ORIGINAL ARTICLE

ROLE OF TUMOUR NECROSIS FACTOR IN PATHOGENESIS OF RADICULAR CYST

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Background: The radicular cyst is very common odontogenic cyst of the jaws, which is usually associated with a tooth with necrotic pulp. 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. The wall of the cyst contains epithelial cells, macrophages, fibroblasts and other cells. TNF is one of inflammatory mediators, which is produced by macrophages and monocytes. This study was carried out to investigate the role of tumour necrosis factor in the pathogenesis of radicular cyst, which is by far the commonest cystic lesion of the jaws. **Methods:** Explants from 20 radicular cysts were cultured in vitro to grow the epithelial cells. However, the cultures were rapidly contaminated with fibroblasts and it was impossible to grow the epithelial cells separately. Therefore, the proliferative effect of Tumour Necrosis Factor (TNF) was studied on mammalian epithelial cells**. Results:** TNF at low concentration had a proliferative effect on the epithelial cells, which may play some role in pathogenesis of radicular cyst. **Conclusion:** TNF stimulated the epithelial cell proliferation in low concentration and inhibit the proliferation in higher concentrations. These two effects may have some implications in the pathogenesis of radicular cyst.

Keywords: Tumour necrosis factor, epithelial rest cells of Malassez, macrophages and radicular cyst

INTRODUCTION

The 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.[1] It has been shown that radicular cysts arise from the activated and dividing cell rests of Malassez.[2] The cyst formation requires proliferation of the epithelial rest cells of Malassez present in the periodontal ligament. Proliferation of epithelial rest cells of Malassez essential event in the formation of radicular cyst.

The great majority of radicular cysts are lined with stratified squamous epithelium with rete pegs.[3] It was shown that epithelial rests are residual cells, which are found in the connective tissue of periodontal ligament, which are still vital, and undergo mitosis.[4] It has been accepted that the lining of stratified squamous epithelium of periapical granuloma or periapical cysts arises from epithelial rests of Malassez.[5]

The endotoxins can activate macrophages, resulting in the production and release of collagenase that been found in several chronic inflammatory lesions including periodontal disease.[6] Tumour necrosis factor (TNF) is a multifunctional cytokine secreted predominantly by monocytes and macrophages. Macrophages are present in the cyst wall of the radicular cyst. The purpose of the study was to investigate if this factor has some driving effect on epithelial cells proliferation, which could be an essential factor in the genesis of the 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. The epithelium was carefully detached from capsule and finely chopped. The fragments were placed in tissue culture flask with 1.5 ml of Minimum Eages Medium (MEM) 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 obtained from Hola, Department of Chemical Pathology, University College and Middlesex School of Medicine.[1] The cells were cultured to use for the experiments. The Proliferation of the epithelial cells was measured by Methyline Blue staining method and by uptake of 3H-thymidine.

The dilutions of TNF tested were 0.390625, 0.78125, 1.5625, 3.125, 6.25, 12.5, 25 and 50 ηg/ml.

RESULTS

The result showed that TNF had proliferative action at the lower concentration. This proliferation occurred in a biphasic manner with maximal stimulation at concentration of 3.125 ηg/ml. Significant stimulation was found at concentration as low as 0.390 ηg/ml. However, the concentration of 25 and 50 ηg/ml, TNF had a cytotoxicity effect (Table-1).

Similar results were obtained as above. The results by this method showed TNF had proliferative action on the cells in a biphasic manner, with maximum stimulation occurring at the concentration of 3.125 ηg/ml. Significant stimulation was found at the lowest concentration used (0.390 ηg/ml). Again, TNF had cytotoxicity effect at 25 and 50 ηg/ml (Table-2).

Table-1: Effect of TNF using methylene blue method

Concentration (ηg/ml)	Mean	SD
Control t=0	0.215	0.012
0.00000	0.655	0.014
0.390625	0.730	0.013
0.78125	0.765	0.021
1.5625	0.782	0.017
3.125	0.852	0.027
6.25	0.790	0.009
12.50	0.658	0.018
25.00	0.571	0.021
50.00	0.507	0.010

Table-2: Effect of TNF using 3-H Thymidine uptake method

Concentration/ml	Mean	SD
Control=0	9098.20	453.22
0.00000	9187.40	496.55
0.390625	10188.00	684.03
0.78125	10673.00	208.98
1.5625	10647,75	352.29
3.125	11651.20	352,29
6.25	10819.00	707.05
12.50	9510.00	386.83
25.00	8836.60	621.88
50.00	7497.80	756.70

DISCUSSION

Although 20 ridiculer cysts were cultured in order to grow the epithelial cells, but this proved to be impossible. Out of 20, 10 cysts did have epithelial outgrowth from the explants, however they were rapidly taken over by fibroblasts. This is because fibroblasts grow much faster than the epithelial cells. After a few days, these rapidly proliferating fibroblasts surrounded the explants to such an extent that it was not possible for epithelial cells to be separated. Although attempts were made to remove the fibroblasts, this was not successful. There was difficulty in growing the epithelial cells from radicular cyst. They found that in radicular cysts culture, they had more fibroblast growth as compared to dentigerous cyst, keratocyst and residual cyst. They suggested that the reason why radicular cyst epithelium did not grow well in culture, due to cytokines from the inflammatory cell infiltrate found in cyst wall in vivo were absent in vitro.[8] It was shown that inflammatory radicular cysts have higher concentrate of TNF-alpha in the cystic fluid in comparison to odontogenic keratocyst.[9] The presence of TNF has some role in the pathogenesis of radicular cyst. This is confirmed by this study, which shows that cytokines like tumour Necrosis factor do stimulate epithelial cell proliferation in lower concentration. Other reasons quoted are:

- They found that many of cysts lacked surface epithelium. This may be during transport (operation theatre to laboratory), pr during tissue processing. Nevertheless, as stated, epithelial discontinuities were in the cysts of the jaws.[10]
- A loss of viability of cell may take place during the time, the specimen is in transit to laboratory
- Failure to remove the rapidly growing fibroblasts from cultures at frequent intervals
- As it was not possible to grow the cyst epithelial cells. It was necessary to use another epithelial cell line.

The epithelial proliferation in periapical granuloma is more frequent with untreated than with treated root canals.[11] Cysts were induced cysts in monkey by leaving the root canals open to the oral environment.[12]

The result in this study has shown that low levels of TNF stimulated epithelial cells proliferation. However high concentration of TNF had inhibitory effect on proliferation of epithelial cells. This may be a fortuitous control mechanism, which may explain why cysts do not continue to grow indefinitely. Even in 3rd world countries where treatment may not be sought, large uncontrolled odontogenic cyst are not found, unlike ameloblastoma and squamous cell carcinoma where the cells are neoplastic and do not appear to require extraneous growth factors.

CONCLUSION

TNF stimulated the epithelial cell proliferation in low concentration and inhibit the proliferation in higher concentrations. These two effects may have some implications in the pathogenesis of radicular cyst. Further studies are recommended to investigate to ensure the proliferation of cyst epithelium, investigate the cellular interaction between fibroblast and the epithelial cells and to test the isolated factors from the fibroblast conditioned media on cyst epithelial cells, and to study the synergy between the various cytokines. This study may also help to understand the pathogenesis of apparently autonomous development follicular and keratocysts.

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