E. Pakyz and J. Fried. 1990. Pyrimethamine: An approach to the development of a male contraceptive. **Proc. Nat. Acad. Sci. USA**. 87: 1431-1435.

Cosentino, M.J., W.Y. Chey, H. Takihara and A.T.K. Cockett. 1984. The effect of sulfasalazine on human male fertility potential and seminal prostaglandins. **J. Urol.** 132: 682-686.

Fukushima, T., Y. Hamada, M. Komiyama, Y. Matsuno, C. Mori and I. Horii. 2007. Early changes in sperm motility, acrosome reaction, and gene expression of reproductive organs in rats treated with sulfasalazine. **Reprod. Toxicol.** 23: 153-157.

Horimoto, M., Y. Isobe., Y. Isogai and M. Tachibana. 2000. Rat epididymal sperm motion changes induced by ethylene glycol monoethyl ether, sulfasalazine, and 2,5-hexandione. **Reprod. Toxicol.** 14: 55-63.

Hoyt, J.A., L.F. Fisher and D.K. Swisher. 1995. Short-term male reproductive toxicity study with sulfasalazine in the rat. **Reprod. Toxicol.** 9: 315-326.

Jolanda, C.B., S. Marij, U. Elaine, C.R. Johannes, J.C.E. Marinus, L. Jan, S.E.L. Joop, S.M. Nick, A.P.S. Eric and P. MS-T. Regine. 2009. Low folate in seminal plasma is associated with increased sperm DNA damage. **Fertil. Steril.** 92: 548-556.

Kalla, N.R., S.K. Saggar, R. Pari and U. Mahta. 1997. Regulation of male fertility by pyrimethamine in adult mice. **Res. Exp. Med.** 197: 45-52.

Levi, A.J., A.M. Fisher, L. Hugues and W.F. Hendry. 1979. Male infertility due to sulphasalazine. **Lancet.** 314: 276-278.

Linares, V., M.L. Albina, M. Belles, E. Mayayo, D.J. Sanchez and J.L. Domingo. 2005. Combined action of uranium and stress in the rat. II. Effects on male reproduction. **Toxicol. Lett.** 158: 186-195.

Marmor, D. 1995. The effects of sulphasalazine on male fertility. **Reprod. Toxicol.** 9: 219-223.

O'Moran, C., P. Smethurst, C. Dore and A.J. Levi. 1984. Reversible male infertility due to sulphasalazine: Studies in man and rat. **Gut.** 25: 1078-1084.

Pholpramool, C., V. Verawatnapakul, S. Ruchirawat and L.M. Lewin. 1990. Mode of action of the antifertility sulphonamides lack of effects on folate metabolism. **Contraception.** 42: 667-675.

Riley, S.A., J. LeCarpentier, V. Mani, M.J. Goodman, B.K. Mandal and L.A., Turnberg. 1987. Sulphasalazine induced seminal abnormalities in ulcerative colitis: Results of mesalazine substitution. **Gut.** 28: 1008-1012.

Uche-Nwachi, E.O. and A.E., Caxton-Martin. 1998. Sulaphdoxine-pyrimethamine embryopathy in Wistar rats. **Kaibogaku Zassashi.** 73: 135-139.

World Health Organization (WHO). 1983. **Protocol MB-50, a Method for Examining the Effect of the Plant Extracts Administered Orally on the Fertility of Male Rats**. Geneva.

Wu, F.C.W., R.J. Aitken and A. Ferguson. 1989. Inflammatory bowel disease and male infertility: effects of sulfasalazine and 5-aminosalicylic acid on sperm fertilizing capacity and reactive oxygen species generation. **Fertil. Steril.** 52: 842-845.

Young, S.S., B. Eskenazi, F.M. Marchetti, G. Block and A.J. Wyrobek. 2008. The association of folate, zinc and antioxidant intake with sperm aneuploidy in healthy non-smoking men. **Hum. Reprod**. 23: 1014-1022.