General Urology / Experimental Investigation Genel Üroloji / Deneysel Araştırma

The comparative analysis of medical and surgical castration on rat prostate apoptosis and glandular atrophy

Sıçan prostat dokusunda medikal ve cerrahi kastrasyonun apoptoz ve glandüler atrofiye etkisinin kıyaslamalı analizi

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Abstract

Objective: The aim of the study is to compare the effect of surgical and medical castration on prostate tissue apoptosis.

Materials and methods: Rats were either surgically or medically castrated. Apoptosis and glandular atrophy in the prostate was assessed via Bax antibody immunohistochemical staining method. 30 adult (10 weeks old) Sprague Dawley Rats weighing about 250 gr were evaluated in three different groups for two weeks. Group (A) consisted of cyproterone acetate (CPA) administered rats (n:12) and Group (B) consisted of surgically castrated rats (n:12). The third group of six rats was the sham operated control group. Histopathologic and immunohistochemical apoptotic activity and glandular atrophy of the prostate was evaluated at the end of the second week.

Results: Significant glandular atrophy was seen in six rats in the medically castrated Group (50%) and in all 12 rats of the surgically castrated (100%). Prostatic atrophy in the surgically castrated group was more significant than the medically castrated group (Fischer's exact test p=0.014). 58.3%, 100% and 0% of the rats in Group A, B and the control group respectively had an increased apoptotic staining in the prostate. Apoptosis in the surgically castrated group was more significant (Fischer's exact test p=0.037) than the medically castrated group.

Conclusion: In this study we found that surgical castration is superior to medical castration in inducing apoptosis in the prostate of the rat.

Key words: Apoptosis; medical castration; surgical castration.

Özet

Amaç: Çalışmanın amacı cerrahi kastrasyon ile medikal kastrasyonun prostat doku apoptozuna etkilerinin kıyaslanmasıdır.

Gereç ve yöntem: Bax antikoruyla immünohistokimyasal boyama yöntemiyle prostat dokusundaki apoptoz ve glandiler atrofiyi değerlendirmek için gruplara ayrılmış sıçanlar cerrahi veya medikal olarak kastre edilmişlerdir.10 haftalık 250 gram ağırlığında 30 adet erişkin Sprague Dawley Sıçan üç ayrı grupta iki hafta boyunca değerlendirilmiştir. Grup (A) da siproteron asetat verilen 12 sıçan, grup (B) de orşiyektomi yapılmış 12 sıçan bulunmaktadır. Üçüncü grupta ise (C) yalancı olarak opere edilen kontrol grubu bulunmaktadır. İkinci haftanın sonunda immünohistokimyasal ve histopatolojik olarak prostatlardaki apoptotik aktivite ve glandüler atrofi değerlendirilmiştir

Bulgular: Medikal kastrasyon yapılan 6 sıçanda (%50) ve cerrahi kastrasyon yapılan 12 sıçanda (%100) pozitif glandüler atrofi saptanmıştır. Prostatik atrofi cerrahi kastre edilenlerde medikal kastre edilenlere göre yüksek bulunmuştur. A, B ve kontrol gruplarında prostatik apoptotik boyanma artışı sırasıyla %58.3, %100 ve %0 bulunmuştur. Apoptoz artışı cerrahi kastre edilenlerde medikal kastre edilenlere kıyasla anlamlı şekilde yüksek bulunmuştur (Fischer's exact test p=0.037).

Sonuç: Bu çalışmada cerrahi kastrasyonun sıçan prostat dokusundaki apoptotik etkisi medikal kastrasyona göre yüksek bulunmuştur (Fischer's exact test p=0.014).

Anahtar sözcükler: Apoptoz; cerrahi kastrasyon; medikal kastrasyon.

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Introduction

Eucaryotic cells have four known properties; they divide for proliferation, produce specific features with differentiation, and enlarge with hypertrophy or suicide with programmed cell death. The latter seems to be extremely important for normal embryologic development, hormonal effect, reproduction forms and homeostatic maintenance of adult tissues. Like other cellular processes, apoptosis/programmed cell death is a well regulated response. Inappropriate activation of apoptosis or defects in this process is encountered in the benign and malignant diseases of the body including the genitourinary system. The tissue that gives the best understanding on genitourinary apoptosis has been the prostate. Apoptosis in the prostate of the rodents can be established by castration. John Kerr was the first one to show apoptosis in the prostate after castration.[1] Lee,[2] and Kyprianou and Isaacs[3], described the differentiation of cells in the regressing prostate tissue. Recently there are increased efforts towards the design of pharmacological agents that would target the apoptotic components of the prostate epithelial cells and significantly improve the therapeutic response in prostate cancer and benign prostate hyperplasia (BPH), while minimizing systemic toxicity.[4-7]

In our study castration was achieved either surgically or medically. The subsequent apoptosis in the prostate tissue was investigated by staining the BAX protein using immunohistochemical techniques. BAX protein prevents the process of cell death suppression that is provided by Bcl-2 gene. Its over expression causes cell death by either apoptosis or necrosis depending on the caspase involvement.[8] This has been shown by addition of purified Bax protein to isolated mitochondria, which results in release of cytochrome c.[9] The release of cytochrome c is prevented by the antiapoptotic protein Bcl-2 and Bcl-X_L.[10] Bax mediates necrotic cell death in a caspase independent manner. This is achieved by release of cytochrome-c involving disruption of the mitochondrial membrane potential, increased production of reactive oxygen species and Adenosine-5'-triphosphate (ATP) depletion through loss of oxidative phosphorilation.[11]

The aim of this study is to compare the effect of surgical and medical castration on prostate apoptosis. This has been demonstrated in the tissue with Bax antibody immunohistochemical staining.

Materials and methods

30 adult Sprague Dawley Rats weighing average 250 gr and 10 weeks old were separated into three groups and were evaluated for two weeks. Medically castrated 12 rats were included in group A while surgically castrated 12 rats were included in group B. The third group of six rats was used as the control group. First group of rats was administered depot cyproterone acetate (CPA) intramuscularly on the first day of the study and on the first day of the second week. Each rat was administered 30mg of CPA oily depot solution. The second group underwent orchiectomy on the first day of the study. The third group underwent a sham operation (scrotal incision). At the end of the second week the rats were sacrificed with 75 mg/kg penthotal and their bladder, prostate and seminal vesicles were removed en bloc.

Histopathology

The pathologist blindly evaluated the specimens without knowing which one belonged to a certain study group. Prostates were laid in the Phosphate buffered saline (PBS) solution, cut longitudinally and fixed in buffered formalin to be placed on slides. Inflammatory infiltrate, edema, fibrosis, basal cell degeneration, hyperplasia and endothelial cell intensity was examined after staining the slices randomly with Hemotoxilen Eosine (HE).

Immunohistochemistry

Initially tissue slices were kept at 57°C for 24 hours, then washed with cold xylene at 30°C and alcohol in 20°C and then distilled to remove the paraffin. They were incubated for 15 minutes in hydrogen peroxide, washed twice with triss buffered saline (TBS), then buffered with citrate in a microwave oven and left to be cooled in room temperature. To prevent non-specific background staining, Ultra V Block (protein blocking) was applied to the slides. Ultra V Block was let to flow off the slides, without washing, and bax antibody with the dilution of 1/100 was dropped on them to be incubated for one hour. The slides were washed four times with TBS and incubated until growing colored with chromogeneous AEC (AEC preparation; one drop of chromogeneous AEC is dropped in 1ml substrate, washed with distilled water). Later slides were kept in Mayer's Hemotoxilen for contrast staining and washed with distilled water to be covered by Ultramount.

Slides were examined under light microscope. Bax positivity was assessed with granular type cytoplasmic staining. Normal Bax staining, increased staining, strongly increased staining were symbolized respectively with (N), (+) and (++). Glandular component decrease, cystic formation and epithelial flattening were considered as the presence of atrophy.

This was classified in, no atrophy (-), mild atrophy (+) and atrophy (++). Results were also stratified as either having apoptosis or not and this was also repeated for atrophy.

Differences in groups were evaluated statistically with Fisher exact's test. A p value <0.05 was considered to be statistically significant.

Results

Staging of Apoptosis and Atrophy

Prostate specimens from the control group rats showed no atrophy (Fig. 1a) and apoptotic staining was normal in all rats (Fig. 1b). In the medically castrated six rats (50%) mild glandular atrophy was visible (Fig. 2a). On the other hand nine (75%) surgically castrated rats (Fig. 3a) had visible mild glandular atrophy three (25%) had significant atrophy. Prostates of five (41.7%) rats from group A had no increased apoptotic activity and seven (58.3%) rats had increased apoptotic staining (Fig. 2b). In the surgically castrated group the apoptotic activity was increased in four (33%) and strongly increased in eight (67%) rats (Fig. 3b).

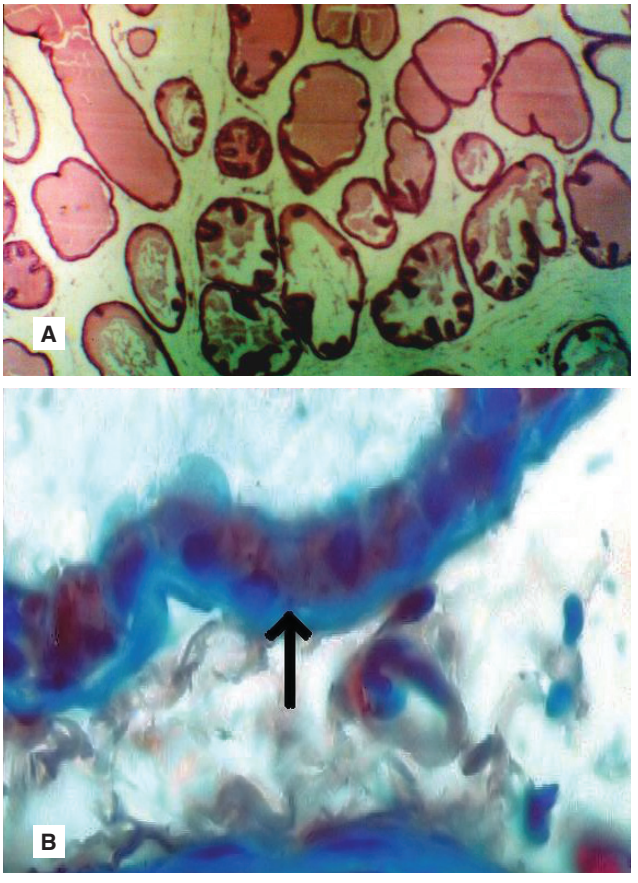

Figure 1 (a) Control group prostate tissue depicting no apoptotic staining. (b) Control group prostate tissue depicting no glandular atrpohy.

Presence of Apoptosis and Atrophy

Another way we analyzed our results was separating them as either having apoptosis and atrophy or not (Table 1). Positive glandular atrophy was observed in six (50%) of the antiandrogen administered rats and in all (100%) surgically castrated rats. The number of rats in which prostatic atrophy developed was significantly greater in the surgically castrated group than the medically castrated group (p=0.014). Five rats in the CPA administered group had normal apoptotic staining (41.7%) similar to those of control group while seven rats had increased apoptotic staining (58.3%). On the other hand all rats that had an orchiectomy had increased apoptotic activity in the prostate (100%) (Table 2). Apoptosis in rats which had undergone orchiectomy was found to be significantly higher than the two other groups (p=0.037). All results are summarized in Tables 1 and 2.

Discussion

Prostate is dependent on androgens all life long. Normal physiologic testosterone levels maintain epi-

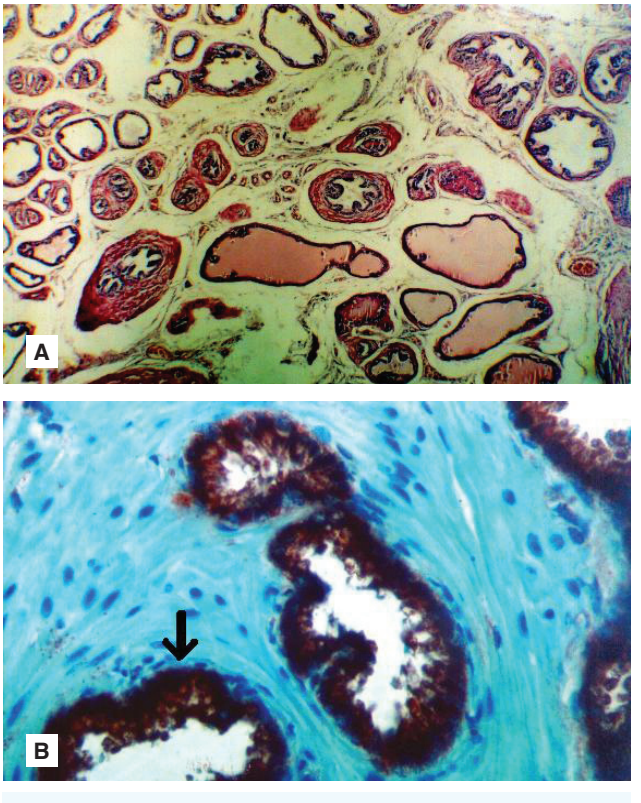

Figure 2 (a) Mild glandular atrophy in the medically castrated group. (b) Increased apoptotic staining in the medically castrated group.

thelial /stromal interaction.[12] Withdrawal of testicular androgen (castration) induces apoptosis that renders the prostate as a rudimentary gland. However; when testosterone is readministered, the prostate gland proliferates to return to its original state. It is also well known that testosterone has an angiogenic effect on the prostate gland.[13-14] Castration causes apoptosis within the luminal cells and death of approximately 80% of epithelial cells of the prostate gland.[15] Subsequent remaining prostatic glandular tissue contains both ductal luminal cells and basal cells. However, the ratio of these cells is affected. It is known that the normal luminal cell/basal cell ratio in non castrated animals is 13/1 however after castration this ratio becomes 1/3.

In this present study we compared the effect of medical and surgical castration on the rate of glandular atrophy and apoptosis. According to our results surgical castration has a significant effect on apoptosis (100%) and atrophy (100%) when compared with the control group and medically castrated rats. The apoptotic and glandular atrophy intensity was significantly higher in the surgically castrated group. This overwhelming effect of surgical castration over medical castration is further confirmed by no atrophy and apoptosis being observed in 50% and 41.7% of the medically castrated rats consecutively. Apoptosis is a result of complex relationships between chemical signals and finally vascular regression. In our study the rate of apoptosis was evaluated qualitatively by immunohystochemical staining. In the literature we found that similar immunohystochemical evaluation techniques have been used as a way of qualitative measurements.[16]

Androgen deprivation therapy is a well established treatment for prostate cancer and surgical castration is one of the well known options. Studies comparing the effect of CPA and surgical castration on survival are limited.[17] In a multicenter, three-armed, randomized study,the effects of short-term (2 weeks) and continuous addition of CPA to buserelin to those of orchiectomy in patients with advanced prostate cancer were compared. In this study it was concluded that the short-term or continuous addition of CPA to buserelin did not improve treatment results compared to orchiectomy only.[18]

Most of the published papers in this field are comparative studies usually made with bicalutamide. In androgen dependent Dunning R3327PAP rat prostate adenocarcinoma, castration keeps the tumor volume and stromal epithelial cells stable, furthermore when 50 microgram estradiol benzoate is added, tumor volume decreases with death of its cells.[19] In our experimental study we have found that surgical castration is superior to CPA in inducing apoptosis and atrophy in the normal prostate. Further experimental comparative studies are necessary to determine if surgical castration is superior to medical castration in prostate cancer.

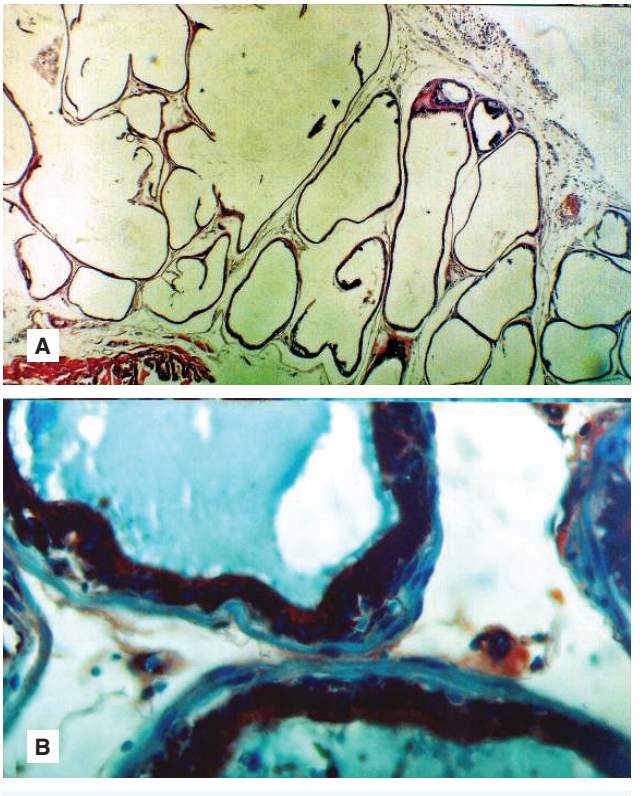

Figure 3 (a) Strongly increased apoptotic staining in the surgically castrated rats group. (b) Cystic atrophy in the surgically castrated rat group.

Table 1. Glandular atrophy rates in study groups

Glandular atrophy	-	+/++ (total) [%]
Antiandrogen	6 (50%)	6/0 (6) [50%]
Orchiectomy	0 (0%)	9/3 (12) [100%]

Glandular atrophy in the castration group is superior to the antiandrogen group (statistically significant, Fischer's exact test; p=0.014)

Table 2. Apoptotic intensity in study groups

Apoptotic staning	Normal	+/++ (total) [%]
Antiandrogen	5 (41.7%)	7/0 (7) [58.3%]
Orchiectomy	0 (0%)	4/8 (12) [100%]

Castration group's apoptotic staining is more dense than that of the antiandrogen group (statistically significant, Fischer's exact test; p=0.037)

Anglin et al suggested that in addition to decreasing smooth muscle tone, adrenoceptor antagonists (ARA), doxazosin and terazosin, induce apoptosis in benign and malignant prostate epithelial cells, as well as prostate smooth muscle cells.[20] In the combination of ARA with finasterid the rate of apoptosis in the prostate is comparable with untreated prostate tissue.[21-22] ARAs effect on progression and long-term therapy of BPH and potentially prostate cancer is through apoptosis. The positive effect of castration on the prostate is also through the apoptotic activity on androgen dependent cells. The combination and comparison of the two treatments in forming apoptosis in the prostate may be a further subject of research.

Our study is not devoid of limitations. First, it can be argued that, medical castration was performed only with CPA and no other known antiandrogen regimen (e.g. bicalutamide) was used. Bicalutamide is a non-steroid antiandrogen which does not suppress Luteinizing-hormone releasing hormone (LHRH) agonists and has no parenteral administration forms. These two drugs can be used together to gain maximal androgen blockage. On the other hand CPA is a steroid antiandrogen which can suppress LHRH and can be administered parenterally. Second, additional groups containing longer evaluation periods than the existing groups could give an idea about the influence of androgen deprivation time on apoptosis and atrophy. Third, the blood CPA levels were not measured. However we do know that a 300mg dose should be administered weekly to a 70 kg male inorder to gain maximal stable blood levels.[23] On the other hand we administered 30 mg CPA to 250 mg weighing rats which we think is more than sufficient. Under these circumstances we did not find it necessary to measure the blood levels of CPA. Nevertheless, our data strongly supports the superiority of surgical castration over medical castration in forming apoptosis and glandular atrophy in the prostate.

Finally in this study we found that apoptotic stage in the surgically castrated rat prostate tissue was superior to that of medically castrated ones. Therefore the same question should be asked in prostate cancer patients.

Our study suggests that apoptotic and glandular atrophic stage and rate in the surgically castrated rats are superior than medically (CPA) castrated rats. Further studies may be conducted to evaluate the clinical significance of the degree of apoptotic stage in the prostate.

References

1. Colombel MC, Buttyan R. Hormonal control of apoptosis: The rat ventral prostate gland as a model system. Methods Cell Biol 1995;46:369-85.
2. Kerr JFR, Searle J. Deletion of cells by apoptosis during castration-induced involution of the rat prostate. Virchows Arch Aht B Zellpathol 1973;13:87-102.
3. Bruchovsky N, Lesser B, Van Doorn E, Craven S. Hormonal effects on cell proliferation in rat prostate. Vitam Horm 1975;33:61-102.
4. Lee C. Physiology of castration induced regression in the rat prostate. Prog Clin Biol Res 1981;75A:145-59.
5. Kyprianou N. Isaacs JT. Activation of programmed cell death in the rat ventral prostate after castration. Endocrin 1988;122:552-62.
6. Chuang YC, Tu CH, Huang CC, Lin HJ, Chiang PH, Yoshimura N et al. Intraprostatic injection of botulinum toxin type- A relieves bladder outlet obstruction in human and induces prostate apoptosis in dogs. BMC Urology 2006;18:6-12.
7. Ditonno P, Battaglia M, Selvaggio O, Garofalo L, Lorusso V, Selvaggi FP. Clinical Evidence Supporting the Role of Lonidamine for the Treatment of BPH. Rev Urol 2005;7 (suppl 7):S27-S33.
8. Wadsworth TL, Carroll JM, Mallinson RA, Roberts CT Jr, Roselli CE. Saw Palmetto Extract Suppresses Insulin-Like Growth Factor-I Signaling and Induces Stress-Activated Protein Kinase/c-Jun N-Terminal Kinase Phosphorylation in Human Prostate Epithelial Cells. Endocrinology 2004:145:3205-14.
9. Crescioli C, Ferruzzi P, Caporali A, Mancina R, Comerci A, Muratori M, et al. Inhibition of Spontaneous and Androgen-Induced Prostate Growth by a Nonhypercalcemic Calcitriol Analog. Endocrinology 2003;144:3046-57.
10. Green DR, Reed JC. Mitochondria and apoptosis. Science 1998;281:1309-12.
11. Marzo I, Brenner C, Zamzami N, Jürgensmeier JM, Susin SA, Vieira HL et al. Bax adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. Science 1998;281:2027-31.
12. Zamzami N, Brenner C, Marzo I, Susin SA, Kroemer G. Subcellular and submitochondrial mode of action of Bcl-2-like oncoproteins. Oncogene 1998;16:2265-82.
13. Xiang J, Chao DT, Korsmeyer SJ. BAX-induced cell death may not require interleukin 1_-converting enzyme-like proteases. Proc Natl Acad Sci USA 1996;93:14559-63.
14. Berry S, Isaacs JI. Comparative aspects of prostatic growth and androgen metabolism with aging rat versus dog. Endocrinology 1984;114:511-20.
15. Shabsigh A, Chang DT, Heitjan DF, Kiss A, Olsson CA, Puchner PJ et al. Rapid reduction in blood flow to the rat ventral prostate after castration. Prostate 1998;36:201-6.

16. Abdel-Latif AM, Abuel-Ela H A, El-Shourbagy SH. Increased caspase-3 and altered expression of apoptosis associated proteins, Bcl-2 and Bax in lichen planus. J Biomed Sci 2009;16:16.
17. Franck-Lissbrant I, Häggström S, Damber JE, Bergh A. Testosterone stimulates angiogenesis and vascular regrowth in the ventral prostate in castrated adult rats. Endocrinology 1998:139:451-6.
18. Klinj JG, de Vooght HJ, Studer UE, Schröder FH, Sylvester R, De Pauw M. Short term versus long term addition of cyproterone acetate to buserelin therapy in comparison with orchidectomy in the treatment of metastatic prostate cancer. European Organization for Research and Treatment of Cancer- Genitourinary Group. Cancer 1993;72(12 Suppl):3858-62.
19. Isaacs JT, Lundmo PI, Berges R, Martikainen P, Kyprianou N and English H.F. Androgen regulation of programmed cell death of normal and malignant prostatic cells. J Androl 1992;13:457-64.
20. Anderson J. The role of antiandrogen monotherapy in the treatment of prostate cancer. BJU Int 2003;91:455-61.
21. Westin P, Brändström A, Damber JE, Bergh A. Castration plus estrogen treatment induces but castration alone supresses epithelial cell apoptosis in an androgen sensitive prostate adenocarcinoma. Br J Cancer 1995;72:140-5.
22. Anglin IE, Glassman DT, Kyprianou N. Induction of prostate apoptosis by α1-adrenoceptor antagonists: mechanistic significance of the quinazoline component. Prostate Cancer Prostatic Dis 2002;5:88-95.
23. ANDROCUR Depot (Cyproterone acetate solution) Product Monograph. 2007. Submission Control No.: 112966.

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