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Comparison of the pharmacokinetic profiles of two locally administered doxycycline gels in crevicular fluid and saliva

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Abstract

Background: Controlled-release delivery systems enable the clinician to extend the half-life period of locally administered antibiotics in gingival crevicular fluid (GCF) significantly. The aim of this split-mouth study was to compare the pharmacokinetic profile of two different doxycycline gels (DOXY and ATRI) for topical subgingival application. Pharmacokinetics of both doxycycline gels were analyzed in GCF and saliva.

Methods: In 10 patients suffering from severe periodontitis, 10 pairs of contralateral defects (pocket depth $\geqslant$5 mm/bleeding on probing or $\geqslant$6 mm) were randomly assigned either to the first application of DOXY or ATRI. Fourteen days after the topical application of the first antibiotic gel, the application of the second gel in the contralateral defect took place. Clinical examinations at baseline showed no significant differences between sites treated with DOXY and ATRI. Samples of GCF and saliva were drawn baseline, 2, 5 and 24 h after application, 2, 3, 4, 7, 9 and 11 days after application. Separation and quantitative measurement of both doxycycline-gels was performed with HPLC- and UV-detection at $\lambda = 260$ nm.

Results: In saliva specimens, time-dependent changes of mean doxycycline concentration were almost identical for both doxycycline-gels and declined from a maximum 2 h after application (ATRI: 6653.90 $\pm$ 3096.14 μg/ml; DOXY: 5386.60 $\pm$ 1542.02 μg/ml [arithmetic mean $\pm$ SEM]) to zero values 9 days after application. In crevicular fluid specimens, sites treated with ATRI exhibited a faster decrease of mean doxycycline concentration (1085.30, 264.00, 273.94, and 258.00 μg/ml measured 2, 5, 24, and 48 h after application) than sites treated with DOXY (1388.38, 1300.40, 803.73, and 235.10 μg/ml). The faster decrease of ATRI compared with DOXY could not be proved to be statistically significantly different.

Conclusions: Both doxycycline gels showed pharmacokinetics of controlled-release delivery systems.

Key words: controlled-release device/biodegradable; doxycycline/doxycycline-free amine, pharmacokinetics; topical antibiotic delivery systems/biodegradable

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Introduction

Successful therapy of periodontitis essentially requires the reduction of microbial levels of the bacteria that are responsible for the periodontal infection within the oral cavity (Drisko 1996, Van Winkelhoff et al. 1996). To eliminate bacterial infections, antibiotics are administered either locally or systemically (Slots & Rams 1990). Periodontal infections are characterized by the accumulation of bacterial pathogens mainly within periodontal pockets. Therefore, local delivery of antibotic agents directly into the periodontal pocket as a possible treatment strategy has been investigated by numerous authors (Goodson et al. 1983, Addy & Fugit 1989, Minabe 1989, Tonetti et al. 1990, Unsal et al. 1994). The main advantage of this local route of application is the possibility to increase the concentration of the antimicrobial agent in the pocket and thereby in the gingival crevicular fluid (GCF), whereas the systemic concentration of the antibiotic can be

kept comparatively low at the same time (Lie et al. 1998). To achieve this, different systems have been developed to deliver a variety of antimicrobial substances to the bacterial pathogens within periodontal pockets (Greenstein & Polson 1998).

Antibiotic agents that are administered in the form of a liquid or gel have been shown to be cleared ultimately from the pocket by normal crevicular fluid flow. Oosterwaal et al. (1990) demonstrated that the half-life of a fluorescein gel deposited into periodontal pockets was 12.5 min (Oosterwaal et al. 1990). In order to overcome the problems associated with crevicular fluid flow clearance, Goodson et al. (1983) loaded 0.5 mm diameter extruded ethylene vinyl acetate fibers with 25% tetracycline HCl. Following fiber placement into periodontal pockets, GCF levels of tetracycline were shown to be sustained at levels in excess of 1500 μg/ml for 10 days. Once the fiber was removed, the half-life of the residual tetracycline was 4.5 h. Serum tetracycline during such treatment was close to undetectable levels (0.1 μg/ml) for the entire 10-day period that the fibers were in place (Goodson et al. 1983).

Stoller et al. 1998 described the pharmacokinetic properties of a biodegradable controlled-release delivery system containing doxycycline hyclate (DH) (8.5% w/w) (Atridox). The components of the system were contained in two separate syringes that have to be mixed just prior to use. One syringe contained DH and the other the polymer of polylactic acid, poly(D–L-lactide) dissolved in a biocompatible carrier, *N*-methyl-2-pyrrolidone (NMP). The constituted product had a concentration of 8.5% w/w DH. When the DH was injected into the periodontal pocket, the NMP carrier that was highly miscible in an aqueous environment was replaced by water, which caused the polymer to return to its solid state. As the polymer degraded, the doxycycline was released into the pocket environment (Stoller et al. 1998). Stoller and coworkers only described the pharmacokinetic properties of Atridox-Gel that was retained with the pockets with the help of a periodontal dressing. Pharmacokinetics of this system without the application of a periodontal dressing have not been described yet.

The aim of this split-mouth study was to compare the pharmacokinetic profile of two different doxycycline gels (DOXY (Vivadent Ivoclar, Schaan, Liechtenstein) and ATRI (Atridox, CollaGenex Pharmaceuticals Inc., Newtown, PA, USA)) for topical subgingival application. Pharmacokinetics of both doxycycline-gels were analyzed in GCF and saliva. The clinical efficacy of both doxycycline gels in terms of attachment gain as well as their pharmacokinetics have already been investigated separately in previous studies (Stoller et al. 1998, Eickholz et al. 2002, Kim et al. 2002).

Materials and Methods

Patients

The study population consisted of 10 patients (seven males, three females) with a mean age of 56.04 $\pm$ 11.15 years. Inclusion criteria for participation in the study were two contralateral single-rooted teeth with persisting or recurring pockets of similar probing depth (PD) and shape (PDs $\geqslant$ 5 mm and bleeding on probing (BOP) or PD $\geqslant$ 6 mm) after thorough mechanical treatment (non-surgical or surgical) of cases of severe chronic periodontitis. Patients with generalized persisting deep periodontal pockets were excluded from the study. Patients were recruited during regular supportive maintenance. Time elapsed after completion of active periodontal treatment varied from 6 months (minimum) to 4 years (maximum). If PD measurements during routine examinations revealed at least two contralateral teeth with PD $\geqslant$ 5 mm and BOP the patients were informed about the procedures, risks, and benefits of the study and informed written consent was obtained. The time between the recall appointments varied between 2 months (shortest interval) and 6 months (longest interval). The study was approved by the Institutional Review Board for Human Studies of the University of Heidelberg.

Randomization

The 10 pairs of contralateral defects (pocket depth $\geqslant$ 5 mm/BOP or $\geqslant$ 6 mm) were randomly assigned either to the first application of DOXY or ATRI. Fourteen days after the topical application of the first antibiotic gel, the application of the second gel in the contralateral defect took place.

Pharmacokinetic evaluation

GCF samples were obtained from test sites at baseline and throughout the course of the study. Prior to collecting GCF, the teeth were isolated, dried, and supragingival plaque was removed. Filter paper strips (Periopaper, Oraflow Inc., Plainview, NY, USA) were then placed subgingivally for a period long enough to collect 0.15–0.70 μl of GCF. Because the amount of GCF that was produced within a defined interval differed from collection site to collection site, a standard time for GCF collection was not used. GCF was collected from the site where the gel had been applied (test GCF sample) and also from the opposite site of the same periodontium (control GCF sample). Upon removal, the quantity of fluid on the strip was recorded. The absolute quantification of GCF on the filter paper strip was performed using an electronic device (Periotron 8000, Oraflow Inc.).

After chewing for 10 min on a paraffin block (Vivacare, Ivoclar/Vivadent AG), 5 ml samples of saliva were collected. Saliva samples were sent to the laboratory for further investigation. Then, GCF and saliva samples were collected at 2 and 5 h, and 1, 2, 3, 4, 7, 9, and 11 days after subgingival application of the two doxycycline gels.

Clinical examination and therapy

Immediately before the baseline sampling of GCF, plaque index (PlI) and gingival index (GI) (Löe 1967) were scored as well as PD and vertical attachment levels (CAL-V) were measured to the nearest 0.5 mm using a periodontal probe (PCPUNC 15, Hu-Friedy, Chicago, IL, USA) at six sites of the respective test teeth. Thereafter, the test teeth were kept dry using cotton rolls and the tip of the DOXY/ATRI carpules was inserted into the pocket and DOXY/ATRI gel pressed into the pocket (Eickholz et al. 2002) (DOXY: 14% doxycycline-free amine in a polyethylene glycol-lactid/glycolid copolymer gel. The carpules were weighed to the nearest 1.0 mg before and after instillation of DOXY/ATRI gel to assess the instilled amount of gel using a precision balance. For both gels, the excess amount of material that had flown out of the pocket after the instillation was collected with the help of a periodontal curette and laid on the balance together with the emptied

carpule (Sartorius research R300S, Sartorius GmbH, Göttingen, Germany). There was no additional mechanical disruption of the subgingival biofilm before or immediately after the application of both doxycycline-gels.

Laboratory assays

The GCF collected on the filter strips was analyzed for doxycycline concentration utilizing reverse-phase high-performance liquid chromatography (HPLC; pump: L-6200 A intelligent [Merck Hitachi, Mountain View, CA, USA]) and UV-detection ($\lambda = 260$ nm; detector: L-7455 [Merck Hitachi]). For the HPLC analysis, a symmetry shield RP 8 column (5 μm 3.0 $\times$ 150 mm [Waters Omega Engineering Inc., Stamford, CT, USA]) was used. The injected volume was 100 μl, with water–acetronitrile–70% $HClO_4$ (699:298.5:2.5 v/v), Na_2EDTA (0.6 mmol/l) and oxalate (5 mmol/l) as a mobile phase. The flow rate was adjusted at 0.7 ml/min.

The results of this assay were expressed in μg of doxycycline per ml of GCF. The concentration of doxycycline in saliva was also determined by HPLC. The applied method has been described in detail in a previous study (Kim et al. 2002).

Results

The clinical parameters of the sites treated subgingivally with the two doxycycline-gels are given in Table 2. The mean amounts of doxycycline gel administered subgingivally were 88 mg (ATRI) and 27 mg (DOXY) (Table 1).

Precision and linearity

The quantitative analysis of doxycycline exhibited an inter-assay variability between 2.69% and 3.66% (depending upon concentration). For the intra-assay precision, the coefficient of variability ranged between 1.31% and 1.46%. Between 50 and 1000 μg/ml, a linear relationship between estimated and real concentration could be found ($r = 0.998$).

Pharmacokinetics

The time-dependent changes of the mean doxycycline concentrations in GCF and saliva are given in Tables 2–4 and illustrated in Figs 1–3. The release of doxycycline peaked at 2 h both in GCF (ATRI: 1085.30 $\pm$ 227.55 μg/ml; DOXY: 1388.38 $\pm$ 371.40 μg/ml) and in saliva (ATRI: 6653.90 $\pm$ 3096.14 μg/ml, DOXY: 5386.60 $\pm$ 1542.02 μg/ml). Within the first 2 days after application, GCF samples that were obtained from control sites also exhibited doxycycline concentrations that can be considered as clinically effective (Table 3, Fig. 2). Clinical efficacy at control and test sites was defined as a concentration that was higher than the minimal inhibitory concentration $(MIC)_{50}$ for *Actinobacillus actinomycetemcomitans* in biofilms. The faster decrease of ATRI in GCF samples compared with DOXY could not be proved to be statistically significantly different.

Table 1. Clinical parameters and applied amount of doxycycline

Patient no.	Tooth a = ATRI d = DOXY	PD (mm)	CAL-V (mm)	GI	PII	Amount of applied doxycycline gel (g)
1	a: 14	6	8.5	2	2	0.0612
	d: 25	6	7.5	2	0	0.0726
2	a: 15	7.5	11.5	0	0	0.1996
	d: 24	7	9	2	0	0.0334
3	a: 14	6	7.5	2	0	0.2836
	d: 25	5	6.5	2	0	0.0115
4	a: 22	5	8.5	2	2	0.0161
	d: 13	5	6.5	2	0	0.0464
5	a: 24	7.5	8.5	2	0	0.0846
	d: 14	7	12	2	0	0.0099
6	a: 14	8	11	0	2	0.0494
	d: 23	7	7.5	2	2	0.0113
7	a: 15	6	7	2	3	0.0337
	d: 21	9	13	2	1	0.0105
8	a: 14	8	7.5	2	1	0.0876
	d: 24	6	6	2	2	0.0101
9	a: 14	7.5	8	2	0	0.0386
	d: 25	8	7.5	2	0	0.0432
10	a: 13	6	5	2	0	0.0206
	d: 21	5	4	2	0	0.022
arithmetic mean	a:	6.75	8.30	1.60	1.00	0.088
	d:	6.50	7.95	2.00	0.50	0.027
SD	a:	1.06	1.87	0.84	1.16	0.087
	d:	1.35	2.73	0.00	0.85	0.021

CAL-V, vertical attachment level; PD, probing depth; GI, gingival index; PII, plaque index, SD, standard deviation.

Table 2. Doxycycline concentration in GCF

Evaluation time	*n*	Arithmetic mean (μg/ml)		SEM (μg/ml)	
		ATRI	DOXY	ATRI	DOXY
baseline	10	0.00	0.00	0.00	0.00
2 h	10	1085.30	1388.38	227.55	371.40
5 h	10	264.00	1300.40	68.58	433.90
day 2	10	273.94	803.73	76.42	201.91
day 3	10	258.00	235.10	182.27	86.33
day 4	10	116.85	229.80	64.15	91.22
day 5	10	105.96	132.20	43.75	62.36
day 8	10	114.13	33.30	63.73	11.74
day 10	10	46.73	72.30	18.49	41.34
day 12	10	8.25	19.00	5.61	7.93

SEM, standard error of the mean.

Discussion

Due to the infective nature of periodontitis, different antimicrobial agents have been used to support mechanical

Table 3. Doxycycline concentration in GCF (control)

Evaluation time	*n*	Arithmetic mean (μg/ml)		SEM (μg/ml)	
		ATRI	DOXY	ATRI	DOXY
baseline	10	0.00	0.00	0.00	0.00
2 h	10	251.47	264.43	106.21	165.91
5 h	10	62.90	120.63	24.41	36.01
day 2	10	46.32	89.81	26.96	62.14
day 3	10	34.46	19.10	21.96	6.36
day 4	10	12.70	14.38	6.11	5.98
day 5	10	13.60	12.28	7.47	6.98
day 8	10	4.20	9.30	2.80	4.75
day 10	10	0.00	9.67	0.00	5.17
day 12	10	0.00	10.76	0.00	5.57

SEM, standard error of the mean.

Table 4. Doxycycline concentration in saliva

Evaluation time	*n*	Arithmetic mean (μg/ml)		SEM (μg/ml)	
		ATRI	DOXY	ATRI	DOXY
baseline	10	0.00	0.00	0.00	0.00
2 h	10	6653.90	5386.60	3096.14	1542.02
5 h	10	1469.30	1995.30	515.43	585.45
day 2	10	343.10	403.00	122.04	169.39
day 3	10	186.40	145.50	77.93	68.24
day 4	10	66.50	86.20	37.97	44.04
day 5	10	28.30	33.10	21.45	19.17
day 8	10	7.80	17.30	7.80	14.11
day 10	10	0.00	11.10	0.00	11.10
day 12	10	0.00	0.00	0.00	0.00

SEM, standard error of the mean.

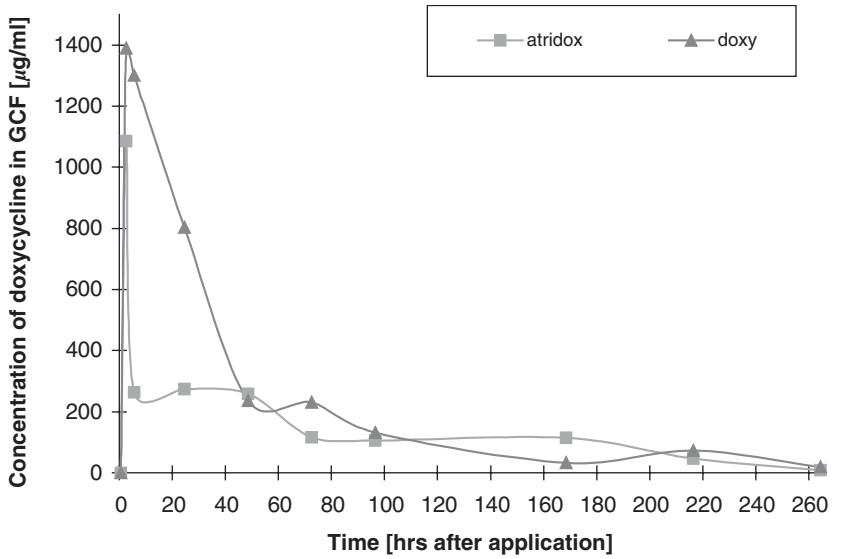

Fig. 1. Time-dependent changes of doxycycline in GCF (test site) after application of ATRI and DOXY gel.

debridement (Löe 1967, Hellden et al. 1979, Goodson et al. 1983, Joyston-Bechal et al. 1984). Up to now, there are mainly five antimicrobial agents used in local delivery systems: tetracycline hydrochloride, doxycycline, minocycline, metronidazole and chlorhexidine (Greenstein & Polson 1998). The local delivery systems that were applied in this study contained doxycycline as effective antimicrobial agent. Doxycycline is a synthetic tetracycline compound whose main advantages over tetracycline hydrochloride are increased oral resorption, prolonged serum half-life, and decreased gastrointestinal side-effects (Pascale et al. 1986).

Calculation of the mean doxycycline concentration in GCF and saliva showed significant inter-individual variability. The release of doxycycline peaked at 2 h both in GCF and in saliva. Clinical efficacy at both control and test sites was defined as a concentration that was higher than the MIC_{50} for *A. actinomycetemcomitans* in biofilms. Within the first 2 days after application, GCF samples that were obtained from control sites, also exhibited doxycycline concentrations that can be considered as clinically effective. In the GCF samples taken from the test sites, the concentration of doxycycline was higher than the MIC_{50} for *A. actinomycetemcomitans* in biofilms for a period of 10 days after the application of both doxycycline gels (Table 2, Fig. 1) (Slots & Rams 1990, Eick et al. 2002).

It is well known that a subgingival biofilm represents a well-organized structure capable of resisting antibiotic penetration and that with the disruption of this biofilm prior to topical application of a local antibiotic optimal antibiotic efficacy can be achieved (Tonetti 1997). Since the goal of this study was the comparison of the pharmacokinetic profiles of two different doxycycline gels, the clinical situation of topical application of the antibiotic gels had to be standardized as much as possible. Therefore any manipulations within the periodontal pocket were excluded from the treatment protocol. To assess the clinical efficacy of the doxycycline concentrations that could be measured in saliva and GCF specimens, we always considered the MICs for periodontal pathogens in biofilms as landmarks. Suspected periodontal pathogens have a susceptibility to doxycycline ranging from 0.1 to 2.0 μg/ml (Mombelli & van Winkelhoff 2001). In biofilms, the necessary MICs are about 50 times higher (Anwar et al. 1992, Cargill et al. 1992, Brown & Gilbet 1993, Vorachit et al. 1993). Thus, a single dose of the investigated doxycycline gels (ATRI and DOXY) provides sufficient antimicrobial activity up to 10 days in the most cases and fulfills the conditions for a ''controlled-release device''. The release rate is not only

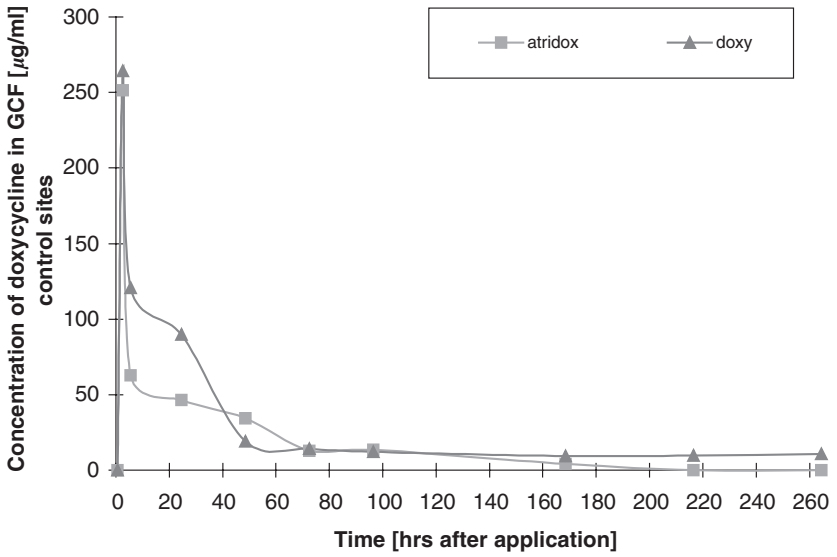

Fig. 2. Time-dependent changes of doxycycline in GCF (control site) after application of ATRI and DOXY gel.

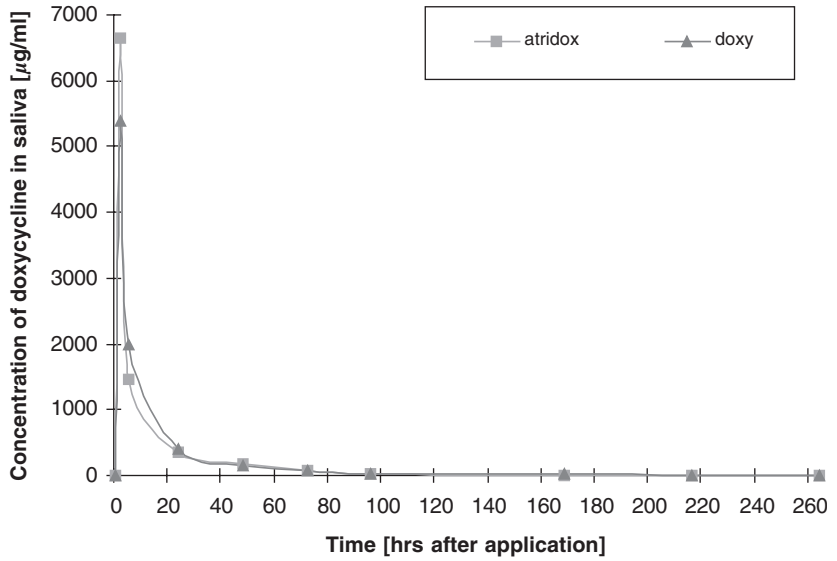

Fig. 3. Time-dependent changes of doxycycline in saliva after application of ATRI and DOXY gel.

determined by the local drug concentration, but is also influenced by the degradation rate and the barrier properties of the drug carrier. In the local delivery systems that were applied in this study, the initial drug concentrations in the carrier were 8.5% (ATRI) and 14% (DOXY) per weight doxycycline-free amine. Although this available drug concentration in the carrier is lower than that of tetracycline HCl used at 25% loading in tetracycline fibers (Actisite, Proctor and Gamble, Cincinnati, OH, USA), the MIC of doxycycline for suspected periodontal pathogens was exceeded for at least 10 days after application of the gel.

These conclusions are supported by several clinical studies that could prove the efficacy of locally administered doxycycline in terms of gain of attachment (Eickholz et al. 1993, Polson et al. 1997, Garrett et al. 1999). In two multicenter clinical trials designed to study the efficacy of this doxycycline containing drug delivery system, reduction of PD of 1.3 mm and clinical attachment gain of 0.8 mm were obtained at month 9 (Garrett et al. 1999). Our results confirm the high inter-individual variability of doxycycline concentration in GCF after local delivery of this antibiotic that has been observed by other authors before (ATRI) (Stoller et al. 1998). One of the main methodological differences between the cited study by Stoller et al. and our own research was that Stoller et al. tried to enhance retention of ATRI within the periodontal pocket with the help of periodontal dressings. This difference could explain the observation that we found a doxycycline concentration in saliva during the first 3 days after application that was 100–1000 times higher than in the study of Stoller et al.

The mean amounts of doxycycline-gel administered subgingivally were 88 mg (ATRI) and 27 mg (DOXY). Since the contralateral defects were of similar size and shape, the fact that substantially more ATRI gel than DOXY gel was administered can only be explained with the considerably lower viscosity of ATRI gel compared with DOXY gel at the time of application.

Within the limitations of this study the following conclusions may be drawn:

(i) With the local delivery systems for doxycycline that were investigated in this study (ATRI and DOXY), it was possible to maintain mean doxycycline levels within the GCF that exceeded 46.73 µg/ml for at least 10 days. Thus, both biodegradable doxycycline gels may be classified as ''controlled-release devices''.

(ii) The antibiotic effect seems to be limited mainly to the subgingival sites of application of the doxycycline gels (ATRI and DOXY).

(iii) The doxycycline-gels (ATRI and DOXY) possess the pharmacokinetic and clinical properties to deliver efficacious levels of antibiotics to the periodontal pocket and to maintain these levels for at least 1 week without the need of further drug retention by a periodontal dressing.

(iv) The time that was needed to collect a GCF volume sufficient for a precise laboratory analysis showed a continuous increase from baseline to 12 days after application of both doxycycline gels (Figs 4 and 5). This observation can be interpreted as a sign of a decreasing production of GCF, which itself is a clinical indicator for reduction of inflammation.

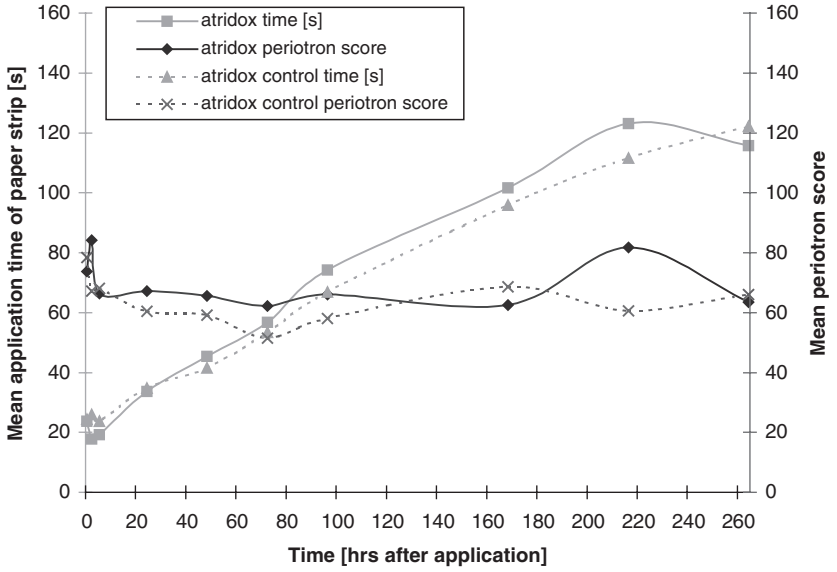

Fig. 4. Mean application time of paper strips/mean periotron score: time-dependent changes for ATRI gel.

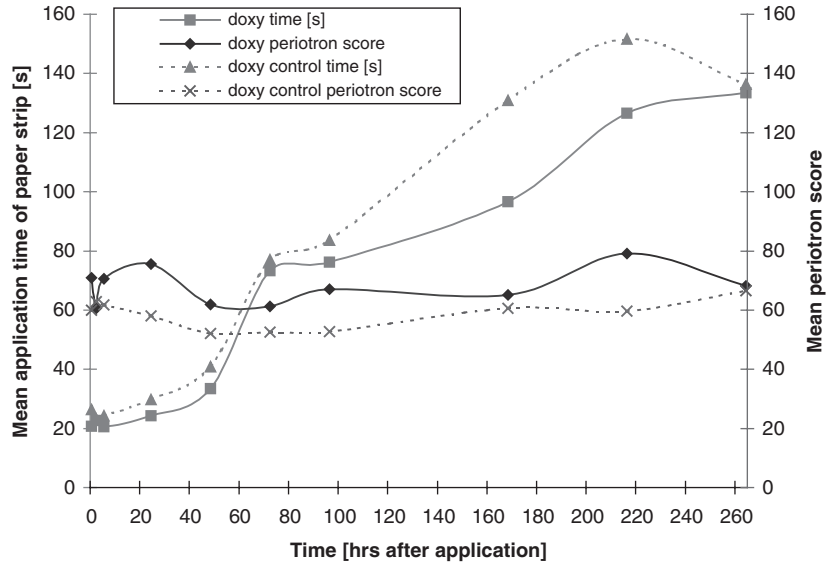

Fig. 5. Mean application time of paper strips/mean periotron score: time-dependent changes for DOXY gel.

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