ANTIMICROBIAL EFFICACY OF AZTHREONAM AGAINST PERIODONTAL PATHOGENS

Dr. Shilpi Gupta
Senior Resident
Department of Periodontology
UP RIIMS & R, Saifai, Etawah.

Co-Authors –

1.) **Dr. Vandana A. Pant**,
Professor, Deptt. of Periodontology, BBDCODS, Lucknow.

2.) **Dr. Vivek Govila,**
Dean, Head and Professor, Deptt. of Periodontology, BBDCODS, Lucknow.

3.) **Dr. Vikas Verma,**
Associate Professor, Deptt. of Periodontology, UP RIIMS & R Saifai, Etawah.

4.) **Dr. Saurabh Bhalla**,
Senior Resident, Deptt. of Dentistry, UP RIIMS & R, Saifai, Etawah.

5.) **Dr. Abhishek Gaur**
Senior Lecturer, Saraswati Dental College, Lucknow.

INTRODUCTION- Chronic periodontitis is an infectious disease resulting in inflammation within the supporting tissue of the teeth, progressive attachment loss, and bone loss, and is characterized by periodontal pockets formation and or recession of the gingiva.[1]In recent years, various host response modulation therapies and local drug therapies have been developed to block the pathway responsible for periodontal tissue breakdown..[2]Both non surgical and surgical therapy are applicable in the treatment of periodontal disease. However, mechanical therapy itself may not always reduce or eliminate the infection at the base of the pocket, within the

gingival tissue and in both structures inaccessible to periodontal instruments.[3]To overcome the drawback of this therapy, systemic and local drug delivery of antimicrobials was initiated to enhance non-surgical therapy by serving as an adjunct to scaling and root planing.Adverse effects such as drug toxicity, acquired bacterial resistance, drug interaction, and patient compliance limit the use of systemic antimicrobials.[4]Therefore to over-ride these short comings, local deliveries of antibacterial agents into periodontal pockets have been extensively studied. This mode of drug delivery avoids most of the problems associated with systemic therapy limiting the drug to its target site and hence achieving a much higher concentration.[5]

Various materials are already being used as local drug delivery. A new drug Azthreonam, the first monobactam, has been used extensively in the treatment of a variety of infections caused by gram-negative pathogens in medical field. It includes treatment of urinary tract, lower respiratory tract and intra-abdominal infections, as well as septicemia, endometritis, pelvic cellulitis, skin and skin structure infections due to aerobic gram (-)ve organisms. It is concluded that azthreonam can be used with confidence in the single drug treatment of susceptible aerobic gram (-)ve pathogens. In the treatment of mixed infections, or those of unknown etiology, however, combination therapy is recommended to ensure coverage of gram (+)ve and anaerobic bacteria.[11]

Minimal side effects has been reported so far including- infection site reaction i.e. rash and rarely toxic epidermal necrolysis, diarrhea and drug induced eosinophilia.[12]

So a thought had come to evaluate the efficacy of this drug in the dental field also.

AIMS AND OBJECTIVES –

- To evaluate the efficacy of Azthreonam against *P. intermedia*and *A. actinomycetemcomitans*delivered sub-gingivally in patients with chronic periodontitis.
- To evaluate the effect of Azthreonam in chronic periodontitis clinically, applying clinical parameters like PI, GI and CAL.
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MATERIALS AND METHODS

Patients diagnosed as suffering from chronic periodontitis having probing depth of 5-8mm aged between 25-45 yrs were included in the study. However subjects who are current smokers and pan masala chewers, suffering from any systemic diseases and are allergic to beta lactam antibiotics were excluded.

CLINICAL AND MICROBIOLOGICAL PARAMETERS-

Prior to scaling and root planing each selected site was subjected to assessment of following clinical parameters.

Plaque Index (Sillness and Loe196)

Gingival Index (Loe and Sillness 1963)

Clinical attachment using UNC-15 periodontal probe

Sud-gingival plaque samples

The clinical parameters and microbiological sampling were assessed at day "0" and on "30" day.

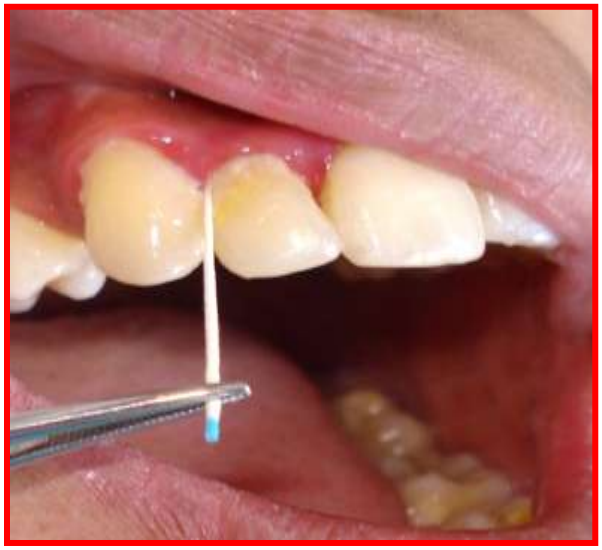
MICROBIOLOGICAL SAMPLING

The bacterial samples were obtained as follows –

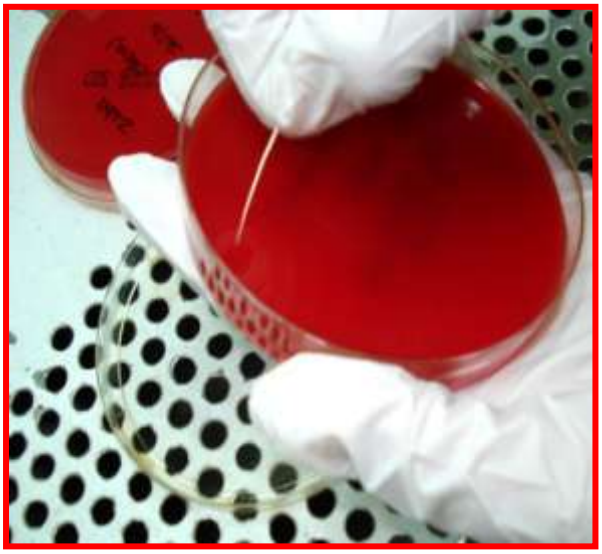
After diagnosis, prior to the start of the treatment, the patients were taken to the Pathology Lab, for obtaining samples. After isolation, the supra gingival plaque was gently removed gently with the help of cotton swab. A sterile paper point (no.30) was introduced in the sulcus as far apically as possible and left undisturbed for 10 seconds. Following this, the paper points were inoculated in duplicates on 5% sheep blood with Columbia agar as base. One plate was incubated at 37^0 C under strict anaerobic conditions by anaerobic gas pack (Himedia) for the growth of anaerobic bacteria while other culture plate were incubated at 37^0 C under aerobic conditions. Both the culture plates were incubated for 48-72 hours. After 72 hours of incubation, sub culture of anaerobic plate was done again on anaerobic blood agar for pure isolation. Final identification was done by Viteck–2 automated identification system using ANC cards. The

growth present on anaerobic culture was compared with aerobic culture. Following organisms were used as controls to check anaerobic conditions-

1) Fusobacterium*nucleatum* - ATCC 25586
2) Bacteroides*vulgatus* – ATCC 8482



Plaque collection with Paper Point for Microbiological Sampling



Fixation of sample

CULTURE CHARACTERISTICS AND IDENTIFICATION OF

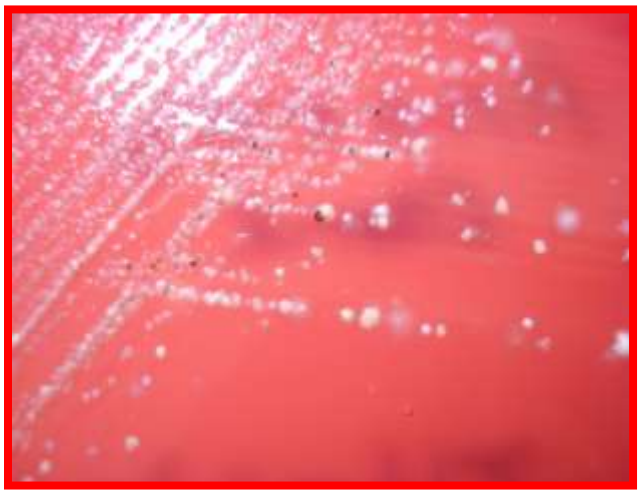
***A. actinomycetemcomitans*–**

- A. a grows slowly on chocolate and blood agar with visible colonies appearing after 48-72 hours.
- Colonies are small, smooth, translucent, non-hemolytic and have slightly irregular edges.
- Fresh clinical isolates are sdherent to the agar and are difficult to identify.

- With prolonged incubation (i.e. 5 to 7 days), colonies may appear as four to six pointed stars.

CULTURE CHARACTERISTICS AND IDENTIFICATION OF *P. intermedia*–

- P. intermedia grows on blood agar with visible colonies appearing after 48-72 hours, trace to heavy growth at 72 hours.
- Colonies are tan to medium brown to black, circular, entire, convex and opaque. Bright pink to brick red flororescence under UV light (365nm).



Anaerobic culture plate

OBSERVATION AND RESULTS-

Clinical and microbiological parameters were noted at baseline and compared between the two groups.

Table 1: Baseline comparison of two groups for clinical parameters (Using Paired "t" test)

SN	Parameter	Group I (n=20)	Group II (n=20)	Significance of

						difference	
		Mean	**SD**	**Mean**	**SD**	**"t"**	**"p"**
1.	PI	1.64	0.17	1.61	0.21	0.497	0.623
2.	GI	1.53	0.19	1.50	0.13	0.607	0.551
3.	CAL	2.93	0.20	3.00	0.20	-1.107	0.277

At baseline, the difference in mean PI, GI, PPD and CAL between two groups was not found to be significant statistically and hence the two groups were matched statistically for clinical parameters ($p>0.05$).

Table 3: Baseline comparison of two groups for Microbial Organisms (Colony count $x10^x$ using Wilcoxon signed rank test)

SN	**Parameter**	**Group I (n=20)**			**Group II (n=20)**			**Significance of difference**	
		Mean	**SD**	**Md**	**Mean**	**SD**	**Md**	**"z"**	**"p"**
1.	*A.actinomycetem-comitans (Aa)*	4.20	1.54	5	4.45	1.23	5	0.550	0.582
2.	*P.intermedia (Pi)*	5.00	0.73	5	5.00	0.73	5	0.059	0.953

At baseline, mean colony counts in both the groups were matched for both the microbes showing no statistically significant difference between two groups ($p>0.05$).

PART I: BETWEEN GROUP COMPARISONS

Plaque Index

Table 4: Comparison of Plaque Index between two groups at different time intervals

SN	Time intervals	Group I (n=20)		Group II (n=20)		Significance of difference	
		Mean	SD	Mean	SD	"t"	"p"
1.	Baseline	1.64	0.17	1.61	0.21	0.497	0.623
2.	Day 30	1.08	0.31	0.87	0.23	2.433	0.021

Statistically no significant difference between two groups was observed at baseline, however, at day 30 mean PI was significantly lower in Group II as compared to Group I ($p<0.001$).

Gingival Index

Table 5: Comparison of Gingival index between two groups at different time intervals

SN	Time intervals	Group I (n=20)		Group II (n=20)		Significance of difference	
		Mean	SD	Mean	SD	"t"	"p"
1.	Baseline	1.53	0.19	1.50	0.13	0.607	0.551
2.	Day 30	1.03	0.28	0.83	0.33	2.067	0.048

Statistically no significant difference between two groups was observed at baseline, however, at day 30, mean GI was significantly lower in Group II as compared to Group I ($p<0.05$).

Clinical Attachment Level

Table 7: Comparison of Clinical Attachment Level between two groups at different time intervals

SN	Time intervals	Group I (n=20)		Group II (n=20)		Significance of difference	
		Mean	SD	Mean	SD	"t"	"p"
1.	Baseline	2.93	0.20	3.00	0.20	-1.107	0.277
2.	Day 30	2.74	0.26	2.11	0.31	6.964	<0.001

Statistically no significant difference between two groups was observed at baseline, however, at day 30, mean CAL was significantly lower in Group II as compared to Group I ($p<0.001$).

MICROBIOLOGICAL OBSERVATIONS-

Table 8: Comparison of Mean Colony Count of *A. actinomycetemcomitans (Aa)* between two groups at different time intervals(Values are exponents to 10)

SN	Time intervals	Group I (n=20)			Group II (n=20)			Significance of difference (Wilcoxon signed rank test)	
		Mean	SD	Md	Mean	SD	Md	z	p
1.	Baseline	4.20	1.54	5	4.45	1.23	5	0.550	0.582
2.	Day 30	3.31	1.05	4	2.73	0.93	3	0.559	0.582

At baseline mean colony count was equal in both the groups, however, at all the subsequent time intervals, mean colony count was lower in Group II as compared to Group I but the difference was not statistically significant ($p>0.05$) at any time intervals.

Table 9: Comparison of Mean Colony Count of *P. intermedia(Pi)* between two groups at different time intervals (Values are exponents to 10)

SN	Time intervals	Group I (n=20)			Group II (n=20)			Significance of difference (Wilcoxon signed rank test)	
		Mean	SD	Md	Mean	SD	Md	z	P
1.	Baseline	5.00	0.73	5	5.00	0.73	5	0.059	0.953
2.	Day 30	3.82	0.59	4	2.94	0.71	3	2.873	0.003

At baseline mean colony count was equal in both the groups, however, at all the subsequent time intervals, mean colony count was lower in Group II as compared to Group I.

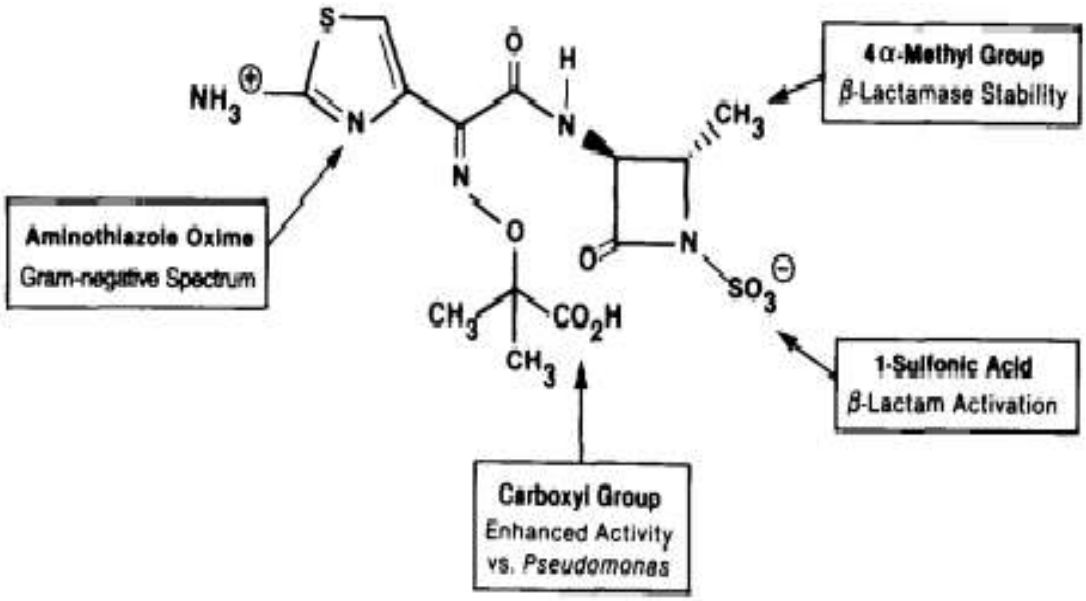
All the groups showed statistically significant reduction in relation to Plaque index, Gingival index, Clinical attachment level and Microbial count.

DISCUSSION-

Periodontitis, a chronic inflammatory disease, begins with a microbial infection, followed by a host-mediated destruction of soft tissue caused by hyperactivated or primed leukocytes and the generation of cytokines, eicosanoids, and matrix metalloproteinases that cause clinically significant connective tissue and bone destruction[13]. *Aggregatibacteractinomycetemcomitans* and*Prevotellaintermedia*are implicated in the pathogenesis of periodontitis . These bacteria are able to produce virulence factors that act locally within the sulcus, and result in tissue destruction[14,15]. Invasiveness and ubiquitous intraoral distribution may be the main reasons for

the reported observation of rather poor results after conventional, merely mechanical treatment of periodontal infections[16]. Thus, adjunctive systemic antibiotics have been advocated in order to suppress the organism in subgingival plaque[17].

Azthreonam (sq,26,776) is a new synthetic monocycle β lactam[6,7]antimicrobial agent hich is originally isolated from cromobacteriumviolaceum. It was approved by the U.S Food and drug Administration (FDA) in 1986. Azthreonam is sensitive down to 100 $ngml^{-1}$ produced by a range of bacterial species.[8]



Structural activity of Aztreonam

The sulfonic acid side chain activates the β-lactam ring and the carboxyl group enhances the activity against certain types of bacteria.[10]Its molecular structure differs from that of older β-lactam antibiotics in the simplicity of the nucleus. This difference makes it suitable for patient ho are allergic of penicillin.[7]It is more potent than moxa-lactam, cefoperazone, cefamandole, cefoxitin or amikacin.It is about 50% bound to human serum protein.It has a high degree of β-lactamase stability and excellent activity against aerobic and gram (-)ve bacteria.

It has a similar effect to that of penicillin in such that it binds to penicillin binding protein-3 inhibiting the synthesis of cell wall of bacteria.[9]

In brief ; upon entering the membrane of the gram (-)ve bacteria, it binds to penicillin binding protein-3 and causes formation of elongated structures in the cell wall causing restriction to cell division and induces cell death. What favors monobactam over penicillin is that monobactam is not a substrate for β-lactamases. That makes it resistant to hydrolysis caused by this enzyme and effective against bacteria that produces it.[7]

The β-lactam ring in the middle gives it the characteristics of a β-lactam antibiotic. The side chains gives the molecule other characteristics such as four α-methyl group which gives and offers stability against β-lactamase.[7]

In our study the adjunctive use of azthreonam when administered sub gingivally has shown a better results in terms of plaque index, gingival index and clinical attachment level as compared to scaling and root planing alone in chronic periodontitis subjects. However, a longitudinal studies should be carried out in future for better corroboration of results.

FUTURE DEVELOPMENT-

Monobactams as all kinds of β-lactam antibiotics faces the risk of multi-resistant bacteria. This requires continues researching for new and more effective antibiotics. This might be achieved by exploiting new methods of improving the biosynthesis of the β-lactams to produce new antibiotics with improved properties. Improving the current monobactams is also one of the goals for future development.

CONCLUSION-

The intensive research that started after the discovery of penicillin led to thediscoveries of other important antibiotics.Azthreonam which is a synthetic monobactam is one of the most important monobactam available today. Its importance is due to its high effectiveness, low toxicity and good pharmacokinetic properties.

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