Department of Conservative Dentistry[1], Gynecologic Hospital[2], Faculty of Medicine, Friedrich Schiller University, Jena, and Jenapharm GmbH & Co. KG[3], Jena, Germany

Influence of sexual steroids on cell functions of PMNL in the gingival sulcus

G. Klinger[1], S. Glänzer[1], B. Sigusch,[1] G. Klinger[2] and W. Römer[3]

Clinical experience confirms the influence of sexual steroids on the periodont under several clinical conditions. The mechanisms of the noticed effects are not all completely understood. In this paper, phagocytes from gingival crevice fluid of 39 patients with different forms of periodontitis and 18 healthy persons without periodontal disease were examined. Phagocytic activity was assessed *in vitro*. Simultaneously to phagocytic examination, 17β-estradiol or dienogest were added to the samples in different concentrations, in order to see whether a difference existed between phagocytosis in the presence or absence of sexual steroids. Phagocytosis was significantly reduced in patients with periodontitis. It was found that phagocytosis was raised significantly by 13% in the group with periodontal disease under the influence of 17β-estradiol. The administration of dienogest did not change the phagocytosis capacity significantly. In the healthy group, neither addition of 17β-estradiol nor addition of dienogest caused any difference.

1. Introduction

Clinical experience confirms the influence of sexual steroids on periodont under several clinical conditions, such as menstrual cycle, hormonal contraception, or gravidity [1–4]. However, the mechanisms of action of the noticed effects are not completely understood. It is known in detail that the phagocytosis capacity in gingiva is regulated by endogenous and exogenous factors. For example, observing pH alterations in the gingival sulcus, the addition of nicotine or the dependence upon age are responsible for the reduction of the phagocytosis capacity in healthy individuals [5]. Patients with periodontitis are characterized by an enhanced number of PMNL on the one hand, and a reduced function of phagocytosis on the other hand [6–9]. It also seems to be proven that the uptake of hormonal contraceptives influences immunologic reactions of periodontal tissues as well as the composition of the subgingival plaques [10–13]. At present, it is being discussed that the development of atherosclerosis is, in part, mediated by inflammatory reactions [14, 15]. Hitherto, we know that estrogens have antiatherosclerotic properties and exert these effects by nonhormonal processes. Furthermore, several authors have reported that the gingiva is characterized by the existence of receptors for sexual steroids [16]. It has so far been assumed and partly confirmed by clinical studies that hormonal contraceptives with estrogen and progestogen in low concencentrations do not trigger inflammatory properties [17]. Norderyd et al. [18] found less bleeding from the gums in women aged over 50 under the influence of estrogen supplementation. It seemed necessary to examine the influence of increased concentrations of sexual steroids, in order to see whether there was a change in PMNL parameters like phagocytotic capacity.

Thus, the aim of our investigations was the analysis of the gingival sulcus fluid to characterize the cellular function *phagocytosis* of female individuals on hormonal contraceptives compared to a control group. It was also of interest to know, whether the addition of sexual steroids, such as endogenous 17β-estradiol and synthetic dienogest known as a 19-norprogestin [19, 20] to the sulcus fluid caused any changes in these parameters. For this reason, these drugs were added in relatively high concentrations to gingival sulcus fluid containing PMNL cells harvested both from healthy persons and patients with periodontitis.

2. Investigations and results

The investigation was carried out in 18 healthy persons (controls) and 39 patients, all from the patient population of the Conservative Dentistry, Department of Periodontology of the Friedrich Schiller University in Jena. They suffered from various kinds of periodontitis. The adult periodontitis (AP) group was comprised of 15 patients with moderate bone loss. The rapidly progressive periodontitis (RPP) group comprised 16 patients with long histories of severe destruction of periodont including intrabony lesions. Eight patients with juvenile periodontitis (JP) had typical alveolar bone loss (incisors and first molars) and also long histories of disease. The healthy group included 18 persons without radiographic evidence of bone loss or evidence of periodontal disease.

Gingival crevicular fluid cells (CF cells) were obtained from 2–3 teeth with probing depths corresponding to the criteria of described disease [1, 21–24]. Prior to washing, the areas were dried. Sulcular fluid was collected using the method by Skapski and Lehner [25], modified by Sigusch et al. [26]. In each case 15 sequential washings of the gingival crevice were performed with phosphate buffered saline (PBS) using a 5-µl conventional pipette. Washes were collected in 1-ml Eppendorf tubes and centrifuged at 2,000 r.p.m. for 10 min. Cells were washed twice with PBS and counted using Neubauer chamber with trypan blue exclusion as an index of cell viability.

Suspensions containing CF cells were adjusted to a concentration of 10^6 cells/ml.

Candida albicans were used as indicator particles to determine the number of phagocytes contained and adhering. 0.2 ml suspension of heat-killed *C. albicans* were mixed with 0.2 ml pooled human AB serum for opsonization in a moist chamber for 30 min. at 37 °C. The opsonized *C. albicans* were washed twice with PBS and suspended in 1 ml PBS + fetal calf serum (20%). This suspension (10 µl) was added to 50 µl of a suspension of crevicular cells. A microscopic slide with two circles was covered with 50 µl of a mixture in each circle. The slides were placed in a moist chamber at 37 °C for 30 min. Phagocytosis was stopped by draining the supernatant and covering the slide with 50 µl staining solution of 0.2% Eosin Y and 0.4% Trypan blue both dissolved in PBS. The slide was immediately examined using a Zeiss light microscope "Jenamed histology" at a magnification of 1,000. *C. albicans* which are ingested can be discriminated from adhering ones.

Phagocytosis was calculated by observation of 100 viable cells, quantified between leucocytes with ingested and leucocytes with adhering *C. albicans.*

To determine the influence of sexual hormones on phagocytosis capacity, 17β-estradiol or dienogest were added to the samples in different concentrations; 17β-estradiol in concentrations from 10 to 100 μM, dienogest in concentrations ranging from 0.05 to 1 mM.

Results were expressed as the mean ± standard deviation. The statistical significance was evaluated by the t-test for paired samples [27].

Table 1 shows number of cells and viability in different groups before administration of hormonal substances. The number of CF cells was significantly higher in all periodontitis groups (AP = 1.7 ± 0.4; RPP = 2.1 ± 0.5; JP = 1.8 ± 0.3) than in the control group (0.9 ± 0.3 × 10^6/ml). Viability was significantly reduced in all groups with periodontal disease (AP = 81.8 ± 5.7; RPP = 78.8 ± 4.7; JP = 77.4 ± 4.7). In the healthy control group, viability was 86.1 ± 3.1%.

Phagocytosis capacity before giving sexual steroids was reduced in all periodontitis patients in relation to the control group at 73.1% ± 3.9. In the AP group, phagocytosis was 59.9% ± 4.4, in the RPP group 42.0% ± 4.8, in the JP group 45.1% ± 5.9.

Furthermore, 35 patients received hormone medication, 4 patients got higher concentrations than mentioned before und were not included in the evaluation.

17β-Estradiol was administered to 9 controls and 17 patients, dienogest was given to 9 controls and 18 patients.

In patients with periodontitis, 17β-estradiol dosage resulted in phagocytosis capacity being significantly elevated from 45.7 ± 8.0% up to 51.5 ± 10.2%. A difference of 13% was found. The results permit the conclusion that concentrations below 50 μM have only little influence on phagocytosis capacity. Otherwise, with higher concentrations than used in this study, no greater effect is to be seen.

Therefore, the administration of dienogest did not change the phagocytosis capacity in a statistically significant way. In the healthy group, there was no difference either by addition of 17β-estradiol or by addition of dienogest (Table 2).

Table 1: Number of cells, viability, and phagocytotic capacity of sulcus cells from healthy subjects and patients with different forms of periodontitis

	Number of subjects	Number of cells × 10^6/ml	Viability (%)	Phagocytotic capacity %
Healthy subjects	18	0.9 ± 0.3	86.1 ± 3.1	73.1 ± 3.9
Patients with AP	15	1.7 ± 0.4*	81.8 ± 5.7*	59.9 ± 4.4*
Patients with RPP	16	2.1 ± 0.5*	78.8 ± 4.7*	42.0 ± 4.8*#
Patients with JP	8	1.8 ± 0.3*	77.4 ± 4.7*	45.1 ± 5.9*#

* p = <0.05 (correlation to the control group)
*# p = <0.05 (correlation to group with AP)

Table 2: Phagocytotic capacity before and after 17β-estradiol or dienogest in healthy subjects and patients with periodontitis

	Number of subjects	Phagocytotic capacity (%)		
		before medication	with 17β-estradiol	after medication
Control group	9	73.7 ± 3.6		75.4 ± 3.7
Patients with periodontitis	17	45.7 ± 8.0	*	51.5 ± 10.2
		before medication	with dienogest	after medication
Control group	9	72.4 ± 3.9		72.3 ± 3.5
Patients with periodontitis	18	51.2 ± 9.7		53.2 ± 8.8

* p = <0.05

3. Discussion

The combined estrogen-progestin contraceptive pill (OC) has been one of the mainstays of fertility control for four decades. During that time, the mechanisms by which steroids provide effective contraception and induce metabolic changes as well as adverse reactions have been extensively studied. In this connection, the influence of macrophages and other immunocompetent cells in phagocytic processes by which cells bind and internalize relatively large particles into vacuoles is a part of the host defense against invading microorganisms like virus and bacteria, and is necessary in the removal of dead cells and cell debris during tissue remodeling and inflammation. Associations have been found between phagocytotic activities in gingival sulcus fluid taken from female patients with peridontitis in comparison with healthy persons. The results also demonstrate the interaction of selected sexual steroids given as oral contraceptives (Valette) and altered phagocytosis capacity investigated in gingival sulcus PMNL cells obtained from female healthy persons and patients with periodontitis. Whereas the phagocytotic activity in gingival sulcus of healthy persons was not influenced by hormonal contraceptives, an increased phagocytotic activity in patients with periodontitis was registered.

Taking together the *in vitro* results, the OC component estrogen could be responsible for a positive action on phagocytosis detected in PMNL obtained from gingival sulcus fluid. In particular, increased concentrations of estrogens should be able to stimulate nongenomic effects on cellular processes. Previously, estrogens such as 17β-estradiol or ethinyl estradiol have been known for radical scavenging and antioxidant properties [28, 29] and possess an important function in the mechanisms of action of host defense [30, 31]. Thus, the justification as a protective agent against inflammatory diseases in healthy women could depend not only on its effectiveness as a prophylactic agent but also on its other beneficial health effects, such as its possible antiinflammatory effects. The phagocytosis capacity of CF cells is influenced by selected sexual steroids. For example, the number of PMNL cells and their phagocytotic activity are regulated by the uptake of hormonal contraceptives containing ethinyl estradiol and dienogest or desogestrel.

4. Experimental

4.1. Chemicals

17β-Estradiol as well as dienogest (17α-cyanomethyl-17β-hydroxy-4,9-estradien-3-one) were obtained from Jenapharm GmbH & Co. KG (Jena, Germany). Ethanol, 96%, p.a. was supplied by Laborchemie Apolda GmbH (Apolda, Germany).

4.2. Preparation of stock solutions and samples

17β-Estradiol and dienogest were reconstituted with ethanol to obtain 20 mM stock solutions. Working solutions were prepared by dilution with de-ionized distilled water.

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Prof. Gisela Klinger
An der alten Post 4
D-07740 Jena