Role of Topical Medication in Prevention of Post-extubation Subglottic Stenosis

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Abstract Iatrogenic laryngotracheal injury is the most serious complication of endotracheal intubation since this method of establishing airway was first described by Eugene Bouchut in 1858. Even today, subglottic stenosis is the most dreaded complication of intubation. This animal study is focused on the host tissue response to intubation induced injury resulting in subglottic stenosis and methods to prevent this complication. To assess the role of topically applied Mitomycin-C and Triamcinolone Acetonide in wound healing process following post-extubation subglottic injury. Prospective Randomized block, single-blinded, experimental study. Forty New-Zealand white rabbits were block randomized and allocated into 4 groups based on the type of topical medication that was applied post-extubation. Further these groups were subdivided into 3 subgroups based on the time of sacrifice (4, 6 and 12 weeks) to study the histopathological changes that occurred in a temporal sequence at the subglottis. It was observed that the rabbits in the control group and those that received Mitomycin-C only had more respiratory distress compared to those treated with Triamcinolone Acetonide. Statistically significant histopathological changes were observed in all the 4 groups. Mitomycin-C applied topically did not alter the wound healing process following post-extubation injury in the subglottis. Triamcinolone Acetonide significantly altered wound healing in

the subglottis and prevented occurrence of respiratory distress.

Keywords Post-extubation laryngotracheal injury · Subglottic stenosis · Mitomycin-C · Steroids · Triamcinolone

Introduction

Laryngotracheal injuries (LTI) has been the main concern since Eugene Bouchut (1818–1891) described his technique of establishing and maintaining airway by endotracheal intubation (ETI) at the French Academy of medical sciences in 1858 [1]. He was so highly criticized by the members of the academy that he abandoned this procedure. William McCewen (1880) revived this technique that was later popularized by Joseph P O'Dwyer (1890) [1–3]. Technological advances that aided better visualization of laryngotracheal airway, better quality and design of the endotracheal tube along with the training in performing ETI has revolutionized the way airway intubation is done and made it a fairly safe procedure.

Today, endotracheal intubation is the preferred method for establishing and maintaining airway. There are many complications that have been reported due to this procedure. The most challenging of them, especially in children and in neonates is subglottic stenosis. Schweiger et al. [4] reported an incidence post-intubation subglottic stenosis (PISGS) in children to be around 11.38%. This incidence of subglottic stenosis (SGS) is definitely high and the authors have emphasized the need to identify the risk factors that could alter the wound healing process and prevent SGS [4]. Many drugs have been evaluated and have been described to modulate wound healing in the

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upper airway [5, 6]. Thus there is a need to identify drugs that could modify the wound healing process in post-extubation laryngotracheal injury (PELTI) and prevent the formation of laryngotracheal stenosis (LTS).

Aim of the study

To assess the role of topical applied Mitomycin-C (MC) and Triamcinolone Acetonide (TA) in wound healing process following post-extubation subglottic injury.

Materials and methods

This study was done at the Central Animal Facility for Toxicology and Developmental Research at Sri Ramachandra University, Chennai, India. Institutional animal ethics committee approval was obtained.

In an earlier study, a rabbit model for inducing laryngotracheal injuries following endotracheal intubation was established. In this study it was observed that New-Zealand white rabbits weighing 2.0–2.5 kg, when intubated by direct laryngoscopic method under sedation with a 4.0 mm cuffed Portex endotracheal tube (Smiths Medicals, Dublin, OH 43017, USA) for 4 h had significant subglottic injury which later had histopathological evidence of subglottic stenosis. All rabbits thus intubated had survived for 12 weeks post-extubation. The group B animal model of laryngotracheal intubation induced injuries that was established and standardized as described in our earlier article was used in the present study [7]. Forty New-Zealand white rabbits were intubated with a no 4.0 cuffed endotracheal tube for 4 h with hourly movement of the tube.

Further, these 40 rabbits were block randomized and allocated into 4 groups based on the topical medication that was applied as shown in Table 1. The medication was locally sprayed using a 23-gauge spinal needle under direct visualization using a laryngoscope on Day 0, end of 1st, 2nd and 3rd week post-extubation. There were 12 rabbits each in group A, B and C and 4 rabbits in group D (control group). The rabbits in the group A, B and C were further randomly divided into 3 subgroups based on the time of at

the end of 4th, 6th and 12th week post-extubation when they were humanly sacrificed. This was done to study and compare the temporal sequence of wound healing in all these 3 groups. Post-extubation the rabbits were monitored for the onset and progress of respiratory distress (increased work of breathing/stridor). Post sacrifice the larynx and trachea was harvested and serial sections were done at the level of the subglottis that was histopathologically (HPE) evaluated by a senior pathologist who was blinded to the type of topical medication received by the rabbit's post-extubation. The HPE features examined in each section were.

Mucosal changes

Mucosal ulcerations, mucosal scabs, mucosal metaplasia.

Sub-mucosal changes

Sub-mucosal thickening, sub-mucosal vascular thickness, sub-mucosal fibrosis.

Cartilage changes

Perichondritis, cartilage deformity.

The scoring system was standardized. The HPE sections were examined at 12°, 3°, 6° and 9° clock position per continuous high power field for mucosal, sub-mucosal and cartilage pathology i.e. mucosal ulceration, mucosal scabs, mucosal metaplasia, sub-mucosal vascular thickness, sub-mucosal fibrosis, perichondritis and cartilage deformity. This scoring grade was done per cross-section recorded on a grade of 0–3 by a single senior pathologist who was blinded to the nature of injury and medication received by the rabbit whose histopathological slide is being examined. (Grade-0 was no significant pathology, grade-1 was 1–25%, grade-2 was 26–50%, and grade-3 was more than 51%). All these scores and measurements were statistically analyzed using IBM SSPS, Statistics for Windows, Version 20, Armonk, New York. Nonparametric test i.e. K-independent sample test using Kruskal–Wallis test was applied to identify any statistically significant difference in the scores and measurements when comparing the sub-groups i.e. comparing the statistical difference between subgroups

Table 1 Block randomized groups based on medication applied topically

	Group A	Group B	Group C	Group D
Number of rabbits	12	12	12	4
Type of medication	Mitomycin-C (MC) (0.4 mg/ml)	Triamcinolone acetonide (TA) (1 mg/ml) + MC (0.4 mg/ml)	TA (1 mg/ml)	Isotonic Saline Control
Amount of medication	1 ml	(0.5 ml + 0.5 ml) = 1 ml	1 ml	1 ml

Table 2 Preclinical observation across all groups

	Group A	Group B	Group C	Group D
Total number of rabbits	12	12	12	4
Signs of airway obstruction ^a	6	2	1	3
Onset of respiratory distress	3rd week—3, 4th week—3	3rd week—2	4th week—1	3rd week—3
Number of week at sacrifice (sub-groups)				
4 weeks	4	4	4	—
6 weeks	4	4	4	—
12 weeks	4	4	4	4

^a All rabbits survived the intended period of time before sacrifice

in A, B, C, D sacrificed at 4th week. Similar statistical analysis was done between groups at 6th and 12th week of sacrifice. If there were any statistically significant results that were obtained in the above test, then 2-Independent sample test using Mann–Whitney *U* test was applied to compare the statistical difference between the two sub-groups.

Results

In this study, all the forty rabbits that were intubated and treated with topical medication, survived the intended period of observation according to the group and sub-group they belonged to. These rabbits were humanely sacrificed at the end of the period of observation. During this period, preclinical observations on the sign and symptoms for respiratory distress are shown in Table 2.

Out of the twelve rabbits in group A (MC), six rabbits had signs and symptoms of airway obstruction in the acute as well as the chronic phase of wound healing. Three out of four rabbits in group D (Control) had signs and symptoms of airway obstruction.

Statistical analysis of the histopathological scores of the 3 sub-groups at the level of subglottis in group A, B and C at 4th week, 6th week and group A, B, C and D at 12th week are shown in Tables 3, 4 and 5 respectively. The

histopathological pictures of the cross-section at the level of subglottis at 4th, 6th and 12th week are shown in Figs. 1 and 2.

Discussion

It has been reported that temporary LTI occurred in 62–94% of intubations [8]. A number of factor influence the severity of injury and the progress to formation of SGS. The most critical risk factor for PESGS in neonates and children is the size/diameter of the tube [9, 10]. In a multicentric study that was conducted across seven neonatal intensive care units, Contencin et al. [10] have reported that 5 out of 247 post-intubated neonates showed evidence of respiratory distress that recovered with medical therapy. These authors identified that the size of the endotracheal tube that was used for intubation as the major risk factor for formation of SGS [9, 10].

Identification and prevention of PESGS is the need of the hour. One must be familiar not only with the risk factors but also the use of drugs that could modulate wound healing so as to prevent the occurrence of SGS. Hirshoren and Eliashar [5] have comprehensively reviewed the literature and summarized the role of drugs that modulate wound healing. Of the many drugs used for modulating wound healing, corticosteroids and MC are the commonly

Table 3 Histopathological scores at the level of subglottis at 4th week

	Group A (MC) Mean ± SD	Group B.(MC + TA) Mean ± SD	Group C (TA) Mean ± SD	Kruskal–Wallis Test (<i>p</i> value)	Mann–Whitney <i>U</i> test		
					Group A and group B <i>p</i> value	Group A and group C <i>p</i> value	Group B and group C <i>p</i> value
Mucosal ulceration	1.75 ± 0.82	0	0	0.005	0.013	0.013	1.000
Mucosal scab	1 ± 0	0	0	0.004	0.008	0.008	1.000
Sub-mucosal thickening (μm)	56.36 ± 17.19	23.45 ± 4.57	15.8 ± 5.01	0.012	0.021	0.021	0.083
Sub-mucosal fibrosis	1.5 ± 0.5	1.25 ± 0.5	0	0.014	0.495	0.013	0.011

p value of less than or equal to 0.05 was considered significant

Table 4 Histopathological scores at the level of subglottis at 6th week

	Group A (MC) Mean $\pm$ SD	Group B (MC + TA) Mean $\pm$ SD	Group C (TA) Mean $\pm$ SD	Kruskal–Wallis test	Mann–Whitney <i>U</i> test		
					Group A and group B <i>p</i> value	Group A and group C <i>p</i> value	Group B and group C <i>p</i> value
Mucosal metaplasia	1 $\pm$ 0	0.25 $\pm$ 0.5	0	0.017	0.040	0.008	0.317
Sub-mucosal thickening (μ m)	90.43 $\pm$ 46.98	39.71 $\pm$ 25.20	18.55 $\pm$ 5.87	0.023	0.083	0.021	0.083
Sub-mucosal fibrosis	2 $\pm$ 0.81	0.25 $\pm$ 0.5	0	0.011	0.025	0.013	0.317

p value of less than or equal to 0.05 was considered significant

Table 5 Histopathological scores at the level of subglottis at 12th week

	Group A Mean $\pm$ SD	Group B Mean $\pm$ SD	Group C Mean $\pm$ SD	Group D Mean $\pm$ SD	Kruskal– Wallis test (<i>p</i> value)	Mann–Whitney <i>U</i> test			
						Group A and group B <i>p</i> value	Group A and group C <i>p</i> value	Group B and group D <i>p</i> value	Group C and group D <i>p</i> value
Sub-mucosal thickening	86.16 $\pm$ 56.82	15.77 $\pm$ 4.17	17.5 $\pm$ 2.66	84.22 $\pm$ 42.40	0.01	0.021	0.021	0.773	0.021
Sub-mucosal fibrosis	2.75 $\pm$ 0.5	0.75 $\pm$ 0.5	0	2.25 $\pm$ 0.95	0.006	0.015	0.011	0.405	0.013

p value of less than or equal to 0.05 was considered significant

Fig. 1 Picture of Hematoxylin and eosinophil stains at the level of subglottis, at 4th, 6th and 12th week, the figures are marked A for group A (MC), B for group B (MC + TA), C for group C (TA) and D for control group

Fig. 2 Picture of Masson Trichrome stains at the level of subglottis, at 4th, 6th and 12th week, the figures are marked A for group A (MC), B for group B (MC + TA), C for group C (TA) and group D for controls

Table 6 Animal studies for efficacy of Mitomycin-C in LTS

Sl. no.	References	Animal; number	Type of injury	Dose of MC	Results
1.	Eliashar et al. [21]	Canine, 60	Direct trauma	0.2 mg/ml	Beneficial
2.	Correa et al. [22]	Canine, 10	CO2 laser injury	1%	Reduced subglottic collagen formation
3.	George et al. [23]	Pigs, 26	Single stage laryngotracheal reconstruction	0.5 mg/ml	Prevent the liquefactive necrosis Promote neo-chondrification, Improved graft incorporation
4.	Eliashar et al. [24]	Canine, 16	Induced LTS	0.5 mg/ml	Does not prevent stenosis
5.	Cincik et al. [25]	Rabbits, 4 in each sub-group	Standardised trauma	0.4 mg/ml	Reduce fibrosis
6.	Roh et al. [26]	Rabbits, 26	Laser injury	0.4 mg/ml	Prevents progression of posterior glottis stenosis
7.	Roh et al. [27]	Canine, 12	Stripping of vocal cords	1.0 mg/ml	Minimise anterior glottic stenosis
8.	Roh et al. [28]	Rabbits, 60	Diode laser	0.4 mg/ml 1.0 mg/ml	Significant risk of airway obstruction
9.	Roh et al. [29]	Rabbits, 60	Laser injury	1.0 mg/ml	No benefit to prevent stenosis
11.	Shvidler, et al. [30]	Ferrets, 20	Simulated intubation injury	–	No benefit to prevent stenosis
12.	Iniñiguez-Cuadra et al. [31]	Rabbits, 18	End to end anastomosis	0.2 mg/ml, 0.5 mg/ml	Not effective, can provoke opposite effect
13.	Present study	Rabbits, 40	Post intubation injury	0.4 mg/ml	Does not alter the wound healing process

used drugs in clinical practice. This study has compared the role of these two drugs individually as well as in combination on the wound healing process in the PESGS with special reference to the subglottis.

Corticosteroids act on all the three phases of wound healing and hence have a definitive role in modulation of wound healing [11, 12]. Human studies on the efficacy of using corticosteroids in SGS are largely limited by case reports. Ratna et al. [13] in a prospective study in 150 patients observed that corticosteroids did not have any beneficial effects in protection of the airway, but, rather resulted in doubling the pulmonary complications.

Initial prospective experimental studies in animal using the combination of steroids along with antibiotics was found to be beneficial for preventing SGS [14, 15]. Aerosolised Dexamethasone was found to be effective in reducing oedema after acute subglottic injury in a ferret animal model when given in the immediate post-injury period at 2, 4 and 6 h [16].

MC is an antimicrobial drug which has both anti-metabolic as well as antiproliferative properties. It inhibits fibroblast proliferation and hence modulates wound healing. It is also suggested that MC inhibits wound healing by down regulating the gene expression for extracellular matrix proteins [17]. The biological effect of topical MC is influenced by the concentration and exposure time [18–20]. The role of MC in LTS has been studied in various experimental animal models as shown in Table 6. As can be seen in Table 6, earlier studies have shown that MC has

a beneficial effect in reducing fibrosis and stenosis but later studies have shown that MC does not have any beneficial effect in preventing or reducing LTS.

In this study there was significant reduction in preclinical symptoms of respiratory distress and histopathological evidence of subglottic stenosis in the group treated with TA as compared to the group treated with TA and MC and the group treated with MC only. It was observed that there was no statistically significant modulation of wound healing in the group treated with MC as compared with the control group. Thus it was inferred that MC sprayed topically in the dose of 0.4 mg/dl did not alter the wound healing process as compared to TA. TA when sprayed topically in the immediate post extubation period in multiple doses at weekly interval modulated wound healing in such a way as to prevent the formation subglottic stenosis. Thus, extrapolating to clinical scenario it is suggested that further studies using TA in the form of topical inhalations in high risk children in the immediate post extubation period will be useful.

Conclusion

The best way to manage acquired/iatrogenic laryngotracheal stenosis is to prevent its occurrence. Identifying the high risk patients and using drugs that can modulate wound healing in the immediate post-extubation period to prevent stenosis is the need of the hour. Mitomycin-C in a dosage

of 0.4 mg/ml, applied topically in 4 or more sittings does not seem to alter the progress of the healing process and does not prevent post-extubation SGS in rabbits as evidenced in this study. Triamcinolone Acetonide applied topically has shown to be a better modulator of wound healing in the laryngotracheal airway following intubation induced injury compared to Mitomycin-C.

Compliance with ethical standards

Conflict of interest None.

Ethical approval Institutional animal ethics committee approval was obtained prior to the start of the study. “All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.” “All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted.”

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