ROLE OF INTERLEUKIN-1 IN PATHOGENESIS OF RADICULAR CYST

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Background: Interleukin-1 (IL-1) is one of the cytokines produced by macrophages, monocytes and dentritc cells. Macrophages are present in apical granuloma and the wall of the radicular cyst. This cytokine causes the cyst expansion and is involved in proliferation of fibroblasts in the cyst wall and stimulate the fibroblasts to produce more prostaglandin. Radicular cyst is the most common cyst of the jaws which is usually associated with necrotic pulp of the tooth. The cyst formation requires proliferation of the epithelial rest cells of Malassez present in the periodontal ligament. Proliferation of epithelial rest cells of Malassez is an essential event in the Pathogenesis of radicular cyst. Objective of the study was to investigate the effect of IL-1 on epithelial cell proliferation which is an important factor in the pathogenesis of radicular cyst. **Methods:** The cyst walls of 20 radicular cysts were removed and were cultured *in vitro* to grow the epithelial cells. The culture were rapidly contaminated and dominated by growth of fibroblasts. Therefore another cell line was used for the experiments. **Results:** The result showed that proliferation was stimulated with increased in a biphasic manner with maximum stimulation at 1.25 ηg/ml, beyond this concentration proliferation was decreased. **Conclusion:** IL-1 had a proliferative effect on epithelial cells at low concentrations which may be playing a role in evoking an inflammatory reaction and stimulating the epithelial cell rests of Malassez to proliferate to form radicular cyst.

Keywords: Interleukin-1, radicular cyst, epithelial cell rests of Malassez, Epithelial cell proliferation

INTRODUCTION

The radicular cyst is an inflammatory cyst that results due to infection extending from the pulp into surrounding periapical tissues. It may be apical radicular cyst or lateral radicular cyst. The epithelial lining is derived from; epithelial cell rests of Malassez in the periodontal ligament. The exact mechanism of development of cyst is debatable.[1] Radicular cyst is common in the jaws due to the proliferation epithelial cells rests of Malassez. It arises at a focus of inflammation in the periodontal ligament caused by pulpal necrosis of an associated tooth. This most commonly occurs periapically, but if the tooth has a lateral root or accessory pulp canal, the cyst may occur laterally.[2] It has been shown that radicular cysts arise from the activated and dividing cell rests of Malassez.[3]

Interleukin-1 (IL-1) is produced by macrophages, monocytes and dentritc cells. Cyst wall contains macrophages and this cytokine is released by them. Both radicular and residual cyst fluids contained IL-1α, and other cytokines and the concentrations of these cytokines were significantly higher in the radicular cyst fluids than those in the residual cysts.[4] The most important role in the growth of these lesion have proinflammatory cytokines TNF-alpha, IL-I, and IL-6 that can be secreted by macrophages, monocytes and other cells of the immune system and can participate in skeletal homeostasis including osteoclasts formation, and bone resorption in the jaws.[5]

Interleukin-1α plays an important role in cyst expansion of the cyst by its direct effect on fibroblast proliferation and bone resorption and by stimulating prostaglansin synthesis in stromal fibroblasts of the cyst capsule. They also showed that several macromolecular bone resorbing factors have been identified as cytokines, including tumour transforming growth factor, IL-1 and TNF account for much of bone resorbing activity attributed to osteoclast activating factor (OAF) produced by mononuclear leucocytes and myeloma cells. Interlkeukin-1 in particular is potent stimulator of prostaglandin and collagenase synthesis by connective tissues. Odontogenic cysts synthesise a macromolecular factor *in vitro* with osteolytic activity which has the charactreristic of cytokine IL-1. They also quoted that stimulation of fibroblast and osteoblast proliferation also suggest IL-1 activity.[6]

The objective of this study was to investigate the effect of IL-1 on epithelial cell proliferation which is an important factor in the pathogenesis of radicular cyst.

MATERIAL AND METHODS

The walls of 20 cysts were collected from patients who were operated upon under either local or general anaesthesia. Each specimen was divided into two parts, one part was fixed in the formal saline and sent for histological examination to confirm that the walls were of Radicular cyst and the other part was placed in sterile Hanks Balance salt solution, containing sodium bicarbonate, penicillin and streptomycin.

The cyst wall was divided into two parts; one part was placed in a sterile test tube containing trypsin and collagenase in phosphate buffer saline. And epithelium was carefully detached from capsule and finely chopped. The fragments were placed in tissue culture flask with 1.5 ml of Minimum Eagles Medium supplemented with 10% foetal calf serum.

The second part of the tissues was minced and the fragments were placed in tissue culture flask with 1.5 ml of MEM supplemented with 10% foetal calf serum. It was found that the cultures were rapidly contaminated with fibroblast and it proved to be impossible to separate the two cell types.

The mammalian epithelial cell line (GPK) was cultured to use for the experiments. The proliferation of epithelial cells was measured by methyline blue staining and by uptake of 3H-thymidine.

Concentrations of Interleukin-1 used were 0.078125, 0.15625, 0.3125, 0.625, 1.25, 2.25, 5.00, and 10.0 ηg/ml.

RESULTS

The IL-1 Proliferation was measured by methylene blue staining method. The result showed that proliferation was stimulated with increased in a biphasic manner with maximum stimulation at 1.25 ηg/ml, beyond this concentration proliferation was decreased (Table-1).

Table-1: Effect of Interleukin-1 using methylene blue staining method

Concentration ηg/ml	Mean	SD
Control t=0	0.191	0.010
Control	0.632	0.017
0.078125	0.649	0.018
0.15625	0.726	0.019
0.3125	0.751	0.011
0.625	0.769	0.011
1.25	0.819	0.032
2.50	0.967	0.033
5.00	0.844	0.018
10.0	0.806	0.016

The IL-1 Proliferation measured 3 H-thymidine uptake. Interleukin-1 stimulated proliferation of the epithelial cells in a biphasic manner with maximum stimulation at 1.25 ηg/ml and proliferation was decreased beyond 1.25 ηg/ml (Table- 2)

Table-2: Effect of Interleukin-1 using 3 H-thymidine uptakes

Concentration ηg/ml	Mean	SD
Control t=0	9972.67	472.22
Control	9517.00	812.96
0.078125	11624.75	360.24
0.15625	11515.50	464.11
0.3125	11910.50	569.44
0.625	12431.25	307.07
1.25	22093.33	3409.76
2.50	15180.50	381.56
5.00	12946.00	283.45
10.0	11581.50	412.63

DISCUSSION

All radicular cysts were cultured to obtain the epithelial cells but this proved to be impossible. Ten out of 20 cysts did have epithelial outgrowth from the explants, however they were rapidly taken over by the fibroblasts and it was not possible for the epithelial cells to be separated. Although attempts were made to remove the fibroblasts, this was not successful. It was shown that there was failure to grow the epithelial cells from radicular cyst. They found that in the radicular cysts cultures, they had more fibroblast growth as compared to dentigerous cysts, keratocysts and residual cysts. They suggested that the reasons why radicular cyst epithelium did not grow well in culture, due to the cytokines from the inflammatory cell infiltrate found in the cyst wall *in vivo* were absent *in vitro*.[7] This is confirmed by this study, which shows the cytokines such as Interleukin-1 do stimulate epithelial cell proliferation in lower concentrations. Proliferation of epithelial cells is an essential factor for the genesis of the radicular cyst and source of growth factors fro epithelial cells may be from periapical granuloma itself which forms before the cyst develops. This is a site of acute inflammation and must contain cytokines such as IL-1, tumour necrosis factor and prostaglandin E_2.

The result in this study has shown that IL-1 stimulated epithelial cells proliferation. This may be an important finding as the IL-1 has been found in the cyst. From this study we can hypothesise that during the initiation and formation of a radicular cyst, IL-I has some driving role in proliferation of epithelial rest cells of Malassez, expansion and growth of the radicular cyst.

CONCLUSION

The results indicated that IL-1 had a proliferative effect on epithelial cells at low concentrations which may be playing a role in evoking an inflammatory reaction and stimulating the epithelial cell rests of Malassez to proliferate to form radicular cyst.

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