

ORIGINAL ARTICLE

The Effect of Radiotherapy on Propylene Glycol-Induced Cholesteatoma in Rats

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OBJECTIVE: Radiotherapy has been an alternative therapy in benign pathologies with epithelial and connective tissue hyperplasia. The aim of this study was to investigate the efficacy of low dose radiotherapy in the inhibition of cholesteatoma in an experimental cholesteatoma model in rats.

MATERIAL AND METHODS: Thirty-five Wistar albino rats were used to form 3 groups: Group I (n=20; bilateral cholesteatoma induction with propylene-glycol), Group II (n=10; unilateral cholesteatoma induction with propylene-glycol and unilateral intratympanic saline injection), Group III (n=5, control group without injection). The effect of radiotherapy was evaluated according to the histopathological parameters.

RESULTS: The results indicated that low dose radiotherapy was ineffective in the management of cholesteatoma in this histopathological study.

Cholesteatoma of the middle ear is an accumulation of exfoliated keratin produced by stratified epithelium which often overlies a connective tissue stroma^[1]. In the studies investigating the histopathological aspects of cholesteatoma, a higher proliferation rate and inflammatory reaction have been demonstrated with respect to the normal stratified epithelium^[2]. A number of pharmacologic agents with anti-inflammatory effect have been used to inhibit the proliferation of cholesteatoma in experimental models with varying results^[3-8].

Radiotherapy has been used in benign pathologies with epithelial and connective tissue hyperplasia, such as nasopharyngeal angiofibroma and keloid formation. The aim of this study was to investigate the efficacy of low dose radiotherapy in the inhibition of cholesteatoma in an experimental cholesteatoma model in rats.

MATERIALS AND METHODS

In this experimental study, 35 healthy Wistar albino rats weighing 180 to 220 grams were used. The animals were anesthetized with 150 mg/kg ketamine hydrochloride and 25 mg/kg xylazin hydrochloride. As

for induction of cholesteatoma animals were treated in 3 different groups. In Group I consisting of 20 animals, a solution of 100% propylene glycol (PG) of 0.2 mL and 0.1 mL solution of gentamicin were injected transtympanically into the middle ears bilaterally. PG was used to induce cholesteatoma. Gentamicin was injected for infection prophylaxis. In Group II with 10 animals, the same combination of solutions were instilled in the right ears, while left ears were treated with 0.1 mL solution of gentamicin and 0.2 mL solution of saline as a control. Three consecutive injections with a 5-days-interval were performed for each treated ear. In order to evaluate the effect of radiotherapy on the normal middle ear mucosa, a third group, Group III was formed with 5 rats without any injection into the middle ears.

After a follow-up period of 1 month, a low dose radiotherapy of 10 Gy was applied on the left temporal bones in Group I and Group III; and on the right and left temporal bones of Group II. Total dose of radiotherapy for each individual ear was 10 Gy, delivered by Theratron 780-C Cobalt-60 (AECL, USA). In order to avoid dose reduction, the irradiated surface was covered with tissue equivalent bolus material of 0.5 cm.

In the third month after the end of radiotherapy course, the animals were deeply anesthetized with the

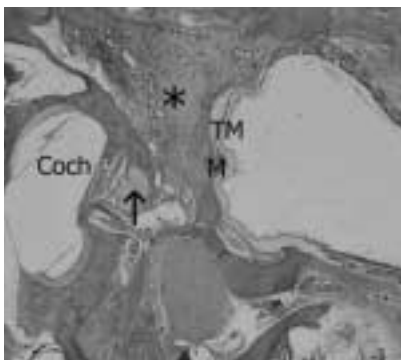


Figure-1: Granulation tissue formation (+++): total obliteration of the tympanic cavity (Magnification x 4) (TM: tympanic membrane, M: malleus, Coch: cochlea, arrow: stapedial artery, asterisk: middle ear).

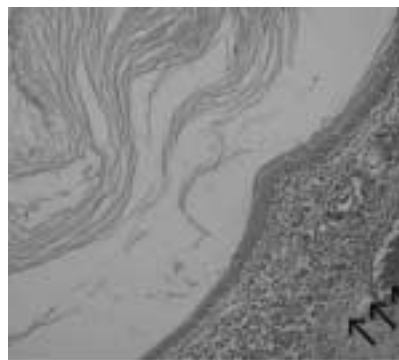


Figure-2: Granulation tissue formation (++) : partial obliteration of the tympanic cavity with granulation tissue over the osseous tissue (arrows) (Magnification x 20).

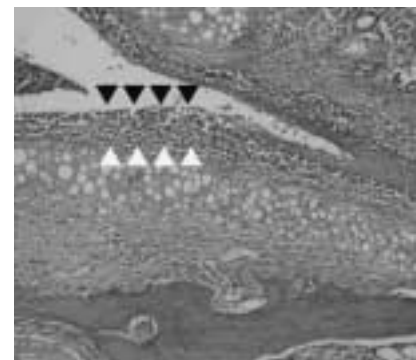


Figure-3: Granulation tissue formation (+): limited granulation tissue under the mucosal surface (white arrow heads) with squamous metaplasia (black arrow heads) (Magnification x 20).

same anesthetic protocol given above and killed by decapitation. The temporal bones were removed from the skull, fixated in 10% formaline, decalcified and embedded in parafin. Light microscopic evaluation was performed in the temporal bones sectioned at 4 μ m and stained with hematoxylin-eosin.

In histopathologic evaluation; the degree of granulation tissue formation, mucosal changes in the middle ear and eustachian tube, changes in the bony walls of tympanic cavity, histopathological changes in the tympanic membrane and ossicles were evaluated.

When evaluating the degree of granulation tissue formation, following criteria was used: (+++), total obliteration of the tympanic cavity by granulation tissue (Figure-1); (++) , partial obliteration of the tympanic cavity by granulation tissue (Figure-2); (+), limited granulation tissue under the mucosal surface of the tympanic cavity (Figure-3); (-), no granulation tissue formation. Mucosal changes in the cavum tympani and eustachian tube (epithelial metaplasia, glandular metaplasia, cholesteatoma formation and keratinization), osseous changes in the surrounding bony walls of the tympanic (destruction or new bone formation) cavity, histopathological changes in the tympanic membrane (fibrosis, calcification and epithelial in-growth) and ossicular changes (destruction, new bone formation and tympanosclerosis) were all evaluated as present (+) or absent (-).

Statistical analysis were performed in subgroups according to the ears: PG treated ears, PG treated and irradiated ears, saline treated and irradiated ears, untreated ears, untreated and irradiated ears. Fisher's exact test and Chi-Square test were used for statistical evaluation. Differences were considered significant at $p < 0.05$.

RESULTS

Six rats were died of PG aspiration. In histopathological evaluation, large perforation on the

tympanic membrane were found in 3 ears. Thus, 15 ears were not included in the study. and excluded from the study. The included ears were re-classified according to the agent(s) used in the intratympanic injection and the presence/absence of radiotherapy application. The histopathological results of these re-classified groups were presented in Table 1.

With regard to the degree of granulation tissue formation and the presence of squamous metaplasia and cholesteatoma, the comparison of the irradiated and non-irradiated ears were given in Table 2. Statistical analysis revealed no statistically significant difference between the groups ($p > 0.05$).

DISCUSSION

Inflammation and hyperplasia in connective tissues are the basic etiological mechanisms in the development of cholesteatoma. Thus a number of anti-inflammatory agents has been used to inhibit these inflammatory reactions. Smith^[6] used topical 5-fluorouracil in the management of cholesteatoma. He claimed that topical 5-fluorouracil in the management of hyperkeratosis, cholesteatoma was almost 100% effective with very few side effects. Two other studies confirmed this conclusion^[5,7]. A number of other therapeutic agents were also studied in the management of cholesteatoma. Pownell et al.^[4] used cyclophosphamide, an immune suppressor and anti-inflammatory agent. Jove et al.^[3] administered isotretinoin, a synthetic vitamin A analogue. White et al.^[8] tried hyaluronic acid for its antiproliferative effect. However, all these latter studies, designed on experimental cholesteatoma models, revealed no significant effect on cholesteatoma formation.

With its suppressive effect on tissue proliferation, radiotherapy has been a therapeutic alternative in some benign lesions with epithelial and connective tissue

Table-1: Histopathological findings in re-classified groups. (PG: propylene glycol; RT: radiotherapy, PS: physiologic saline)

	GROUPS	PG	PG+RT	PS+RT	UNTREATED	UNTREATED +RT
	n (Ears)	15	21	9	5	5
Middle ear mucosa	Granulation tissue	14 (93.3%)	18 (85.7%)	2 (22.2%)	–	–
	Glandular metaplasia	15 (100%)	15 (71.4%)	–	–	–
	Squamous metaplasia	7 (46.7%)	12 (57.1%)	–	–	–
	Squamous metaplasia (eustachian tube)	7 (46.6%)	5 (23.8%)	–	–	–
	Cholesteatoma	5 (33.3%)	6 (28.6%)	–	–	–
Bone	Osteoneogenesis	15 (100%)	16 (76.1%)	–	–	–
	Destruction	15 (100%)	15 (71.4%)	–	–	–
	Ossicular changes	11 (73.3%)	8 (38.1%)	–	–	–
Tympanic membrane	Fibrosis	11 (73.3%)	14 (66.6%)	–	–	–
	Calcification	9 (60%)	10 (47.6%)	–	–	–
	Epithelial ingrowth	1 (9.5%)	1 (4.7%)	–	–	–

Table-2: Statistical comparison of groups with and without radiotherapy treatment on the basis of granulation tissue formation, squamous metaplasia and cholesteatoma (* Chi-Square test; **Fisher's exact test)

		PG	PG+RT	p
Granulation tissue formation	–	1 (6.7%)	2 (9.5%)	0.584*
	+	2 (13.3%)	6 (28.6%)	
	++	10 (66.7%)	9 (42.9%)	
	+++	2 (13.3%)	4 (19%)	
Squamous metaplasia		7 (46.7%)	12 (57.1%)	0.736**
Cholesteatoma		5 (33.3%)	6 (28.6%)	1.0**

hyperplasia, such as angiofibroma, cheloid formation [9-13]. Clinical data and in vivo studies suggest that low-dose radiation has anti-inflammatory effects and these contribute to the effectiveness of radiotherapy on benign diseases [14,15]. In a number of studies, inflammatory cell numbers often remained unaffected by radiotherapy suggesting their functional modulation [15-17]. A murine model for chronic granulomatous inflammation has supported this suggestion [18]. The functional modulation has been proposed to oespecially in the endothelial cells and

macrophages [15]. It has been suggested that radiation, even at low doses, may affect the function of endothelial cells in recruiting inflammatory cells to the affected tissue [19-22]. Hildebrandt et al. [18-23] observed that low radiation doses significantly decreased the production of nitric oxide and the expression of inducible nitric oxide synthase by activated macrophages. In their recent experimental study in mice, Schaue et al. [24] indicated that ionizing radiation induces functional modulation of macrophages and neutrophils, and possibly of other inflammatory cells.

The effect of radiotherapy on cholesteatoma has not been studied in the literature. This study is the first to evaluate this issue on experimental cholesteatoma model. The results revealed no significant change in the histopathological findings in the PG treated ears after radiotherapy with regard to those without any treatment. Percentage of cholesteatoma formation, the pathological process of clinical interest, was not significantly different in the ears treated with radiotherapy. In this study, special concern has been focused on the granulation tissue formation, since this pathological inflammatory process is the destructive aspect of the cholesteatoma (Figure 4). However, the degree of granulation tissue formation was statistically similar when the ears treated with radiotherapy and the untreated ears were compared. Considering the effects of low dose radiotherapy on the functional modulation in the inflammatory cells, the inefficacy of radiotherapy on cholesteatoma management might be the result of a lack of power in this study, where the efficacy of radiotherapy in the management of cholesteatoma was studied histopathologically. Histopathological evaluation might be inadequate to investigate the cellular changes in the inflamed tissues surrounding cholesteatoma.

In this study, pure PG was injected transtympanically on the posterosuperior quadrant to induce cholesteatoma formation, since formation of the

cholesteatoma on which the effect of radiotherapy had been aimed regardless of the pathogenetic mechanism. The reason to use pure PG was its high efficacy (85%) in cholesteatoma induction, which was reported by Huang et al.^[25]. However, contrary to the findings of Huang et al., cholesteatoma was formed in 30.5% of the ears in this study (Figure 5 a,b). Additionally, epithelial ingrowth in the tympanic membrane was noted only in 5.5% of the ears (Figure 6). Although this finding seems to confirm the basal cell hyperplasia, the percentage of epithelial ingrowth was notably low when compared to those of literature^[3,25,26].

In the present study, single-layered cuboidal epithelium of the middle ear was demonstrated to change into single-layered or pseudostratified ciliated columnar epithelium under the influence of inflammation in 52.7% of the ears (Figure 7). The rate of glandular metaplasia was 83.3%. In some of these ears, there was so significant glandular metaplasia that differential diagnosis would be indicated to exclude glandular neoplasia. As in the other parts of the body, such as in bronchial mucosa or endocervical epithelium of uterus, it is not surprising that this ciliated columnar epithelium in the middle ear would change into squamous epithelium in reaction to the long-standing mucosal insults, such as inflammation. The end stage of this transition would be cholesteatoma formation secondary to the

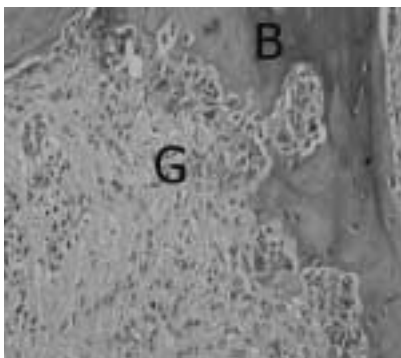


Figure-4: Granulation tissue (G) destruction on bone (B) with pagetoid pattern (Magnification x 40).



Figure 5a: Keratin formation (asterisk) in the middle ear (Magnification x 4) (ME: middle ear, EEC: external ear canal, TM: tympanic membrane).



Figure-5b: Squamous metaplasia in the middle ear mucosa (arrows) with keratin formation in the middle ear (Magnification x 20).



Figure-6: Proliferation of the squamous epithelium of the external ear canal into the tympanic membrane (in-growth) (arrows) (Magnification x 10) (EEC: external ear canal, ME: middle ear, TM: tympanic membrane) .

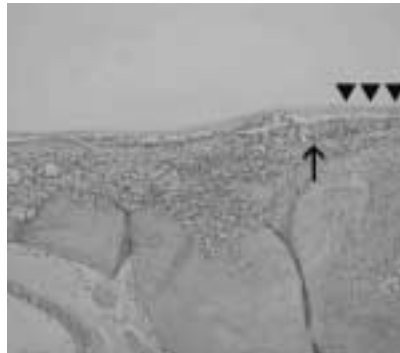


Figure-7: The junction (arrow) between single layered cuboidal epithelium and pseudostratified epithelium with glandular metaplasia (arrow heads) (Magnification x 4).

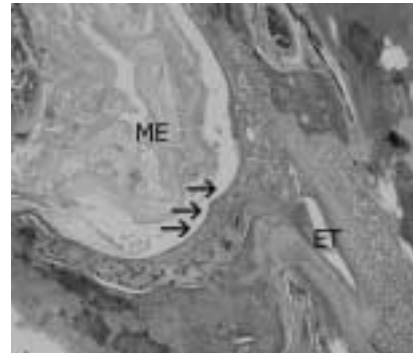


Figure-8: Squamous metaplasia (arrows) in mucosa of eustachian tube (ET) and keratin formation in the middle ear (ME) (Magnification x 4).

keratinization of the squamous epithelium. This was supported in the present study, such that foci of squamous metaplasia and cholesteatoma formations were demonstrated in 52.7% and 30.5% of the PG injected ears (n=36), respectively, in different areas away from the vicinity of tympanic membrane (Figure 2, 3).

The mucosa of eustachian tube was noteworthy. In a healthy middle ear, eustachian tube is covered with pseudostratified ciliated columnar respiratory epithelium. Thus, in case of mucosal inflammation, squamous metaplasia would take place earlier in the mucosa of eustachian tube than in the cuboidal epithelium of the middle ear. In this experimental model, squamous metaplasia was dominantly noted in close proximity of the eustachian tube, that is in 33.3% of the ears (Figure 8).

CONCLUSION

Low dose radiotherapy was not found to be effective in the management of cholesteatoma in this histopathological study.

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