

Effects of Carnoy's Solution and TCA on the Pouch Mucosa of Hamster

Xinguang Han, Cailing Yang

Department of Stomatology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan 450052, China

Abstract: Objective. To investigate the injury of Carnoy's solution and trichloroacetic acid (TCA) on the pouch mucosa of hamster, and provide evidence for these agents to be used in keratocyst treatment. **Methods.** 20 hamsters were employed as research objects, and divided into 4 groups randomly: Carnoy's group, 20% TCA group, 35% TCA group, and control group. The cheek pouches of hamster were treated with freshly prepared Carnoy's solution, 20% TCA and 35% TCA separately for 3 minutes. The specimens were taken immediately, and at 1, 3, 6, 12 weeks after operation. HE staining method, Van Gieson staining method, enzymohistochemistry and Transmission electron microscopy were used to study the effects of these agents on the mucosa. **Results.** Both Carnoy's solution and 35% TCA caused formation of fistula on pouch at the first week. Microscopic observation showed the epithelia formed an unorganized necrosis zone, and cells lose their morphous in Carnoy's solution group. The penetration depth was about 0.3 mm. In both 20% and 35% TCA group, the epithelia cell showed nuclear fragmentation or deliquescence, and internal structure lost, leading to the cell a vacuolar shadow. Arrangement of collagen fibers under epithelia was disordered; and fibroblast was disappearing in all test groups in the first week. From the 3rd week to the 6th week, the collagen fibers and fibroblast in all test groups were hyperplasia. **Conclusion.** Carnoy's solution and TCA have great penetrating power on the mucosa of cheek pouch, and they can destroy the epithelia and subcutaneous tissues. If these solutions could be for keratocyst therapy, they might cure the keratocyst or decrease recurrence rate. [Life Science Journal. 2006;3(2):35-40] (ISSN: 1097-8135).

Keywords: Carnoy's solution; TCA; hamster; cheek pouch; keratocyst

Abbreviations: HE: hematoxylin and eosin; TCA: trichloroacetic acid

1 Introduction

Keratocyst of jaws is a common disease in oral and maxillofacial surgery. One of the major problems is the recurrence following the surgical treatment. To reduce the recurrence rate, attempts have been made by improving surgical techniques, such as removal of super-adjacent mucosa, smoothing of the osseous wall of the cystic cavity, resection of neighboring parts of the mandible, tanning of the epithelial lining of the cyst with Carnoy's solution and marsupialisation^[1]. The results showed resection was found to have the lowest recurrence rate (0%) but the highest morbidity rate^[2]. Simple enucleation was reported to have a recurrence rate of 17% to 56%. Simple enucleation combined with adjunctive therapy, such as the application of Carnoy's solution was reported to have recurrence rates of 1% to 8.7%^[2,3].

As fixative solution, Carnoy's solution and TCA have great penetrating power on tissue. As an adjunctive therapy in treatment cyst, they can re-

duce the recurrence rate. But we did not know clearly: what is the mechanism of Carnoy's solution or TCA on the epithelia of cyst? Could Carnoy's solution or TCA destroy the secondary cyst that resided in fibrous capsule wall? How is the response of epithelia of cyst to the Carnoy's solution or TCA? To answer these questions, we took cheek pouch of hamster as epithelial model of keratocyst, to explore the mechanism of Carnoy's solution and TCA on the epithelia of keratocyst.

2 Materials and Methods

2.1 Experimental animal

20 hamsters were employed as research objects (provided by Institute of Biological Product, Wuhan), and were divided into 4 groups randomly: Carnoy's group, 20% TCA group, 35% TCA group, and control group.

2.2 Management method

Hamsters were anesthetized with pentobarbital sodium 45mg/kg. Then the cheek pouches of hamsters were exposed and treated with fresh prepared Carnoy's solution, 20% TCA and 35% TCA sepa-

rately for 3 minutes. The cheek pouches of hamsters in control group was treated with physiological saline. The specimens were taken in 0, 1, 3, 6, 12 weeks after operation.

2.3 General observation

After management with Carnoy's solution and TCA, the colorations and texture of cheek pouch were observed.

2.4 Epithelial changes

The specimens of cheek pouch were fixed with 10% formalin solution for 24 hours, then flushed with lotic water, dehydrated with gradient alcohol, and immersed in wax and embedded. 5 μ m thickness slices were stained with HE. Observation was carried out under light microscope. The penetration depth of Carnoy's solution was detected with micrometer in 10 \times 10 fields of view.

2.5 Collagen fiber observation

In order to observe the changes of collagen fiber below epithelia, Van Gieson staining^[4] method was used in present study.

2.6 Enzymohistochemistry

7 μ m thickness cryostat sections were made by freezing microtome. Then put the sections into dye vat, and added effective solution 100 ml (0.05 M acetic acid buffer solution 95 ml, β -sodium glycerophosphate 0.5 g, lead acetate 0.5 g, 5% magnesium chloride 5 ml, pH 5.0 - 5.2), 37 $^{\circ}$ C, incubation for 80 min. In negative control group, β -sodium glycerophosphate was substituted by distilled water. After that the sections were put into 2% acetic acid solution for 1 min, and put them into fresh prepared 1% ammonium sulfide solution for 1 min in turn and flushed with distilled water behind every step. Then dehydrated with gradient alcohol, cleared with xylene, and mounted with optical gum, the sections were observed under light microscope.

2.7 Transmission electron microscopy

Specimens of cheek pouch taken in the 1st week and the 3rd week after operation were examined by transmission electron microscope. The specimens were fixed with 2.5% glutaraldehyde solution, dehydrated with gradient alcohol, and embedded with ethoxyline resin. Ultrathin sections were made, stained with uranyl acetate, and observed by transmission electron microscope.

3 Results

3.1 General observation

The mucosa of cheek pouch had obvious changes in appearance and texture after treatment with Carnoy's solution and TCA solution. In Carnoy's solution group, the color of mucosa of

cheek pouch became black, the texture and elasticity lost in earlier stage. In TCA group, the color became white, especially the mucosa treated with 35% TCA. A week later, the mucosa became necrotic and ulcerative. There were 4 hamsters' cheek pouches formed fistulation because of exfoliation of ulcerative tissue (2 cheek pouch 2 hamsters for Carnoy's solution group and 35% TCA group respectively). 3 weeks after operation, the ulceration of mucosa had been recovered. From the 6th week the mucosa of cheek pouch had returned to their normal thickness and elasticity.

3.2 Epithelial changes (Figures 1,2)

The mucosa of cheek pouch was keratotic stratified squamous epithelia. The thickness was about 3 - 5 layers cell with tenuis stratum corneum. In Carnoy's solution group, the epithelia turned black and the architecture could not be identified. According to the color change, the penetration depth was detected about 0.25 mm. In TCA group, the architecture was clear, and no obvious change was found. One week later, the epithelia formed an unorganized zone of necrosis, and cells lost their shape in Carnoy's solution group. The black change reached to the muscular layer with its depth about 0.3 mm and its circumscription was clear. Under the zone of necrosis, the evident inflammatory cell infiltration was found, even the inflammatory granulation tissues were showed. In TCA group, the epithelia cell showed nuclear fragmentation or deliquescence, and internal structure lost, made the cell to be a vacuolar shadow. There were no significant differences in epithelial between 20% TCA and 35% solution.

3 weeks after operation, the epithelia of cheek pouch recovered and architecture was clear in all test groups. The thickness of epithelia was 3 - 5 layers of cell. But in some areas, the thickness reached to 10 layers of cell. 6 weeks later, the epithelia had returned to normal, and there was no significant difference compared with normal epithelia of cheek pouch.

3.3 Changes of collagen fibers (Figures 3,4)

The fresh collagen fibers under epithelia showed normal architecture in all test groups, and showed red with Van Gieson stain. One week later, collagen fibers lost their normal architectures, such as disordered arrangement, and broken or even disappeared fibers in Carnoy's solution group. In TCA group, the arrangement of collagen fibers was irregular, and fibroblast was disappeared. It showed salmon pink in Van Gieson stain. 3 weeks later the collagen fibers and fibroblast in all test groups became hyperplastic. The arrangement of collagen fibers was compact. These changes made

the proper lamina to be augmented. From the 12th week, collagen fibers and fibroblast gradually tended to become normal.

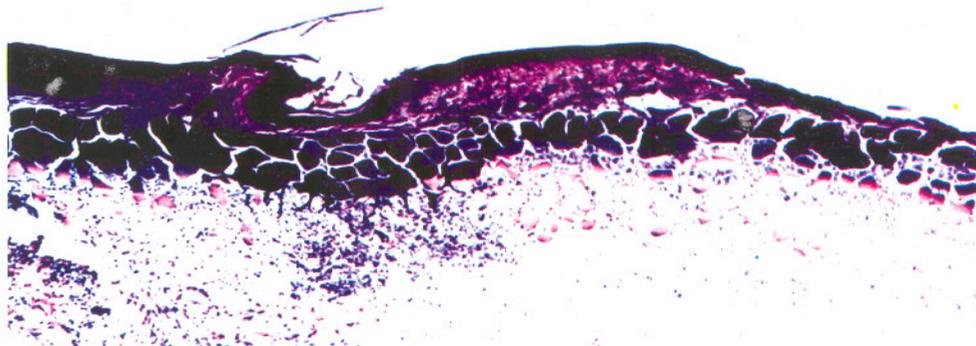


Figure 1. Carnoy's solution group at first week after operation. The figure showed necrosis of epithelia and penetration into muscular layer. (HE×100)

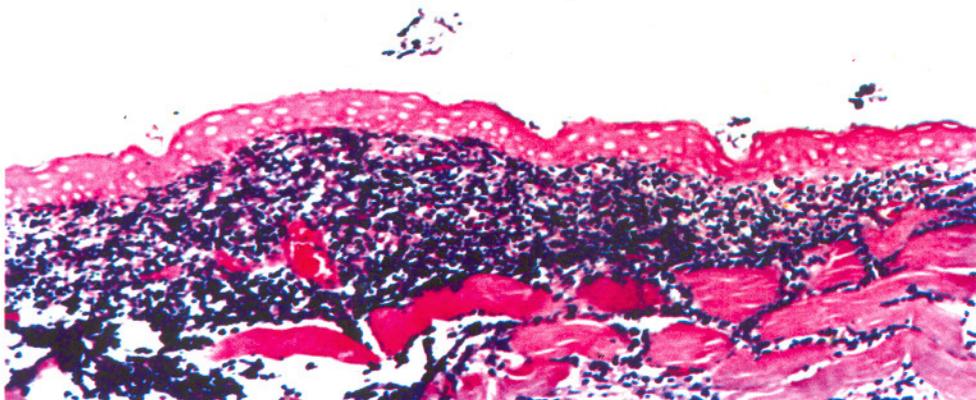


Figure 2. 35% TCA group at first week after operation. The figure showed disappearance of cellular structure, and the vacuolar shadow of the cells. (HE × 100)

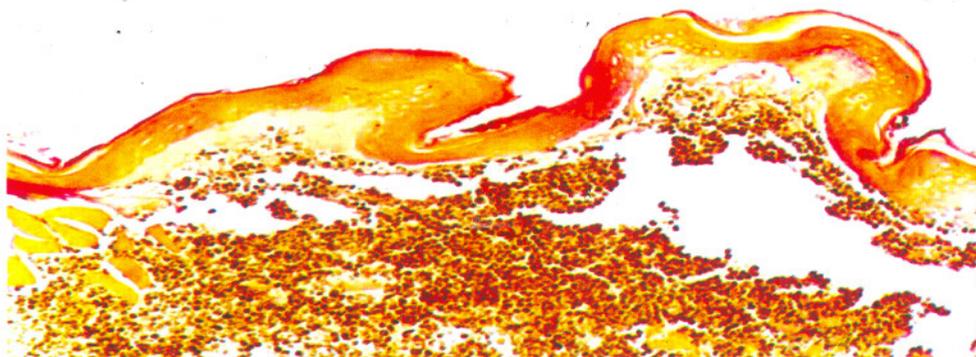


Figure 3. Carnoy's solution group at first week after operation. The figure showed disordered arrangement of collagen fibers, the broken or even disappeared collagen fibers. (VG × 200)

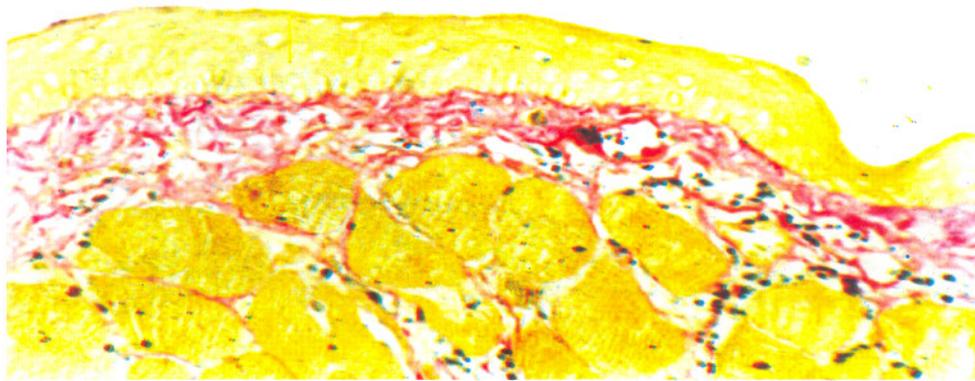


Figure 4. 20% TCA group at first week after operation. The figure showed disappearance of fibroblast. (VG \times 400)

3.4 Enzymohistochemistry (Figures 5,6)

No enzyme activity in epithelial cells was found in all test groups until 3 weeks after opera-

tion; the positive was showed as yellow stain in epithelia of cheek pouch.

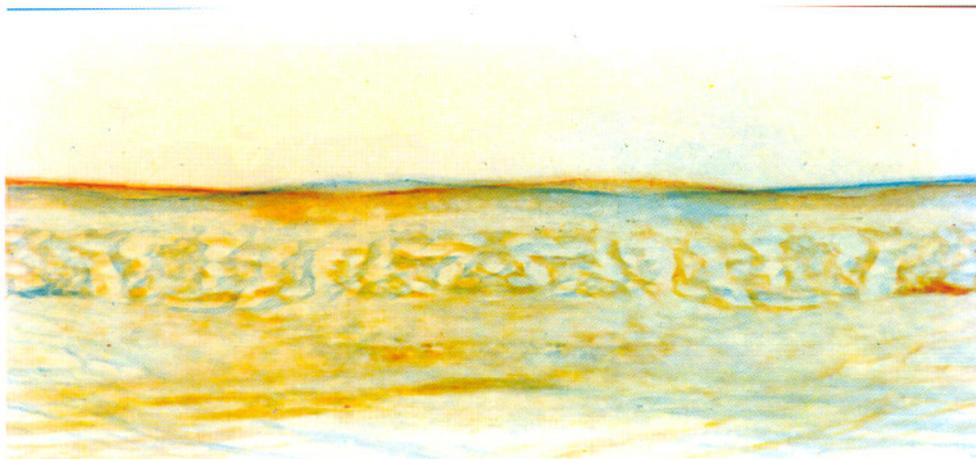


Figure 5. The specimen of Carnoy's solution at immediately time after operation. It showed disappearance of enzymatic activity at epithelial cell. (\times 200)



Figure 6. The specimen of 35% trichloroacetic acid group 3 weeks after operation. It showed enzymatic activity at epithelial cell. (\times 100)

3.5 Transmission electron microscopy (Figures 7,8)

One week after operation, the epithelia of cheek pouch in Carnoy's solution group showed the increased electron density area was unorganized; cellular organelle could not be identified; basement membrane was discontinuous; desmosome and hemidesmosome disappeared. Leakage or vacuole was found in proper lamina; and fibrous structure could not be distinguished. The epithelial changes in TCA group were similar to that of epithelia in

Carnoy's group. But collagen fibers could be recognized. There were no differences between 20% TCA group and 35% TCA group. After 3 weeks, the ultrastructure of epithelial cells could be recognized. In all test groups, the chondrosome in epithelial cell was swelling, the crista was short or disappeared, the heterochromatin was visible in nucleus; desmosome and hemidesmosome were recovered, and basement membrane was integrated. The difference between 20% TCA group and 35% was not significant.

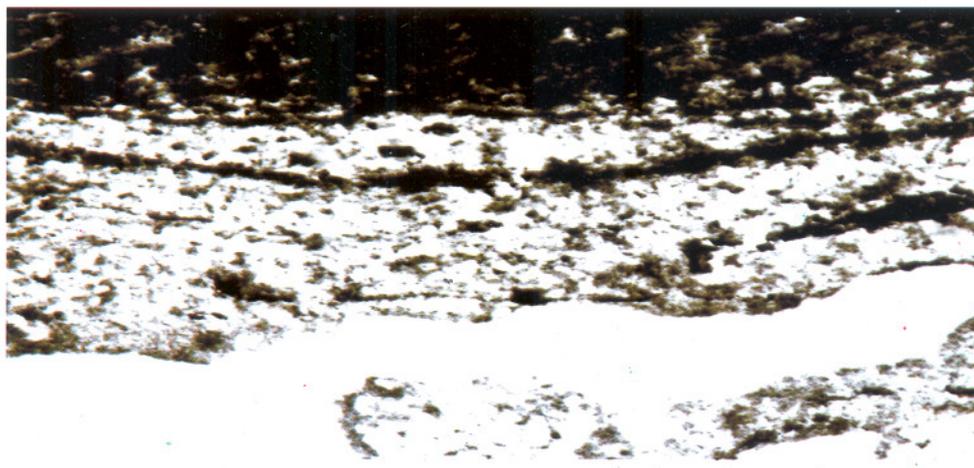


Figure 7. Carnoy's solution group at first week after operation. The figure showed ultrastructure could not be identified. (TEM $\times 10,000$)

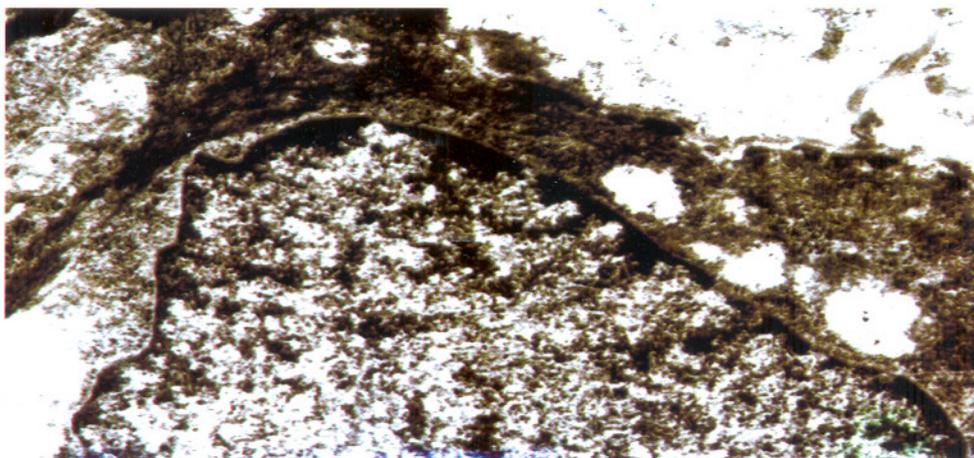


Figure 8. 20% TCA group 3 weeks after operation. The chondrosome of epithelial cell was swelling, and crista was short or disappears. (TEM $\times 15,000$)

4 Discussion

Both Carnoy's solution and TCA are chemical materials with causticity. And Carnoy's solution was usually used as fixative solution, and TCA was

used as peeling solution^[5]. Living tissue would be damaged with these chemical solutions. The degree of injury was relevant to: ① chemical strength of reagent; ② quantity of reagent; ③ exposure chamber and time; ④ strength of penetration; ⑤

mechanism of action^[6].

The mucosa of cheek pouch is cuticular stratified squamous epithelia, whose thickness is 3 – 5 layers of cells. And it is similar to epithelia of keratocyst in origin. So we took cheek pouch as model of keratocyst to investigate the mechanism of Carnoy's solution and TCA in treatment of cyst.

Penetration is one of important factors of Carnoy's solution leading to tissue damage. Some studies showed depth of penetration of Carnoy's solution on nerve was related to time. But the penetrating process was not continuous because of barrier function of perineural epithelia^[7]. In this study, we observed Carnoy's solution's action on mucosa, which resulted in the formation of fistula in cheek pouch and for 3 min the depth of penetration was about 0.3 mm. The difference in depth of penetration on different tissue may be related to their structure.

Some studies showed degree of skin destruction made by TCA was not linear with TCA concentration. However, high concentration of TCA might cause strong reaction and severe clinical pathological changes. The higher concentration is, the deeper the penetration^[7,8]. In this study, the results of 20% TCA and 35% were different. In 35% TCA group, the color change of cheek pouch was greater than that of 20% TCA, and fistula was formed in cheek pouch. These results further demonstrated that effects of 35% TCA on mucosa were greater than 20% TCA.

Proper lamina of mucosa is connective tissue that was mainly constituted by collagen fibers. It has important influence on the epithelial cells. The mucosa as well as its collagen fibers in proper lamina would be damaged when treated with those solutions with penetrating power, such as Carnoy's solution and TCA. The results were supportive for Carnoy's solution and TCA to treat keratocyst, for secondary cyst that resided in fibrous capsule wall would be eliminated by their penetrating power.

Reepithelialization of treatment areas is another question we should pay attention to. The regenerative cells of treatment areas came from the adjacent tissues not the injured zone. It reminded that we should treat lining epithelia of keratocyst thoroughly when we use these solutions to treat keratocyst. Otherwise, any residual epithelia may lead to

recurrence of keratocyst.

The present study demonstrated that Carnoy's solution and TCA had great penetrating power on the mucosa of cheek pouch, and be destroyed the epithelia and subcutaneous tissue. If we use these solutions to treat keratocyst, they maybe cure the keratocyst or decrease recurrence rate after operation.

Acknowledgments

We are grateful to Shichun Xiong (Department of Pathology, School of Stomatology, Wuhan University) for excellent technical assistance.

Correspondence to:

Xinguang Han, M.D.
Department of Stomatology
The First Affiliated Hospital
Zhengzhou University,
Zhengzhou, Henan 450052, China
Email: xinguanghan@sina.com

References

1. Schultz CB, Pajarola GF, and Gratz KW. Therapy and course of recurrent odontogenic keratocyst. A case report. *Schweiz Monatsschr Zahnmed* 2005; 115(6): 554 – 65.
2. Blanas N, Freund B, Schwartz M, and Furst IM. Systematic review of the treatment and prognosis of the odontogenic keratocyst. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000; 90(5): 553 – 8.
3. Zhao YF, Wei JX, Wang SP. Treatment of odontogenic keratocysts: a follow-up of 255 Chinese patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; 94(2): 151 – 6.
4. Gudiene D, Baltrusaitis K, Rackauskas M. Features of elastic tissue staining and its arrangement in the wall of human basilar artery. *Medicina (Kaunas)* 2003; 39(10): 946 – 50.
5. Paul J Carniol, Jyothi Vynatheya, Eric Carniol. Evaluation of acne scar treatment with a 1450 nm midinfrared laser and 30% trichloroacetic acid peels. *Arch Facial Plast Surg* 2005; 7(4): 251 – 5.
6. Carvajal Hugo F. *Burns in Children*. Year Book Medical Publishers Inc. 1988; 3.
7. Frerich B, Cornelius CP, Wietholter H. Critical time of exposure of the rabbit inferior alveolar nerve to Carnoy's solution. *J oral maxillofac Surg* 1994; 52(6): 599 – 606.
8. John D Rachel, Jasmin J Jamora. Skin rejuvenation regimens. A profilometry and histopathologic study. *Arch Facial Plast Surg* 2003; 5(2): 145 – 9.

Received February 15, 2006