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Efficacy assessment of local doxycycline treatment in periodontal patients using multivariate chemometric approach

Liljana Bogdanovska [a,*], Ana Poceva Panovska [a], Natalija Nakov [a], Marija Zafirova [a], Mirjana Popovska [b], Aneta Dimitrovska [a], Rumenka Petkovska [a]

[a] Institute of Applied Chemistry and Pharmaceutical Analysis, Faculty of Pharmacy, University of Ss. Cyril and Methodius, Mother Theresa 47, 1000 Skopje, Former Yugoslav Republic of Macedonia
[b] Department of Oral Pathology and Periodontology, Faculty of Dentistry, University of Ss. Cyril and Methodius, Mother Theresa 47, 1000 Skopje, Former Yugoslav Republic of Macedonia

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ABSTRACT

The aim of our study was application of chemometric algorithms for multivariate data analysis in efficacy assessment of the local periodontal treatment with doxycycline (DOX). Treatment efficacy was evaluated by monitoring inflammatory biomarkers in gingival crevicular fluid (GCF) samples and clinical indices before and after the local treatment as well as by determination of DOX concentration in GCF after the local treatment.
The experimental values from these determinations were submitted to several chemometric algorithms: principal component analysis (PCA), partial least square discriminant analysis (PLS-DA) and orthogonal projection to latent structures-discriminant analysis (OPLS-DA). The data structure and the mutual relations of the selected variables were thoroughly investigated by PCA. The PLS-DA model identified variables responsible for discrimination of classes of data, before and after DOX treatment. The OPLS-DA model compared the efficacy of the two commonly used medications in periodontal treatment, chlorhexidine (CHX) and DOX, at the same time providing insight in their mechanism of action. The obtained results indicate that application of multivariate chemometric algorithms can be used as a valuable approach for assessment of treatment efficacy.

1. Introduction

The efficacy of a given pharmacological treatment is evaluated against a set of predefined variables for which sufficient evidence exists that they can provide a valid and a reliable measure of treatment's benefits. This evaluation results in generation of complex data conventionally analyzed by univariate statistical approach where a single variable is treated independently to the other variables (Rasmussen et al., 2010). Univariate approach could miss important "weak" correlations or could overestimate the importance of certain variables (Francesci et al., 2012). In contrast, application of multivariate chemometric approach for evaluation of treatment efficacy overcomes the limitations of the traditional approach, thus providing possibility of reliable exploration the complex data sets with multiple variables, missing data even with small number of observations (Helmy et al., 2012; Jimenez et al., 2013).

Multivariate chemometric approach comprises of application of versatile algorithms for multivariate data analysis which can be classified in two general categories: unsupervised and supervised. Unsupervised chemometric algorithms such as principal component analysis (PCA) or hierarchical cluster analysis (HCA) are being used for exploratory data analysis (finding patterns of similarity or outliers in complex data sets). Supervised algorithms such as partial least squares (PLS), partial least squares-discriminant analysis (PLS-DA), orthogonal projection to latent structures (OPLS) or orthogonal projection to latent structures-discriminant analysis (OPLS-DA) tempt to determine variables that can discriminate between known classes of data (for example, data before and after treatment) allowing unknown samples to be correctly classified. They require a priori knowledge of the class membership to develop the model (Ivosev et al., 2008; Cova et al., 2013; Gromski et al., 2015). All these algorithms are indispensable as they provide reliable, validated and robust tools for evaluation of treatment efficacy (Durcekova et al., 2011; Wang et al., 2012, Tomita et al., 2014).

Periodontal disease is a chronic inflammatory disorder affecting the tissues supporting the teeth. The main therapeutic approach consists of mechanical treatment of root surfaces followed by local application of antibiotics or antiseptics. Evaluation of treatment efficacy is performed

Abbreviations: DOX, doxycycline; GCF, gingival crevicular fluid; PCA, principal component analysis; PLS-DA, partial least square discriminant analysis; OPLS-DA, orthogonal partial least square-discriminant analysis; CHX, chlorhexidine; HCA, hierarchical cluster analysis; PLS, partial least squares; OPLS, orthogonal partial least squares; PD, pocket depth; CAL, clinical attachment loss; GI, index of gingival inflammation; ALP, alkaline phosphatase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; VIP, variable influence on projection.

* Corresponding author at: Faculty of Pharmacy, Univ. Ss Cyril and Methodius, Mother Theresa 47, POB 36, 1000 Skopje, Former Yugoslav Republic of Macedonia.
E-mail address: lbogdanovska@ff.ukim.edu.mk (L. Bogdanovska).

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Table 1
Data set containing the results obtained by determination of activity/concentration of inflammatory biomarkers in GCF and clinical indices in the control group.

No	ALP (IU/L)	LDH (IU/L)	AST (IU/L)	IL-1β (pg mL^{-1})	TNF-α (pg mL^{-1})	GI (1–3)	CAL (mm)	PD (mm)
1	37.3	263.1	26.7	27.3	35.6	2	2	2
2	24.9	251.0	6.7	31.2	15.6	1	1	1
3	7.3	89.5	10.0	15.6	12.4	1	2	1
4	14.9	36.4	26.7	17.4	18.0	1	1	1
5	29.7	99.5	13.3	31.2	18.7	1	2	1
6	27.3	111.0	13.3	15.6	20.4	1	1	1
7	29.6	384.9	16.7	17.4	21.8	1	1	1
8	17.6	99.9	20.0	35.9	18.5	1	1	1
9	19.0	389.6	16.7	29.0	15.6	1	1	1

using three different types of parameters, namely clinical indices (periodontal pocket depth (PD), clinical attachment loss (CAL) or index of gingival inflammation (GI)), inflammatory biomarkers in gingival crevicular fluid (GCF) such as enzymes alkaline phosphatase (ALP), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), cytokines interleukin-1β (IL-1β) or tumor necrosis factor-α (TNF-α) and drug concentration in GCF (Loos and Tjoa, 2005; Perinetti et al., 2008; Koss et al., 2009; Aimetti et al., 2012; Ertugrul et al., 2013; Spruill et al., 2014). Although the evaluation of periodontal treatment efficacy results in large amounts of data, their analysis and interpretation is commonly performed using the conventional approach. The biggest limitation of the conventional univariate approach is multicollinearity – mathematical coupling of variables, very common in the dental field. If multicollinear variables are used as explanatory variables, it can be very difficult to distinguish their individual relationship with the outcome. This may yield greater uncertainty in the obtained results and requires certain variables to be removed from analysis, although it may not always be clear which variable should be removed. The solution to this problem is the application of multivariate chemometric algorithms such as principal component analysis, partial least squares or their extensions (Tu et al., 2009).

In this study, the efficacy of the local periodontal treatment with DOX is evaluated using a multivariate chemometric approach, consisting of the following steps: i) data overview (determination of the discriminatory power of the selected variables for monitoring disease progression and treatment effects, identification of outliers, determination of correlation among selected variables); ii) classification of data (patients before vs patients after the local treatment with DOX); iii) comparative evaluation of the efficacy between local periodontal treatment with DOX and the local periodontal treatment with CHX.

2. Materials and methods

2.1. Chemicals, materials, standard solutions and quality control (QC) samples

Doxycycline hyclate (certified reference material, CRM) and tetracycline hydrochloride (internal standard, IS) were acquired from Sigma-Aldrich (Germany). Methanol (HPLC grade) was supplied by Carlo Erba (Italy). Sodium acetate and Na_2EDTA (AppliChem, Germany) and calcium chloride (Alkaloid, Macedonia) were of analytical grade. For the local treatment of patients with periodontal disease, 10% DOX controlled – release gel (45 mg doxycycline hyclate/0.5 g gel) was used. Whatmann 3MM chromatography paper strips, 2 × 5 mm (Whatman Lab sales Ltd., UK) were used for GCF collection. Protease inhibitor cocktail was purchased from Sigma Aldrich (Germany). Commercial kits for

Table 2
Data set containing the results obtained by determination of inflammatory biomarkers in GCF and clinical indices in patients suffering from periodontal disease before and local administration of DOX.

No	Before local administration of DOX								No	After local administration of DOX							
	ALP (IU/L)	LDH (IU/L)	AST (IU/L)	IL-1β (pg mL^{-1})	TNF-α (pg mL^{-1})	GI (1–3)	CAL (mm)	PD (mm)		ALP (IU/L)	LDH (IU/L)	AST (IU/L)	IL-1β (pg mL^{-1})	TNF-α (pg mL^{-1})	GI (1–3)	CAL (mm)	PD (mm)
1	**67.8**	**582.8**	**30.0**	**369.8**	**133.2**	**2**	**3**	**4**	26	41.5	226.7	8.3	15.6	21.9	1	2	2
2	65.2	149.8	23.3	211.4	19.9	2	4	2	27	16.6	137.6	20.0	33.6	23.3	1	1	1
3	69.1	356.2	16.7	318.9	25.6	1	2	5	28	6.9	668.8	16.7	24.0	22.9	1	1	1
4	36.7	1141.4	26.7	488.6	37.8	2	3	3	29	22.1	741.6	16.7	89.0	29.0	1	1	1
5	48.4	825.7	21.7	140.1	31.2	2	4	5	30	18.0	429.0	16.7	55.4	15.6	1	2	2
6	48.4	157.9	48.3	131.9	4.2	2	2	2	31	38.7	416.9	16.7	62.5	23.7	1	1	1
7	63.6	530.2	23.3	567.4	19.9	1	2	3	**32**	**24.9**	**53.3**	**2.3**	**31.8**	**7.8**	**1**	**1**	**1**
8	48.4	388.6	56.7	229.0	61.5	3	3	6	33	27.6	315.7	7.8	42.2	17.7	1	2	2
9	23.5	562.1	30.0	275.9	19.6	2	1	3	34	42.8	267.1	15.0	162.0	22.9	1	1	1
10	42.8	1307.3	76.7	225.7	38.7	3	3	4	35	52.5	1003.1	73.3	37.6	19.6	1	2	3
11	63.6	1113.1	122	268.8	29.2	2	4	3	36	38.7	505.9	56.7	95.5	30.6	1	3	2
12	22.1	388.6	38.3	58.8	25.0	3	5	4	37	19.3	1044.3	25.0	146.8	35.2	1	3	2
13	13.8	935.0	3.7	196.2	30.9	3	4	4	38	34.6	1291.2	35.0	136.4	15.6	2	3	1
14	51.1	2319.2	76.7	282.8	22.2	3	5	3	39	31.8	1894.9	103.3	56.9	35.6	1	2	2
15	**48.4**	**1849.7**	**36.7**	**269.2**	**80.2**	**2**	**6**	**5**	40	52.5	918.8	70.0	47.8	23.2	1	2	3
16	62.2	412.8	13.3	23.9	34.9	3	4	2	41	33.2	323.8	16.7	221.8	33.9	1	4	2
17	**73.2**	**1849.7**	**280**	**115.3**	**33.9**	**1**	**5**	**4**	42	66.3	1076.2	30.0	301.4	42.9	1	3	1
18	45.6	627.7	40.0	61.2	47.6	3	3	5	43	24.9	344.0	56.7	156.4	15.6	1	2	1
19	13.8	935.0	31.7	196.2	30.9	2	3	3	44	16.0	429.0	16.7	125.4	15.6	1	2	2
20	51.1	2319.2	76.7	282.8	22.2	2	4	5	45	35.7	416.9	16.7	62.5	23.7	1	2	3
21	48.4	1849.7	36.7	269.2	80.2	2	2	2	**46**	**20.9**	**789.3**	**28.3**	**31.8**	**7.8**	**1**	**4**	**2**
22	62.2	412.8	13.3	23.9	34.9	1	2	3	47	21.7	315.7	7.8	221.8	17.7	1	3	1
23	**73.2**	**1849.7**	**280**	**115.3**	**33.9**	**3**	**5**	**6**	48	38.7	267.1	151.0	162.0	22.9	1	2	1
24	45.6	627.7	40.0	61.2	47.6	2	4	3	49	52.5	283.1	73.3	137.6	19.6	1	3	2
25	71.3	659.9	20.6	101.6	36.0	2	3	3	50	38.7	505.9	56.7	95.5	30.6	2	2	2