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Metronidazole concentrations in plasma, saliva and periodontal pockets in patients with periodontitis

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Abstract

Objectives: Metronidazole is widely used antibacterial compound in the treatment of some types of periodontal disease. Pharmacokinetics of metronidazole in plasma has been well-described but few data exist about penetration f the drug to the gingival crevice fluid. The aim of the present study was to compare the concentrations of metronidazole in plasma, saliva and gingival crevice fluid in patients with periodontitis after multiple administration.

Materials and methods: Eleven patients with severe generalised adult periodontitis participated in the study. Metronidazole, 500 mg, was administered orally two or three times per day for at least 2 days before sample collection. Samples were collected 2 h after last dose. Metronidazole concentrations in all fluids were measured with high-performance liquid chromatography.

Results: Mean drug concentrations in palsma, saliva and crevice fluid were 14.33, 15.15 and 12.86 µg/ml, respectively. Difference between plasma and crevice fluid or between plasma and saliva did not reach statistical significance.

Conclusion: Present study revealed that metronidazole penetrates well into gingival crevice fluid and saliva. Metronidazole concentrations in crevice fluid are about equal to the protein unbound drug concentrations in plasma. Therefore, general pharmacokinetic data of metronidazole can be also applied in the treatment of periodontal disease and in the design of respective treatment regimens.

Metronidazole is an antimicrobial compound with wide spectrum of activity against protozoal infections and anaerobic bacterial infections. Metronidazole was first introduced in the treatment of trichomoniasis in the late 1950s. Its therapeutic use has subsequently been expanded to include anaerobic bacterial infections (Tracy & Webster 1996, Lamp et al. 1999).

At present, metronidazole is one of the most widely used antibacterial compounds in the treatment of some types of periodontal disease such as aggressive periodontitis and chronic progressive periodontitis that does not react favourably to conventional treatment. It is active against most established periodontal pathogens and is frequently used alone or combined with amoxicillin as an empirical treatment of periodontitis (van Winkelhoff et al. 1989, American Academy of Periodontolgy 1996, Elter et al. 1997, Winkel et al. 2001, Slots & Ting 2002). Several clinical studies comparing the clinical outcome of mechanical treatment combined with metronidazole administration to mechanical treatment alone can be found in the literature (van Winkelh-off et al. 1989, Loesche et al. 1992, Berglundh et al. 1998, Winkel et al. 2001,). Dosage of metronidazole follows the same scheme as it is used in soft tissue infections. Dosage schemes are based rather on empirical data of effectiveness than on the pharmacokinetics of drug at the infection site (Slots & Ting 2002).

Pharmacokinetics of metronidasole in plasma has been well described. Oral bioavailability of metronidazole is generally reported >90% (Lau et al. 1992, Lamp et al. 1999). There are also several studies indicating that metroni-

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dazole penetrates well into different tissues, including uterus, pancreatic tissue, central nervous system. In comparison, concentrations in adipose tissue and colonic mucosa were significantly lower than respective plasma or serum concentrations (for a review, see Lamp et al. 1999).

In the treatment of periodontitis the presence of antimicrobial in periodontal tissues is of utmost importance. Few data exist showing that metronidazole penetrates to the gingival crevice fluid in concentrations comparable with those found in plasma. However, these tissue distribution studies have been performed almost two decades ago when precise determination methodology was not available. Bioassay measuring the total bacteriostatic activity of the sample (Van Oosten et al. 1986) or high-performance liquid chromatography (HPLC) with experimentally increased flow rate of gingival crevice fluid (Britt & Pohlod 1986) has been used.

The aim of the present study was to compare the concentrations of metronidazole in plasma, saliva and gingival crevice fluid in patients with periodontitis after multiple administration.

Material and Methods
Patients

The study protocol was approved by the ethics committee of University of Tartu. All patients received detailed description of study protocol and signed written informed consent form before enrolment into the trial. The study was performed in accordance with the Declaration of Helsinki.

Eleven patients (six male, five female) with severe generalized adult periodontitis were selected to the study. All subjects had at least 22 teeth. Mean age of patients was 46.3 ± 12.8 (range 24–60) years. Patients were in good general health and did not took any other medication. Mechanical debridement and root planning under local anaesthesia was finished at least 3 weeks before present trial.

Metronidazole (500 mg tablet, Nycomed SEFA, Põlva, Estonia) was administered orally two or three times daily for at least 2 days before sample collection. Dosage frequency was chosen by clinical investigator depending on the activity and severity of the disease. Number of tablets per day administered to each patient can be found in Table 1. Samples for drug determination were collected 2 h after last dose.

Venous blood was collected by direct venepuncture into tubes containing Li–heparin anticoagulant, immediately centrifuged and plasma separated.

Crevice fluid was collected from eight deepest pockets (clinical pocket depth $\geqslant 6$ mM) with micropippettes and pooled. Automatic 50 µl pipettes (Lenpipet, St. Petersburg, Russia) with adapted and curved tips were used. Crevice fluid collected from each patient was pooled into weighed and labelled plastic microtube per patient. Amount of fluid in tubes was calculated by weighing the tubes after sample collection. Before the gingival fluid was sampled the teeth surfaces were isolated with cotton rolls and saliva injector and air-dried to prevent contamination of sampling area with saliva.

Samples of collected biological material were stored in PCR-clean microtubes (Eppendorf, Hamburg, Germany) without any conservant added at –20°C until analysis.

Assay method

Metronidazole concentrations in plasma, saliva and gingival fluid samples were analysed by a HPLC method. The same procedure was used for preparation of plasma, saliva and gingival fluid samples, for calibration curve construction and for quality control (QC) samples.

Samples were prepared for analyses by extraction with acetonitrile. Samples were thawed in room temperature. Fifty microliter of plasma or saliva was transferred to the 1.5 ml vial; gingival fluid samples were prepared in the sample collection vial. Exact amount of gingival fluid was estimated by weighing the vial before and after sample collection. To this sample 50 µl of 50 µg/ml tinidazole solution was added and vortex-mixed for 10 s. Then 600 µl of acetonitrile was added and the sample was vortex-mixed for 1 min. The resulting sample was centrifuged at $6000 \times g$ for 15 min. at 10°C. Vials were kept frozen overnight at -20°C. From frozen samples 500 µl of organic layer was removed with pipette and transferred to the 1.5 ml vial. The solvent was evapourated to dryness under the stream of air. The sample was reconstituted in 120 µl of mobile phase, vortex-mixed for 0.5 min. and centrifuged at $8000 \times g$ for 10 min. at 10°C. Ninety microliter of this solution was transferred to the autosampler vial.

The chromatographic system consisted of Lichrosorb RP-18 pre-column (Merck, Darmstadt, Germany), Lichrosorb RP-18, 5 µ, 250×3.2 mM column (Merck), and a ultraviolet detector measuring at 318 nm. Mobile phase consisted of acetonitrile–0.01 M phosphate solution (NaH_2PO_4), 15:85 (v:v), flow rate was 0.7 ml/min., column temperature 22–25°C (room temperature).

Analytical method was validated before determination of study samples with regard to the following parameters: specificity, limit of detection and quantification, linearity, precision and accuracy, inter-day variability, intra-day variability, stability in the freezer and stability in the autosampler. The minimum quantifiable concentration (lowest concentration in the calibration curve) was 0.1 µg/ml. The method was linear over concentration range 0.1–50 µg/ml.

Table 1. Number of tablets taken by patients per day and individual and mean $\pm$ SD metronidazole concentrations (mg/l) in plasma, saliva and gingival crevice fluid

Patient no.	No. of 500 mg tablets per day	Plasma	Saliva	Crevice fluid	Crevice fluid/plasma (%)
1	2	10.02	7.58	6.46	64.5
2	3	26.19	30.61	27.75	106.0
3	2	13.68	16.82	12.42	90.8
4	2	14.45	14.70	10.52	72.8
5	3	7.55	7.21	7.18	95.1
6	2	6.33	9.35	4.81	76.0
7	3	13.84	16.59	12.03	86.9
8	2	8.59	8.30	6.87	80.0
9	3	25.82	23.09	27.41	106.2
10	2	12.05	11.93	9.53	79.1
11	3	19.11	20.53	16.47	86.2
Mean		14.33	15.15	12.86	85.78
SD		6.80	7.40	7.99	13.17

SD, standard deviation.

No interfering peaks were registered at the retention times of the drug and internal standard. A measure of goodness of fit of linear regression, r^2, was above 0.9995 in all calibrations showing good linearity. Mean intra-day precision (coefficient of variation in the determination results of QC samples) of the determinations was 5.51–9.19% and mean intra-day accuracy (bias % of QC samples) from 1.20 to 4.53% in all concentrations. Mean inter-day precision of the determinations was 8.03–8.16% and mean inter-day accuracy from − 3.66 to 0.33% in all concentrations.

Statistics

Repeated measures ANOVA was used for analysing the data. Bonferroni's multiple comparison test was used to compare the plasma concentrations with concentrations in saliva and crevicular fluid. Data were analysed with GraphPad Prism 3.0 program (GraphPad Software, San Diego, CA, USA).

Results

All included patients completed the study. All patients declared that they took the drug as prescribed. No adverse events were registered during the study. From all patients all planned samples were collected; the volume of gingival crevice fluid obtained per patient ranged from 8 to 35 μl.

Metronidazole was quantified in all measured samples. Concentrations ranged from 4.8 to 30.6 mg/l. Individual measured concentrations with mean values and standard deviations are presented in Table 1. The highest concentrations were measured in saliva, the lowest in crevicular fluid. However, difference between plasma and crevicular fluid or between plasma and saliva did not reach statistical significance.

Discussion

Our study indicated that metronidazole penetrates well into gingival crevicular fluid and saliva. Therefore, the present study supports the results of some earlier experiments. The main advantages of the present study were the use of patients without any induction of the crevicular fluid flow and the use of validated, specific and sensitive HPLC methodology.

The flow rate of crevicular fluid can differ several times between normal state and patients with periodontitis (Goodson 2003). It is possible that the diffusion process of compounds from the plasma to the fluid depends on the flow rate. As antibiotics are used in the treatment of periodontitis, penetration into crevicular fluid during the increased secretion induced by the disease state is a key factor of effectiveness. In all patients included to the present study severe periodontitis not responding to mechanical debridement alone was diagnosed. This patient population is typical in whom the antibacterial treatment (including metronidazole) is used (Winkel et al. 1997, Slots & Ting 2002).

Assay method used in the present study was developed in our laboratory to measure metronidazole concentrations in samples of different origin with volume 5–50 μl. As a part of larger project (including non-published sponsored trials), full validation of the method according to good laboratory practice principles was performed. This assay was used also to measure metronidazole concentrations during the microdialysis (Karjagin et al. 2004). Therefore, one aim of the study was to compare the metronidazole penetration into gingival crevicular fluid with drug penetration into muscular tissue. Comparisons were not made during this study at the same patients but as results are obtained at the same laboratory using the same assay method, results are well comparable. Our microdialysis study revealed that equilibrium between metronidazole concentrations in muscle and plasma was reached 2–3 h after drug administration and concentrations in muscle accounted about 85% of total plasma concentrations. In the present study, the mean concentration in gingival crevice fluid compared with plasma was 86%. According to literature, the protein binding of metronidazole is 10–15% (Lamp et al. 1999, Tracy & Webster 1996). Therefore, we can conclude that metronidazole concentrations in gingival crevicular fluid are well correlated with protein unbound metronidazole concentrations in plasma.

One limitation of the present study was the single time point for sample collection after drug administration. Collection of crevice fluid can cause gingival tissue irritation or even damage. This probably also influences the crevicular fluid flow rate (Lamster et al. 1985, Goodson 2003). Therefore, single sample collection point was chosen at the time when equilibrium between plasma and tissue was reached. Present study was performed under steady state conditions, which also supports the equilibrium between all distribution compartments. Elimination half-life of metronidazole is about 8 h and 48 h was considered sufficient for reaching the steady state. As it is evident from the results table, the percentage of metronidazole concentration in crevice fluid compared with plasma is not dependent on the plasma drug concentrations.

Significant inter-individual differences in metronidazole concentrations were observed. This can be explained by the different treatment regimen used and different distribution volume for drug which depends on body weight and height (Lau et al. 1992, Lamp et al. 1999).

There are no questions about general effectiveness of metronidazole in the treatment of periodontitis. Problems can be accounted in the treatment of infections caused by strains with increased resistance (Roberts 2002). In this case high concentrations of antimicrobial at the infection site are of critical importance. Present experiment confirmed that metronidazole penetrates well to crevice fluid. High concentrations were measured also in saliva; mean concentration was very similar to those found in plasma. Although saliva does not have access inside the gingival pocket, high antibiotic concentration in saliva promotes to eradicate microbes outside gingival structure and to prevent the spread of infection to non-affected gingival areas.

In conclusion, present study revealed that metronidazole penetrates well into gingival crevice fluid and saliva. Metronidazole concentrations in crevice fluid are about equal to the protein unbound drug concentrations in plasma. Metronidazole distribution to crevice fluid conforms to the distribution of the compound into soft tissues with good circulation, e.g. muscular tissue. Therefore, general pharmacokinetic data of metronidazole established in numerous trials, can be also applied in the treatment of periodontal disease and in the design of respective treatment regimens.

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