INTRATESTICULAR INJECTION OF 20% HYPERTONIC SODIUM CHLORIDE AS A NOVEL CASTRATION METHOD: A PRECLINICAL STUDY

[1]Marta Hendry, [1]Sunaryo Hardjowijoto, [2]Basuki B. Purnomo, [3]Endang Joewarini, [4]Dewa Ketut Meles, [5]Widodo JP

[1]Department of Urology, Faculty of Medicine/Airlangga University, Soetomo Hospital, Surabaya
[2]Subdivision of Urology/Department of Surgery, Faculty of Medicine/Brawijaya University, Saiful Anwar Hospital, Malang
[3]Department of Anatomical Pathology, Faculty of Medicine/Airlangga University, Soetomo Hospital, Surabaya
[4]Faculty of Veterinary/Airlangga University, Soetomo Hospital, Surabaya
[5]Faculty of Public Health/Airlangga University, Soetomo Hospital, Surabaya

ABSTRACT

Objectives: *To demonstrate that intratesticular injection of 20% hypertonic sodium chloride can result in permanent castration and to evaluate serum changes in sodium chloride levels.* ***Material & Method:*** *A total of 40 Wistar rats were divided into 4 groups, consisting of bilateral orchidectomy (n=10), control (n=10) and 2 groups receiving intratesticular injections of 20% sodium chloride (n=10 in each group). Serum testosterone was measured on day 0, day 1, day 15 and day 30. Serum sodium chloride was assessed before injection, at 1 hour and 24 hours after injection. All testicles were harvested for histological examination. One way ANOVA and student t-test were used for statistical analysis.* ***Results:*** *Serum testosterone decreased to castrate levels in the orchidectomy and injected groups with no significant difference ($p>0,05$). Significant rise in serum sodium chloride was found 1 hour post injection ($p\leq0,05$) but after 1 day it decreased significantly ($p\leq0,05$). There was no significant difference in histopathological findings between the 2 injected groups after day 15 and 30 ($p>0,05$).* ***Conclusion:*** *Twenty percent hypertonic chloride injection has the same permanent castration effect with bilateral orchidectomy in rats. The serum sodium chloride changes did not reach the lethal level for rats. Therefore this treatment has a promising potential as a novel and cost-effective castration method with the additional benefit of retaining both testes.*

Keywords: *hypertonic sodium chloride, castration, prostate cancer, intratesticular injection*

Correspondence: Marta Hendry, c/o: Department of Urology, Faculty of Medicine/Airlangga University, Soetomo Hospital. Jl. Prof. Dr. Moestopo 6-8, Surabaya 60286. Phone: 031-5501318.

INTRODUCTION

Castration is a procedure to remove or eliminate the function of either male or female sexual gland. Castration can be performed surgically by removing either both testes in males (bilateral orchidectomy) or both ovaries in females (bilateral oophorectomy) or medically by providing Luteinizing Hormone Releasing Hormone agonist (LHRH agonist). Castration with bilateral orchidectomy is a hormonal therapy first introduced by Huggins in 1941 and up to recently remained the standard therapy for advanced stage prostate cancer.[1] Bilateral orchidectomy can be performed with local anesthesia in outpatients. In addition to its cost-effectiveness, its primary benefit is its direct effect on testicular dysfunction. The reduction in plasma testosterone (castrate level) is reached in 3-12 hours or on average 8,6 hours after the procedure.[2,3] One shortcoming of bilateral orchi-dectomy is that patients rarely accept the fact that their scrotum is empty, which is psychologically disturbing, primarily among younger patients. Besides, this procedure leaves a permanent outcome. Prosthetic implantation may overcome such shortcomings, but the cost required is very high.[1,2]

Castration is performed medically using LHRH agonist. Monthly or three-monthly subcutaneous depot injection may suppress the production of Luteinizing Hormone (LH) and testosterone down to the castrate level, although preceded by an early stimulation phase.[4] This phase, which is also called a biochemical flare, may produce uncomfortable clinical symptoms, such as bone pain that may last during the first 2-3 weeks. In prostate carcinoma patients with extensive metastasis or with symptom of spinal cord compression, this early stimulation may result in acute condition such as paraplegia. LHRH agonist may also result in increased intracranial pressure by stimulating previously-undiagnosed hypophyseal adenoma.[1] Cost of LHRH agonists is high, resulting in high drop out rates. In the USA, the cost of LHRH agonist therapy, as reported by Medicare Program, was $ 761.000.000 in 1997. In the same year, the cost in Sweden was $ 17.000.000, while in Germany $ 142.000.000.[5]

In Indonesia, the newest LHRH agonist, injected three-monthly, costs up to Rp. 3.200.000 per injection. This price is too expensive to bear by the patients in developing countries like Indonesia. It is apparent that today we need effective castration methods, with lower price and with mild psychological effects. One methods is 20% hypertonic sodium chloride (NaCl) injected directly into the testis. In a study with intratesticular injection of 20% hypertonic sodium chloride (NaCl) by Emir in 2007 in Turkey using Wistar strain rats, it was concluded that there was no significant difference in testosterone levels between rats subjected to bilateral orchidectomy and those subjected to intratesticular 20% hypertonic NaCl injection after day 1 and day 30. Pathologically, total necrosis was found in both testes. There was no infection and necrosis on the skin and abscess in the testis.[6] In that study, the authors did not explain complications resulting from use of 20% hypertonic NaCl and resulting reversibility of castration.

Hypertonic NaCl acts through difference in osmotic pressure gradient. Interstitial 20% hypertonic sodium chloride may draw intracellular water outward, resulting in intracellular dehydration, and leading to cell death.[7] Through this mechanism, testosterone-producing Leydig cells experience the same process. Highest risk in the use of 20% hypertonic NaCl is hypernatremia and hyperosmolaremia. Several studies showed that a rise in serum sodium concentration up to 160 mEq/L and serum osmolarity up to 330 mOsm/L can be well-tolerated and has no correlation with significant complications. In animal experiment, fatal hypernatremia may occur if blood sodium increases rapidly from normal to 206 mEq/L.[8]

OBJECTIVE

To prove whether intratesticular 20% hypertonic NaCl injection can be utilized as a novel castration method in rats and prove presence plasma sodium and chloride concentration changes after injection and the emerging castration reversibility.

MATERIAL & METHOD

This was an experimental study using male Wistar strain white rats (*Rattus norvegicus*), using pre-post test control randomized group design. Variables were measured before and after treatments. The rats were divided into 4 groups, each comprising 10 rats. Group I was subjected to bilateral orchidectomy and sacrificed on day 15. Group II received no treatment, and sacrificed on day 15. Group III was injected with intratesticular 20% hypertonic NaCl and sacrificed on day 15. Group IV was injected with intratesticular 20% hypertonic NaCl and sacrificed on day 30. Surgical procedure for bilateral orchidectomy and intratesticular 20% hypertonic NaCl injection was performed on D-0. Prior to the procedure, blood samples was taken as much as 2 ml from each rats in group I, II, and III for measurement of testosterone, serum sodium and chloride concentrations. Blood samples were taken from the heart.

Rats in group I were subjected to bilateral orchidectomy. Prior to the operation, the rats were fasted (except drink) for 5-6 hours. The rats were given with 10 mg/kg BW ampicillin prior to the operation and anesthetized with 40 mg/kg BW ketamine-HCl intramuscularly. Group III and IV were subjected with intratesticular injection of 20% hypertonic NaCl using insulin needle. The rats were anesthetized with ketamine-HCl in a dose of 40 mg/kg BW intramuscularly. The rats were subsequently put on

supine position. 20% hypertonic NaCl was injected to all directions of the testis as much as 0,5 to 1 mL or equal to 1,71 mEq to 3,42 mEq using insulin syringe. Blood samples were taken directly from the heart as much as 1-2 ml prior to the treatment and on day 1, day 15 (groups I, II, III), and day 30 (group IV) to examine testosterone level. Particularly in group II and III, serum sodium and chloride examinations were performed after 1 hour and 1 day post-20% hypertonic NaCl injection.

The rats died after blood samples were taken from heart, so that for the subsequent examination and blood sampling they were replaced with homogeneous rat groups (with the same age and body weight) that were included in the group. Thus, if a group would undergo 4 times blood examination (for example, group III), rats prepared in group III consisted of 40 homogeneous rats (4 x 10 rats).

Rats' testes were evaluated daily. After days 15 and 30, rats receiving 20% hypertonic NaCl infection (group III and IV) and control group without treatment (group II) were sacrificed and subjected to testicular histopathological examination. Data were processed using computer and SPSS application, and analyzed descriptively as well as analytically with a significance degree of 95%. Statistical test used was independent sample t test for 2 variables or Anova test for more than 2 variables if the requirements of parametric test were met.

RESULTS

Descriptive data on bodyweight of the rats (Table 1) in this study had normal distribution and was homogeneous. Comparative tests on rats' bodyweight with Anova test revealed significance value of $p>0,05$, revealing no difference of bodyweight between groups.

Independent t test on days 1 and 15 revealed significance values of 0,06 and 0,08 ($p>0,05$), respectively. This leads to a conclusion that there was no significant difference in testosterone level in control group of bilateral orchidectomy and intratesticular 20% hypertonic NaCl injection, both on examination days 1 and 15.

After day 1 intratesticular injection of 20% hypertonic NaCl, the testosterone level decreased substantially to castrate level and remained low up to day 30, as can be seen in Figure 1.

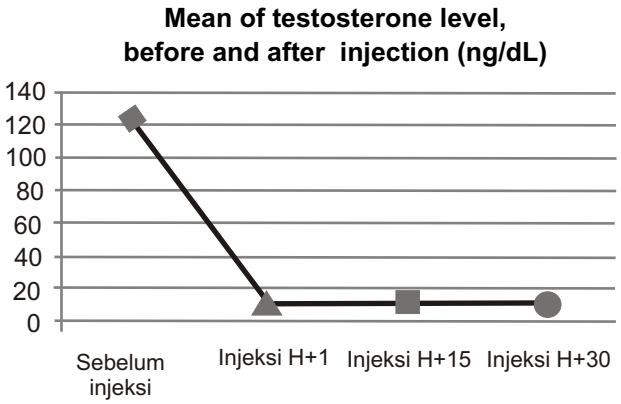

Figure 1. Mean of testosterone level, before and after 20% hypertonic NaCl injection.

One-way Anova test showed that testosterone levels were significantly different ($p<0,05$). To test the difference between observation days, LSD test was performed, as seen in Table 3. It shows that mean of testosterone level before 20% hypertonic NaCl injection was significantly higher than after the intratesticular injection of 20% hypertonic NaCl, either on day 1, day 15, and day 30 ($p\leq0,05$). LSD test revealed that testosterone levels between injected groups after day 1, day 15, and day 30 were not significantly different ($p>0,05$).

Sodium and chloride levels after 1 hour was the highest than that from other examinations, while result of examination after day 1 injection was the lowest, and the difference was statistically significant ($p\leq0,05$). The difference of sodium and chloride levels between time of examinations can be observed with LSD test, which indicated that sodium and chloride levels between before and 1 hour after injection, before and after day 1, and between 1 hour and day 1, were significantly different.

Table 1. Description of mean bodyweight of rats in all groups.

Rats' Group	N	Mean	SD	K. Smirnov	Sig.
Group I (bilateral orchidectomy)	10	234,30	12,95	0,54	0,93
Group II (control without injection)	10	233,30	11,73	0,54	0,94
Group III (NaCl 20% H+15)	10	230,30	9,74	0,67	0,76
Group IV (NaCl 20% H+30)	10	231,90	12,97	0,42	0,99

Table 2. Description of mean testosterone level in bilateral orchidectomy and 20% hypertonic NaCl injection on day 1 and day 15.

Group	N	Day 1		Day 15	
		Mean	SD	Mean	SD
Bilateral orchidectomy	10	11,00	1,33	11,20	2,62
NaCl 20% injection	10	12,50	1,96	13,30	2,36

Table 3. Post-hoc test with LSD on testosterone level in groups, before and after 20% hypertonic NaCl injection on day 1, 15, and 30.

Group	Control before injection	NaCl 20% injection day 1	NaCl 20% injection day 15	NaCl 20% injection day 30
Control before injection	-	0,001*	0,001*	0,001*
Injection NaCl 20% day 1	-	-	0,96	0,96
Injection NaCl 20% day 15	-	-	-	0,93
Injection NaCl 20% day 30	-	-	-	-

Table 4. Mean sodium and chloride levels after 1 hour and 1 day injection of 20% hypertonic NaCl.

Group	N	Na		Cl	
		Mean	SD	Mean	SD
Before injection	10	148,90	7,81	111,20	3,97
After 1 hour	10	159,40	11,69	121,70	8,43
After 1 day	10	136,20	3,65	103,50	3,57

Table 5. Post-hoc test with LSD for sodium level.

Group	Control before injection	1 hour after injection	1 day after injection
Control before injection	-	0,009*	0,002*
1 hour after injection	-	-	0,001
1 day after injection	-	-	-

Table 6. Post-hoc test with LSD for chloride level.

Group	Control before injection	1 hour after injection	1 day after injection
Control before injection	-	0,001*	0,006*
1 hour after injection	-	-	0,001
1 day after injection	-	-	-

Histopathological examination with HE (Haematoxylin Eosin) staining in control testes showed intact seminiferous tubule containing spermatogonia cells in various stages. Leydig cells were found in interstitial tissue. These Leydig cells could easily be differentiated from other cells due to their larger size and round shape, and rich fine endoplasmic reticulum and mitochondria with tubular crystals. In addition to Leydig cells, this interstitial tissue is rich of capillary blood vessels. Testosterone hormone is secreted directly by the Leydig cells (Figure 2).

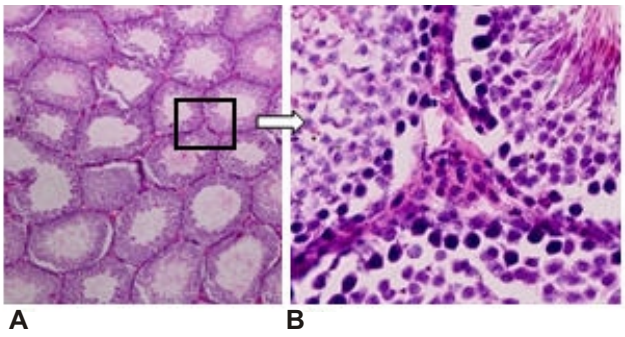

Figure 2. Testicular sections of Wistar rats in control group, Haematoxylin Eosin. A: magnification 100x, B: magnification 400x. Leydig cells are situated between testicular seminiferous tubule and Spermatogonia cells in various stages within seminiferous tubule.

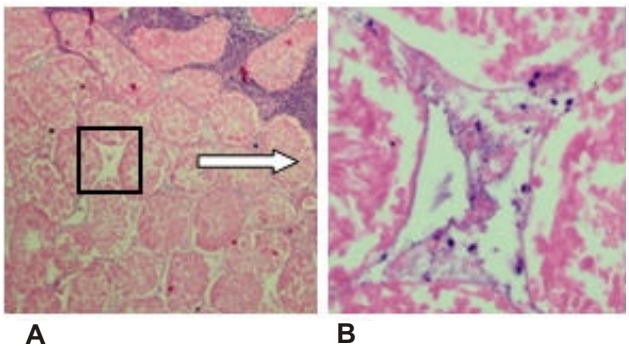

Figure 3. Testicular sections of Wistar rats receiving injection of 20% hypertonic NaCl on day 15 and 30 with HE staining. A: magnification 100x, PMN infiltration into interstitial tissue is apparent. B: magnification 400x. Leydig cells have disappeared and replaced with PMN cells.

Histopathological appearance in the testes of groups receiving 20% hypertonic NaCl injection after day 15 and day 30 was similar, i.e., there was necrosis in all testicular interstitial cells, including spermatogonia cells with relatively intact seminiferous tubule. The interstitial tissue was infiltrated with polymorphonuclear (PMN) inflammatory cells (Figure 3).

One-way Anova test on the rat testicle volume before and after the injection of 20% hypertonic NaCl on day 15 and day 30 showed $p \leq 0,05$, indicating there was minimally one pair of group that was significantly different. To test the difference between days of observation, LSD test was performed as seen in Table 8. It shows that there is significant difference in testicular volume between the groups before and after the injection of 20% hypertonic NaCl injection on day 30. Between the day 15 and 30, there was no significant difference, and as well as between day 15 and before injection.

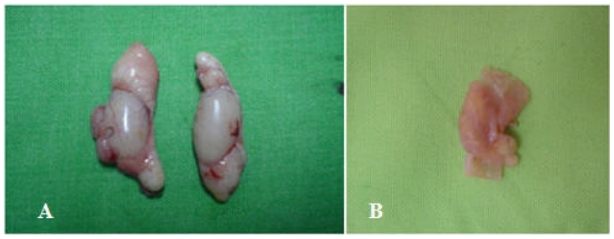

Figure 4. Comparison between control testes (A) and after day 3 of 20% hypertonic NaCl injection.

Table 7. Description of rats' testicular volume (mm3) before and after the injection of 20% hypertonic NaCl injection on day 15 and day 30.

Testicular volume	N	Mean	Standard Deviation
Before 20 % NaCl injection	10	1580,60	0,006*
Day 15	10	1109,00	341,82
Day 30	10	761,90	473,31

Table 8. Advanced or post-hoc test with LSD on testicular volume.

Groups	Before injection	After D+15	After D+30
Before 20 % NaCl injection	-	0,07	0,01*
Day 15	-	-	0,41
Day 30	-	-	-

DISCUSSION

This study of intratesticular 20% hypertonic NaCl injection is aimed to prove whether this procedure can be applied as a novel castration method, whether there were complications such as hypernatremia and hyperchloremia after the injection, and whether the resulting castration was permanent.

There was a sharp decline in testosterone levels down to castrate level on day 1 and remained under such level until day 30 after the injection of 20% hypertonic NaCl ($p \leq 0,05$). This proved that castration with intratesticular 20% hypertonic NaCl is as effectiveness as bilateral orchidectomy.

A similar experiment was performed by Emir L et al.[6] from Turkey in 2007. This study concluded that there was no significant difference between rat testosterone levels produced with bilateral orchidectomy and that with the intratesticular injection of 20% hypertonic NaCl after day 1 and day 30 ($p>0,05$). However, the real castration mechanism remains unclear, whether the castration results from hypertonicity of 20% NaCl or from intratesticular pressure due to the injection. Further studies are required to address this issue.

Serum electrolyte levels revealed that sodium and chloride levels after 1 hour injection was raised to 11 mEq/L and 10 mEq/L, respectively, compared to those before the injection. This increase was statistically significant ($p \leq 0,05$). After 1 day injection, sodium and chloride levels decreased to 12 mEq/L dan 8 mEq/L, respectively, as compared to before injection, and this difference was statistically significant ($p \leq 0,05$). The increase of sodium and chloride levels 1 hour after injection indicated that the entry of this ion into the circulation was rapid and carries a potential risk of acute hypernatremia and hyperchloremia. After injection, the body has adapted to increased sodium and chloride ions. However, the increase of sodium and chloride levels 1 hour after the injection was not fatal. Although the increase of electrolyte concentrations was statistically significant, it did not result in clinical changes in the rats enrolled in this study.

Physical examination during the first 24-hour post-injection revealed no difference. The result was the same as that of the control group. After reviving from anesthesia, the rats were able to eat and drink as usually. This result confirmed other studies, showing that the increase of sodium level up to 160 mEq/L will be well-tolerated by the body and has no correlation with significant complications.[8] The mechanism of sodium level reduction on day 1 was caused by the increase of sodium level in the loop of Henle, which resulted in increased water reabsorption in renal tubules. This finally leads to the reduction of serum sodium, which previously increased suddenly from normal concentration.[9]

Histopathological profiles of the testes injected with 20% hypertonic NaCl after day 15 and day 30 were significantly different compared to histopathological profile of the control group. In injection groups, there was necrosis in all testicular interstitial cells and spermatogonia cells with relatively intact seminiferous tubule. Around the tubule or interstitial area, there were many infiltrations of polymorphonuclear (PMN) inflammatory cells that were confined within tunica albuginea. Infiltration of inflammatory cells replaced the position of necrotic interstitial cells. Infiltration of these inflammatory cells was the response of the tissue to the provided exposure. As a result of this inflammatory response, the dead tissue was demolished and eradicated, opening the way for repair process to replace necrotic area with scar tissue.[10]

This change in the testis is permanent since after day 15 to day 30 there was no cellular regeneration, either from interstitial cells (Leydig cells) or spermatogonia cells, to replace the necrotic cells. Presence of this permanent change was supported by the results of other studies, which found that after day 15 and day 30 the testosterone level remained significantly low (below the castration level) compared to that before injection. Rat testicular volume started to show reduction after day 30. The reduction in volume occurred due to the necrosis of spermatogonia and interstitial cells. However, testicular shape was still present. There was no damage to scrotal skin in groups receiving 20% NaCl injection, a result that was also found by Emir in 2007. This is one of the benefits not found in castration using bilateral orchidectomy. With this novel method, the shape of the scrotum remains normal since it still contains the testes.

The novel castration method produces castration similar to bilateral orchidectomy, even with benefits that both testes remained present and scrotal skin remained normal. There were no cases of fatal hypernatremia and hyperchloremia. The increase of sodium and chloride levels can be overcome by body homeostatic function taking place within 24 hours after the injection. Such increase did not produce significant clinical symptoms. This novel castration method can address the shortcomings in castration methods of bilateral orchidectomy and LHRH agonist, either in terms of cost, time, treatment, or psychological aspects.

CONCLUSION

The injection of 20% hypertonic NaCl can be used as a novel castration method, which is cost-saving, effective, and efficient (in experimental animals).

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