Intratesticular Injection of a Balanced Zinc Solution for Permanent Sterilization of Dogs

Veera Tepsumethanon, DVM*,
Henry Wilde, MD, FACP*, , Thiravat Hemachudha, MD**,

*Queen Saovabha Memorial Institute, Thai Red Cross Society,
(WHO Collaborating Centre for Research on Rabies Pathogenesis and Prevention)
**Faculty of Medicine, Chulalongkorn University

Recipients of a grant from National Science and Technology Development (Thailand)

Five male mature dogs with normal sex organs and normal pre-study semen evaluation received intratesticular injections of a sterile solution of balanced zinc gluconate. Semen without sperm could be collected on days 26, 30, 35 and 51 from 4 dogs. The dogs achieved penile erection but no ejaculation before these days. There were no significant adverse effects and no change in the dog behavior during the time that they were observed. Histopathological findings documented virtually complete fibrosis of seminiferous tubules and Leydig cells on days 60 and 75. This preliminary study concludes that balanced zinc gluconate may be used to sterilize dogs.

Keywords: *Rabies control, Canine population reduction*

J Med Assoc Thai 2005; 88(5): 686-9
Full text. e-Journal: *http://www.medassocthai.org/journal*

Thailand is a rabies endemic country where stray and community dogs are the main vectors. There has been a reduction in the incidence of human rabies during the past decade, largely by the provision of expensive but now readily available post-exposure treatment of animal bite victims. However, the disease remains rampant among the large population of stray dogs. Cultural barriers prevent large scale culling of dogs as has been done in neighboring Malaysia to facilitate sustainable vaccination of at least 70 percent of the dog population as is required for canine rabies elimination. Thus, there is a need for other humane methods that might reduce the dog population. Sterilization of male dogs has traditionally been carried out by surgical castration. This is a relatively simple procedure which, however, requires anesthesia and a veterinary surgeon or experienced technician. Research to develop chemical and hormonal alternatives that can be implemented by para-professional staff have a long and complicated history[1-6]. They may be of value in regions where cultural and religious beliefs prevent canine population control by culling, and where skilled veterinary surgeons are not readily available. Hormonal methods, usually using progesterone derivatives, are expensive and can cause pyometra following long term use. They may still be useful in selected locations[7]. Efforts to find chemicals that sclerose testis started with American pig farmers where the objective was to reduce the bad taste that develops in adult boar meat due to androgen action[8]. Silver nitrate, formalin, 95% ethanol and quinacrine have all been tried[1-5]. They can be injected by farmers, do scar the testis but will not usually stop testosterone production and the bad smell and taste of boar meat[8]. Zinc tannate will destroy seminiferous tubules and, at a higher dose levels, also Leydig cells. However, it was found to be neurotoxic in pigs[2]. Indian veterinarians have experimented with testicular injection of zinc compounds. However, one experimental application resulted in necrosis and infection that required surgical intervention. The project was abandoned (personal communication Dr. S Abdul Rahman, Bangalore). Neutralized Zinc appeared more promising

Correspondence to : Tepsumethanon V, QSMI, 1871 Rama IV Rd, Bangkok 10330, Thailand. E-mail: tepsumethanonv @yahoo.com

than other products previously tried and the authors decided to experiment with the latter in an effort to develop a humane and inexpensive method for stray dog population control. The objective was to develop a method that can be applied by trained para-professionals in a sustainable manner.

Material and Method

Five healthy adult mixed breed dogs aged 1 to 5 years with a body weight of 11 to 16 Kg (mean 13.2 Kg) were selected. Ejaculation was induced in the dogs by manual masturbation and all were shown to have motile sperm in adequate amounts prior to inclusion in the study. They were restrained without sedating and each testis was injected with our sterile solution of balanced zinc gluconate with a concentration of 13.1 mg/mL. It was injected at 0.8-1.0 mL using a tuberculin syringe and 28 gauge, inch needle. The dosage was in accordance with the testicular width (Table 1). Testes were measured before and after injection for 60 days. The body temperature, general appearance and food as well as water intake were recorded for each dog daily for 60 days. Efforts were made to obtain semen on days 21-59. One testis was surgically removed on day 60 and the other on day 75. They were then examined by a pathologist at King Chulalongkorn University Hospital. Dogs were kept for 90 days after the injection and then returned to the municipal dog pound. The study was approved by the ethics committee of the Thai Red Cross Society.

Results

Testis: The dogs did not appear to have much discomfort with the injection itself but testes were inflamed and edematous for 7 days. Some discomfort on manipulating the testis was present on days 1-11 in 3/5 dogs. Two dogs appeared to not have any pain or discomfort. The size of each testis was the largest on days 2-5 after injection. It gradually reduced to normal size within 3-6 weeks. Decreasing of testicular size was then continuous, reaching approximately 75% of the original size by the time the authors carried out the orchidectomies on days 60 and 75.

Behavior: There was no significant change in the behavior of any of the five dogs.

Adverse effects: Edema and swelling of the scrotum were the only significant adverse effects noted. One dog had a small superficial ulceration at one injection site which healed within one week. Body temperatures remained normal during the study (Table 2).

Table 1. Testicular width and dose

Testicular Width (mm)	Zinc dose Administered (mL)
10-12	0.2
13-15	0.3
16-18	0.5
19-21	0.7
22-24	0.8
25-27	1.0

Collection of semen: Efforts were made to obtain semen on days 21-59 but were usually not successful. The animals were either not able to achieve an erection, unable to ejaculate or the ejaculate did not contain any sperm (Table 3).

Pathology: See Table 4 for histological findings. They documented virtually complete scarring of seminiferous tubules and Leydig cells.

Discussion

The authors' preliminary experiment indicated that the zinc compound (balanced zinc gluconate) was

Table 2. Rectal temperatures (Normal canine temperature range 100.5-102.5 F)

Dog No.	Temperature (F)			
	D0	D1-7	D8-60	Remarks
3	101.4	101.0-101.8	100.2-102.2	D48 = 103.0
4	101.2	101.4-102.4	100.2-102.0	-
5	101.2	101.0-101.6	100.0-102.0	D28 = 104.4
6	ND	ND	ND	-
10	102.0	101.0-102.2	100.4-102.8	D20 = 104.8 D21 = 104.6

Table 3. Collection of semen after injection

Dog No.	Collection of semen	
	Day*	Result
3	23, 27	Erection, ejaculate, sperm +2 and 1-3% movement
	28, 29, 34, 36, 38-40	Erection, no ejaculate
	30, 33, 35, 37, 41, 57	Erection, ejaculate, no sperm
4	24-41, 55	Erection, no ejaculate
	35, 39	Erection, ejaculate, no sperm
5	27-28, 33-43	Erection, no ejaculate
	26, 29, 30	Erection, ejaculate, no sperm
6	Not done	Not done
10	21-37	Erection, no ejaculate
	51	Erection, ejaculate, no sperm

* The dogs did not develop erections during days 21-59, except where noted

Table 4. Histopathology

Dog No.	Pathological diagnosis	
	Left testis 8 weeks after injection	Right testis 10 weeks after injection
3	Coagulation necrosis	Old focal hemorrhage
	Histiocytic infiltration and fibrosis	Atrophic seminiferous tubules
4	Atrophic tubules and chronic inflammation	Diffusely hyalinized testis
5	Coagulation necrosis with histiocytic infiltration	Atrophic seminiferous tubules
6	Unremarkable testis except minute areas of cell necrosis	Chronic and organizing orchitis
10	Coagulation necrosis and atrophic tubules	Coagulative necrosis and hyalinized tubules

well tolerated by the animals and that it resulted in virtually complete destruction of sperm and androgen production by testicular structures (seminiferous tubules and Leydig cells). There were no significant adverse effects and no change in the dog behavior during the time that they were observed. The authors conclude that this method may be a useful arm in an overall stray dog population reduction effort. It will, however, only be effective in rabies control if it is accompanied by sustainable vaccination of at least 70 percent of the entire dog population. Surgical sterilization and/or hormonal estrus reduction in female dogs must also be implemented for one active and fertile male dog is able to fertilize females in the entire neighborhood.

Acknowledgements

This study is receiving ongoing support from Thai Biotech Fund which the authors thankfully acknowledge. It is only one component of a large canine rabies control project involving staff from the Thai Red Cross, Chulalongkorn University and the Ministries of Public Health and Agriculture. Mr. Payoongsak Saralamp provided valuable technical help in compounding the zinc product. The authors wish to thank the director of this institution, Professor Visith Sitprija and the Dean of the Faculty of Veterinary Science, Professor Narongsak Chaiyabutr, for their active support of this promising preliminary study. The technical staff of the rabies diagnostic facility at QSMI provided patient and humane care for the experimental animals.

References

1. Fahim MS, Wang M, Sutcu MF, Fahim Z, Youngquist RS. Sterilization of dogs with intra-epididymal injection of zinc arginine. Contraception 1993; 47: 107-22.
2. Migally NB, Fahim MS. Pharmacokinetics of zinc tannate after intratesticular injection. Arch Andol 1985; 13: 129-36.
3. Fahim MS, Fahim Z, Harman JM. Chemical sterilization in the male: rats. Arch ndrol 1983; 11: 261-5.
4. Malaviya B, Chandra H, Kar AB. Chemical occlusion of vas deferens by quinacrine in resus monkeys. Indian J Exp Biol 1974; 62: 560-62.
5. Pineda MH, Reimers TJ, Faulkner LC, Hopwood ML, Seidel GE Jr. Azospermia in dogs induced by injection of sclerosing agents into the cauda of the epididymis. Am J Vet Res 1977; 38: 831-8.
6. Freeman C, Coffey DS. Sterility in male animals induced by injection of chemical agents into vas deferens. Fertil Steril 1973; 24: 884-97.
7. Brooks RI, Pearson AM, Hogberg MG, Pestka JJ, Gray JI. An immunological approach for prevention of boar odor in pork. J Anim Sci 1986; 62: 1279-89.
8. Beery KE, Sink JD, Patton S, et al. Characterization of the swine sex odor components in boar fat volatiles. J Food Sci 1971; 36: 1086-10.

การทำหมันถาวรในสุนัขโดยการฉีดสารละลาย Zinc เข้าในลูกอัณฑะ

วีระ เทพสุเมธานนท์, เฮนรี ไวล์ด, ธีระวัฒน์ เหมะจุฑา

สุนัขเพศผู้จำนวน 5 ตัวที่อยู่ในวัยเจริญพันธุ์ มีอวัยวะสืบพันธุ์ปกติและได้รับการตรวจสอบความเป็นปกติและคุณภาพของน้ำเชื้อและตัวอสุจิ ซึ่งได้รับสารละลาย Zinc gluconate โดยการฉีดเข้าในลูกอัณฑะ พบว่าน้ำเชื้อที่ได้จากสุนัข 4 ตัวตรวจไม่พบตัวอสุจิตั้งแต่วันที่ 26, 30, 35 และ 51 ตามลำดับ (สุนัขตัวที่ 5 ไม่สามารถรีดน้ำเชื้อได้ตลอดการทดลอง) โดยก่อนหน้าวันดังกล่าวมีการแข็งตัวของอวัยวะเพศแต่ไม่มีการหลั่ง ไม่มีผลข้างเคียงที่เป็นอันตรายและการเปลี่ยนแปลงทางพฤติกรรมในสุนัขระหว่างการทดลอง ผลทางพยาธิวิทยาระดับเนื้อเยื่อพบว่า seminiferous tubules and Leydig cells ถูกทำลายอย่างสมบูรณ์ในวันที่ 60 และ 75 การศึกษาเบื้องต้นนี้สรุปได้ว่า Zinc gluconate สามารถใช้ในการทำหมันสุนัขเพศผู้ได้