The effects of lauromacrogol on thyroid tissue in rabbits.
Is this a safe option for the treatment of nodular thyroid disease?

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AIM: *The effects of lauromacrogol as a sclerosing agent were evaluated on rabbit thyroid tissue.*
MATERIAL AND METHODS: *Twelve rabbits were divided into two equal groups. Intra-thyroid injections of 0.1 ml lauromacrogol were administered in the study group and 0.1 ml physiologic serum in the control group. The blood levels of free T3, free T4, TSH, postoperative adhesions and histopathologic differences of the thyroid tissues were evaluated.*
RESULTS: *The values of serum free T3, free T4 and TSH levels did not differ significantly (p>0.05), but the free T3 levels were significantly different in the inter-group analysis (p=0.020). Postoperative macroscopic adhesion scores did not differ significantly (p>0.05). In the histopathologic evaluation, the inflammation and fibrosis scores were significantly higher in the study group (p=0.003).*
DISCUSSION: *In our study, we found two important outcomes. Firstly, tests of thyroid function were not significantly different between the control and study groups. This important finding suggests lauromacrogol can be safely used without the risk of hypothyroidism or hyperthyroidism. Secondly, the Erlich-Hunt Model histopathologic evaluation results revealed that inflammation and fibrosis were significantly increased in the study group. This finding shows lauromacrogol can be effectively used to treat thyroid nodules by means of fibrosis.*
CONCLUSIONS: *Lauromacrogol causes fibrosis in thyroid tissue without significant perithyroidal adhesion formation and functional differences. Treatment of nodular thyroid disease with lauromacrogol may be safe.*

KEY WORDS: Lauromacrogol, Nodule, Thyroid

Introduction

Thyroid diseases are the most frequent endocrine disorder, and the thyroidectomy is the most frequent endocrine surgical procedure [1]. Thyroid surgery is primarily performed for thyroid nodules. The estimated prevalence of palpable thyroid nodules ranges from 3% to 7% [2]. During the past two decades, the widespread use of ultrasonography for evaluation of thyroid and non thyroid neck disease has resulted in a dramatic increase in the prevalence of clinically unapparent thyroid nodules, estimated at 20% to 76% in the general population [3,4].

Most thyroid nodules are benign, but some require treatment for the risk of malignant transformation, cosmetic reasons or subjective symptoms [5]. Surgery is used to treat benign thyroid nodules. Although surgery is an invasive technique, non-surgical, minimally invasive percutaneous modalities have been described, including

ethanol injection, laser, microwave and radiofrequency ablation [6-15].

Lauromacrogol (polidocanol) is a local anesthetic agent. It has a high affinity for vessel endothelium, reacting with endothelium and causing inflammation and sclerosis [11,12]. Lauromacrogol is used to treat some pathologic processes, including thyroid nodules. There is some literature regarding the use of lauromacrogol for the treatment of thyroid nodules [16-18]. However, the physiologic and histopathologic effects of lauromacrogol on the thyroid tissue have not been previously evaluated. In this study, we evaluate the effects of lauromacrogol on rabbit thyroid tissue.

Materials and Methods

This study was performed at the Experimental Animal Production and Research Laboratory of Bezmialem Vakif University and was approved by the local Animals Ethics Committee. All protocols were in accordance with the regulations governing the care and use of laboratory animals in the declaration of Helsinki.

According to power analysis with 0.05 accuracy and 0.95 power, the number of rabbits were determined to be six in each of the groups. Twelve female New Zealand white rabbits (mean weight 3120±75 g; mean age 5.5 months; outbred) were divided into two equal groups. The rabbits were housed singly in standard cages with stainless steel tops and bottoms and woven wire sides. The cage floors were covered with wood shavings that were changed daily. The rabbits were kept at room temperature with adequate ventilation. Water and feeding containers were made of standard plastic with side entrances. The animals were fed pellets designed for small laboratory animals.

The rabbits were starved overnight and anesthetized with Ketamine (Ketalarâ, Parke Davis and Co. Inc.; 40 mg/kg body weight) and xylazine (Rompunâ Bayer Co.; 5 mg/kg body weight).

Before the surgical procedure, blood samples were taken from the ear veins of all subjects and sent to the biochemistry laboratory for free T3, free T4 and TSH levels. The anterior neck was shaved and disinfected with povidone iodine. A 5 cm longitudinal mid-line incision was made. The pretracheal muscles were divided laterally and reached to the thyroid region. Thyroidal lobes were uncovered from para-thyroidal tissues and exposed clearly.

In the study group (n= 6), 0.1 ml lauromacrogol per lobe (aethoxysklerolâ 0.1%, Kreussler Pharma, Germany) was injected intrathyroidally. In the control group (n= 6), the same procedure was performed with 0.1 ml 0.9% physiologic serum (% 0.9 Serum Fizyolojikâ, Eczacıbaşı Co, Turkey).

After the procedure, the muscles and skin were stitched separately with 3-0 polypropylene sutures (Prolene®,

TABLE I - *Adhesion grading according to the Evans Model.*

Grade	Definition
0	No adhesions
1	Spontaneously separating adhesions
2	Adhesions separating by traction
3	Adhesions separating by dissection

TABLE II - *The Erlich-Hunt Model*

Grade	Definition
Grade-0	None
Grade-1	Low density and separated
Grade-2	Low density and in all places
Grade-3	High density but separated
Grade-4	High density and in all places

Kurtsan Co, Turkey). After examination at 3 hours postoperatively, the rabbits were allowed their usual feed. On postoperative days 5, 10, 20 and 30, blood samples were received from all subjects for re-evaluation of free T3, free T4 and TSH levels. The animals were sacrificed on postoperative day 30 by intraperitoneal injection of 200 mg/kg sodium pentothal. The same neck incision was used for re-exploration after widening it superiorly and inferiorly. Postoperative adhesions were evaluated according to the Evans Model (19) (Table I), and thyroid tissue with tracheal segments were resected for histopathologic examination.

The specimens were fixed in 10% formal, dehydrated, and embedded in paraffin wax. Sections were cut at a thickness of 5 mm and stained with hematoxylin and eosin. The tissue was evaluated in terms of the quantity of inflammatory cells, fibroblasts, neovascularization level, and collagen content according to the Ehrlich-Hunt model [20] (Table II).

The primary evaluation parameters were histopathologic evaluation and differences of secretory function of thyroid tissue, and the secondary parameter was the postoperative adhesion formation grades of the groups.

Statistical Analysis

The statistical analyses were performed using IBM SPSS Ver. 19.0. In addition to descriptive statistical methods (mean, standard deviation, and median), we used the Friedman test and post hoc Dunn test for in-group variables comparisons and the Mann Whitney U test for comparisons of inter-group variables.

Results

The values of serum free T3, free T4 and TSH levels of the groups are shown in Table III, IV, and V No statistically significant differences were observed in the in-group analysis (p>0.05), and only the free T3 levels differed significantly in the inter-group analysis (p=0.020).

When we evaluated the postoperative adhesions, the macroscopic adhesion scores did not differ significantly (p>0.05). In the histopathologic evaluation according to the four parameters, inflammation and fibrosis were significantly different (p=0.003) between the two groups (Table VI).

TABLE III - *The serum free T3 levels of the groups*

Serum T3 Levels (pg/ml)	Control Group			Study Group			Mann Whitney U	
	Mean	Standard Deviation	Median	Mean	Standard Deviation	Median	z	p
Preop	5.6167	1.6916	5.0500	5.4333	0.5354	5.5500	-0.401	0.688
Postop Day 5	6.2667	1.2127	6.4000	6.8167	1.6546	6.6000	-0.401	0.688
Postop Day 10	6.3000	2.2018	6.1000	6.6667	0.7448	6.7000	-.0160	0.873
Postop Day 20	6.8333	1.5642	6.7000	7.6500	0.8758	7.8500	-1.129	0.259
Postop Day 30	6.2000	1.6186	6.1000	7.9833	1.2336	8.3500	-1.684	0.092
Friedman chi-square		3.467			11.661			
P		0.483			0.020			

TABLE IV - *The serum free T4 levels of the groups*

Serum T4 Levels (pg/ml)	Control Group			Study Group			Mann Whitney U	
	Mean	Standard Deviation	Median	Mean	Standard Deviation	Median	z	p
Preop	17.9833	3.0993	18.1000	20.0000	3.8766	21.1500	-1.123	0.261
Postop Day 5	17.3000	3.2472	18.2000	18.1500	3.5444	18.9000	-0.480	0.631
Postop Day 10	17.9000	2.3461	17.6500	18.9333	2.8549	19.7500	-0.643	0.520
Postop Day 20	16.3000	4.3308	14.5000	15.3333	3.8702	14.5500	-0.321	0.748
Postop Day 30	17.3667	3.7120	16.5000	16.0500	2.2722	17.1500	-0.642	0.521
Friedman chi-square		0.840			9.176			
P		0.933			0.057			

TABLE V - *The serum TSH levels of the groups*

Whitney U Serum TSH Levels (μIU/mL)	Control Group			Study Group			Mann Whitney U	
	Mean	Standard Deviation	Median	Mean	Standard Deviation	Median	z	p
Preop	0.0012	0.0004	0.0010	0.0017	0.0005	0.0020	-1.682	0.093
Postop Day 5	0.0015	0.0005	0.0015	0.0018	0.0010	0.0015	-0.524	0.600
Postop Day 10	0.0018	0.0008	0.0020	0.0022	0.0008	0.0020	-0.782	0.434
Postop Day 20	0.0018	0.0004	0.0020	0.0023	0.0012	0.0025	-0.777	0.437
Postop Day 30	0.0015	0.0008	0.0010	0.0017	0.0005	0.0020	-0.713	0.476
Friedman chi-square		5.483			3.362			
P		0.241			0.499			

TABLE VI - *The histopathologic evaluation and macroscopic adhesion score results of the groups.*

	Control Group			Study Group			Mann Whitney U	
	Mean	Standard Deviation	Median	Mean	Standard Deviation	Median	z	p
Inflammation	0.5000	0.5477	0.5000	3.3333	0.5164	3.0000	-2.983	0.003
Fibrosis	0.5000	0.5477	0.5000	2.3333	0.5164	2.0000	-2.983	0.003
Neovasc.	0.5000	0.5477	0.5000	0.6667	0.5164	1.0000	-0.561	0.575
Collagen Deposition	0.8333	0.7528	1.0000	0.8333	0.7528	1.0000	0.000	1.000
Macroscopic Adhesion Score	1.8333	0.7528	2.0000	2.5000	0.5477	2.5000	-1.573	0.116

Discussion

Knowing that the majority of thyroid nodules are benign, alternates to surgical treatment have been proposed. Ethanol injection, laser, microwave and radiofrequency ablation are the most popular methods [6-17]. The ethanol injection is very effective when used to treat cystic thyroid nodules [8-12]. Ethanol works as a sclerosing agent via cellular dehydration and protein denaturation that leads to coagulation necrosis and fibrosis. Yasuda et al. reported the cystic nodule volume decreased more than half in 72% of the patients treated with ethanol sclerotherapy [21]. Cho et al. reported 68% of the patients had more than a 50% decrease in thyroid nodule volume after ethanol injection [22]. Others suggest ethanol can be a safe and effective treatment for cystic nodules that are proven to be benign and cause cosmetic or local discomfort.

Tetracycline-based sclerotherapy is one of the sclerosing treatment methods proven effective for cystic lesions. Treece et al. [23] and Goldfarb et al. [24] used tetracycline for the treatment of cystic nodules and suggested the mechanism of action might be the result of tetracycline's low pH.

Ultrasonography guided interstitial laser photocoagulation (ILP) is a non-invasive method of local tissue ablation that was first described to treat advanced cancers. Use of the laser causes a coagulation necrosis by thermal effect, and the necrotic area, which is induced by laser can be controlled unlike with ethanol administration. Studies indicated a need for controlled trials to establish the role of ILP in routine practice [13-15].

Radiofrequency ablation (RFA) is an effective treatment for various malignant tumors such as those of the liver. Recently, RFA was used to treat thyroid nodules and recurrent thyroid cancers. Lee et al. reported the initial experience of RFA for treatment of thyroid nodules. The volume reduction rate in their study was 84%. The major side effect was recurrent laryngeal nerve injury, which occurred in 3.3 % of patients [12].

Lauromacrogol (Polidocanol®, Aethoxysklerol®) is an effective sclerosing agent consisting of 95% hydroxy-polyethoxydodecane and 5% ethyl alcohol. It has a high affinity for vessel endothelium, reacting with the endothelium and causing inflammation and sclerosis. Conrad et al. investigated the efficacy and safety of lauromacrogol on leg varices, noting a low risk of systemic and local complications [25]. Lauromacrogol is used for the treatment of some pathologic processes. Guglielmi et al. used lauromacrogol to treat gastroduodenal ulcer and esophageal variceal bleeding [26], Lopes et al. used it to treat recurrent tracheoesophageal fistulas [27], Ormeci et al. used it for sclerosing liver and spleen cyst hydatic disease [28,29], and Angelini et al. used it for palliative treatment of unresectable esophageal tumors [30]. Kaneko et al. compared different agents for portal vein embolization and found lauromacrogol was the most effective at inducing hypertrophy and was the most suitable agent for portal vein embolization [31].

Although there are many studies in other areas, there is limited literature regarding the use of lauromacrogol for the treatment of thyroid nodules. Kuznetsov et al. studied 37 patients with nodular thyroid disease and found lauromacrogol can be used for hot nodules [32].

Lauromacrogol is known to be an effective and safe sclerosing agent and can be easily used in routine clinical practice. The functional and histopathologic effects of lauromacrogol on thyroid tissue have not been previously evaluated.

In our study, we found two important outcomes. Firstly, tests of thyroid function were not significantly different between the control and study groups. This important finding suggests lauromacrogol can be safely used without the risk of hypothyroidism or hyperthyroidism. Secondly, the Erlich-Hunt Model histopathologic evaluation results revealed that inflammation and fibrosis were significantly increased in the study group. This finding shows lauromacrogol can be effectively used to treat thyroid nodules by means of fibrosis. More adhesion formation was found in the study group, but the result was not statistically significant. It is not surprising to see excess adhesions after sclerosing agent induction, but we did not see any adhesion related complications in the observation period.

Conclusions

In summary, we revealed lauromacrogol causes fibrosis in thyroid tissue without significant perithyroidal adhesion formation and functional differences. Treatment of nodular thyroid disease with lauromacrogol may be safe.

Riassunto

Lo studio è finalizzato al rilevamento degli effetti del lauromacroglo, un agente sclerosante, sul tessuto tiroideo del coniglio, utilizzando 12 conigli divisi in due gruppi di sei ciascuno. Nel gruppo di studio è stato iniettato nel tessuto tiorideo 0,1 ml di lauromacrogolo, ed in quello di controllo lo stesso volume di soluzione fisiologica. Al controllo postoperatorio sono stati valutati il tasso di FT3, FT4, TSH, la presenza di aderenze e le differenze istopatologiche dei tessuti tiroidei.

Non sono risultate differenza significative nel livelli di FT3, FT4, TSH (p>0,05) ma i livelli di , la presenza di aderenze e le differenze istopatologiche dei tessuti tiroidei.

Ai controlli i valori di FT3, FT4 ed TSH non sono risultati significativamente differenti (p>0,05), mentre i livelli di FT3 sono stati significativamente differenti all'interno dei due gruppi (p=0,20). Non sono state riscontrate differenze macroscopicamente significative delle aderenze postoperatorie (p>0,05), ma flogosi e fibrosi sono risultate istologicamente più elevate nel gruppo di studio (p=0,003).

Due sono i risultati importanti dello studio: la differenza non signifcativa dei test di funzione tiroidea tra i due gruppi, suggerendo che il lauromacrogol può essere usato con sicurezza senza rischi di ipo- o ipertiroidismo; la valutazione istopatologica su modello Erlich-Hunt ha dimostrato che flogosi e fibrosi sono significativamente accresciute nel gruppo di studio, indicando che il lauromacrogol più essere usato efficacemente e con sicurezza per il trattamento dei noduli tiroidei per provocare fibrosi, senza significative aderenze peritiroidee e differenze della funzione.

References

1. Gessl A, Lemmens-Gruber R, Kautzky-Willer A: *Thyroid disorders*. Handb Exp Pharmacol, 2012; 214:361-86.

2. Hegedus L: *Clinical practice: The thyroid nodule*. N Engl J Med, 2004; 351:1764-771.

3. Tan GH, Gharib H: *Thyroid incidentalomas: Management approaches to nonpalpable nodules discovered incidentally on thyroid imaging*. Ann Intern Med, 1997; 126:226-31.

4. Ezzat S, Sarti DA, Cain DR, Braunstein GD: *Thyroid incidentalomas: Prevalence by palpation and ultrasonography*. Arch Intern Med, 1994; 154:1838-840.

5. Papini E, Guglielmi R, Bizzarri G, Pacella CM: *Ultrasound-guided laser thermal ablation for treatment of benign thyroid nodules*. Endocr Pract, 2004; 10:276-83.

6. Shemen LJ, Strong EW: *Complications after total thyroidectomy*. Otolaryngol Head Neck Surg, 1989; 101:472-75.

7. Valcavi R, Frasoldati A: *Ultrasound-guided percutaneous ethanol injection therapy in thyroid cystic nodules*. Endocr Pract, 2004; 10:269-75.

8. Sung JY, Baek JH, Kim YS, Jeong HJ, Kwak MS, Lee D, et al.: *One-step ethanol ablation of viscous cystic thyroid nodules*. AJR Am J Roentgenol, 2008; 191:1730-733.

9. Yasuda K, Ozaki O, Sugino K, Yamashita T, Toshima K, Ito K, et al.: *Treatment of cystic lesions of the thyroid by ethanol instillation*. World J Surg, 1992; 16:958-61.

10. Zingrillo M, Torlontano M, Chiarella R, Ghiggi MR, Nirchio V, Bisceglia M, et al.: *Percutaneous ethanol injection may be a definitive treatment for symptomatic thyroid cystic nodules not treatable by surgery: five-year follow-up study*. Thyroid, 1999; 9:763-67.

11. Kim JH, Lee HK, Lee JH, Ahn IM, Choi CG: *Efficacy of sonographically guided percutaneous ethanol injection for treatment of thyroid cysts versus solid thyroid nodules*. AJR Am J Roentgenol, 2003; 180: 1723-726.

12. Lee JH, Kim YS, Lee D, Choi H, Yoo H, Baek JH: *Radiofrequency ablation (RFA) of benign thyroid nodules in patients with incompletely resolved clinical problems after ethanol ablation (EA)* World J Surg, 2010; 34:1488-493.

13. Dossing H, Bennedbaek FN, Karstrup S, Hegedus L: *Benign solitary solid cold thyroid nodules: US-guided interstitial laser photocoagulation. Initial experience*. Radiology, 2002; 225: 53-57.

14. Pacella CM, Bizzarri G, Spiezia S, Bianchini A, Guglielmi R, Crescenzi A, et al.: *Thyroid tissue: US-guided percutaneous laser thermal ablation*. Radiology, 2004; 232:272-80.

15. Spiezia S, Vitale G, Di Somma C, Pio Assanti A, Ciccarelli A, Lombardi G, et al.: *Ultrasound-guided laser thermal ablation in the treatment of autonomous hyperfunctioning thyroid nodules and compressive nontoxic nodular goiter*. Thyroid, 2003; 13:941-47.

16. Kuznetsov NA, Rodoman GV, Sumedi IR, Shalaeva TI, Sviridenko NV, Cherner VA: *Sclerotherapy for the treatment of thyroid functional anatomy*. Khirurgiia, 2010; 8: 11-15.

17. Porenta M, Fettich JJ: *Treatment of thyroid cysts by sclerosation*. Radiobiol Radiother, 1985; 26:249-54.

18. Galvan G, Manzl M, Balcke C, Maier F: *Therapy of thyroid cysts with fine needle aspiration*. Schweiz Med Wochenschr, 1982; 112: 926-930.

19. Evans DM, Mc Aree K, Guyton DP, et al.: *Dose dependency and wound healing aspects of the use of tissue plasminogen activator in the prevention of intra-abdominal adhesions*. Am J Surg, 1993; 165: 229-32.

20. Ehrlich HP, Hunt TK: *The effects of cortisone and anabolic steroids on the tensile strength of healing wounds*. Ann Surg, 1969; 170: 203-06.

21. Yasuda K, Ozaki O, Sugino K, Yamashita T, Toshima K, Ito K, et al.: *Treatment of cystic lesions of the thyroid by ethanol instillation*. World J Surg, 1992; 16:958-61.

22. Cho SH, Lee SH, Jung KY, Woo JS, Back SK, Choi JH, et al.: *Sonography-guided OK-432 sclerotherapy for benign thyroid cysts.* Acta Otolaryngol, 2008; 128:597-600.

23. Treece GL, Georgitis WJ, Hofeldt FD: R*esolution of recurrent thyroid cysts with tetracycline instillation.* Arch Intern Med, 1983; 143:228-32.

24. Goldfarb WB, Bigos ST, Nishiyama RH: *Percutaneous tetracycline instillation for sclerosis of recurrent thyroid cysts.* Surgery, 1987; 102:1096-100.

25. Conrad P, Malouf GM, Stacey MC: *The Australian Polidocanol (aethoxysklerol) study. Results at 2 years.* Dermatol Surg, 1995; 21; 334-36.

26. Guglielmi A, Ruzzenente A, Sandri M, Kind R, Lombardo F, Rodella L, et al.: *Risk assessment and prediction of rebleeding in bleeding gastroduodenal ulcer.* Endoscop, 2002; 34: 778-86.

27. Lopes MF, Pires J, Nogueria Brandao A, Reis A, Morais Leitão L: *Endoscopic obliteration of a recurrent tracheoesophageal fistula with Enbucrilate and Polidocanol in a child.* Surg Endosc, 2000; 33:17-20.

28. Ormeci N, Soykan I, Bektas A, Sanoglu M, Palabiyiko lu M, Hadi Yasa M, et al.: *A new percutaneous approach for the treatment of hydatid cysts of the liver.* Am J Gastroenterol, 2001; 96:2225-230

29. Ormeci N, Soykan I, Palabıyıkoglu M, Idilman R, Erdem H, Bektaş A, et al.: *A new therapeutic approach for treatment of hydatid cysts of the spleen.* Dig Dis Sci, 2002; 47:2037-44.

30. Angelini G, Pasini AF, Ederle A, Castagnini A, Talamini G, Bulighin G. Nd: *YAG Laser versus Polidocanol injection for palliation of esophageal malignancy: Prospective randomized study.* Gastrointest Endosc, 1991; 37:607-10.

31. Kaneko T, Nakao A, Takagi H: *Clinical studies of new material for portal vein embolization: comparison of embolic effect with different agents.* Hepatogastroenterology, 2002; 49:472-77.

32. Kuznetsov NA, Rodoman GV, Sumedi IR, Shalaeva TI, Sviridenko NV, Cherner VA: *Sclerotherapy for the treatment of thyroid functional anatomy.* Khirurgiia, 2010; 8:11-15.

ERRATA CORRIGE

In the paper of Pavicevic, et al. *"Efficacy of Magnetic Resonance urography in detecting crossing renal vessels in children with ureteropelvic junction obstruction"* published in Annali Italiani di Chirurgia 2015; 86:443-49, a must be done correction of the Authors that should be:

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