**In Vitro Activity of Metronidazole against Entamoeba Gingivalis**

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**Rapid Communication**

Entamoeba gingivalis has been the first ever discovered amoeba in humans in the dental plaque [1]. Its role in the periodontal disease remains controversial, but studies found a significant association between *E. gingivalis* and the severity of periodontal disease [1,2]. One clinical study showed a decreased frequency of *E. gingivalis* in patients with periodontal disease receiving metronidazole, compared to untreated patients [1]. However, the in vitro activity of metronidazole against *E. gingivalis* is unknown.

Therefore, we investigated the in vitro activity of metronidazole against *E. gingivalis* ATCC 30927 type strain (American Type Culture Collection, Rockville, USA). Amoeba were cultured in modified liquid medium Peptone Yeast Glucose (PYG) ( Gibco\(^a\), Saint Aubin, France) incubated at 37°C in Hungate tubes (Dutscher, Issy-les-Moulineaux, France) under 2-bar of N\(_2\)/CO\(_2\) (80%-20%). The activity of metronidazole (B. Braun Medical SAS, Boulogne, France) was ensured by testing the reference Methanobrevibacter smithii strain ATCC 35061T DSMZ 861 as previously described [3]. In vitro susceptibility test was carried out by inoculating 105 cells of *E. gingivalis* into 8 mL of fresh medium containing 0.5, 1, 2, 4, 8 or 40 mg/L of metronidazole. Non-inoculated negative controls tubes contained identical concentration of metronidazole, and three inoculated tubes without metronidazole were used as positive controls. All tubes were then incubated at 37°C with agitation. *E. gingivalis* cells were counted after 3-day incubation, every day for 10 days, by using 32x82.5 mm slides (Hycor Biomedical, Penicuik, United Kingdom) and observed using a microscope at X10 and X40 magnifications. The minimal inhibitory concentration (MIC) was defined as the lowest metronidazole concentration inhibiting growth of *E. gingivalis*. Cell viability was assessed after metronidazole treatment by centrifuging cells at 5000Xg for 20 minutes, washing the cell pellet three times with medium, sub-culturing in metronidazole-free medium, and observing cells under the microscope as stated. Experiments were done in triplicate.

The activity of the metronidazole suspension used here, was confirmed by observing growth inhibition of *M. smithii* in agreement with previously reported observations [3]. While negative control tubes remained sterile, positive control tubes containing an initial 104 *E. gingivalis* cells/mL suspension yielded an exponential growth kinetics over 8 days to reach a stationary phase after 8-day incubation (Figure 1). In this system, the doubling time was of two days (Figure 1). The MIC of metronidazole was determined as 4 mg/L as metronidazole concentrations of 0.5, 1 and 2 mg/L did not inhibit the growth of *E. gingivalis* (Figure 1) contrary to concentrations of 4 mg/L, 8 mg/L and 40 mg/L. Subculturing *E. gingivalis* exposed for 10 days in to 4 mg/L metronidazole, failed.

![Figure 1](image.png)

**Figure 1:** Growth monitoring of Entamoeba gingivalis strain ATCC 30927 with metronidazole. The data result from triplicate experiment. Positive control (no metronidazole); ● 1 mg/L of metronidazole; ▲ 4 mg/L; ● 10 mg/L; ● 40 mg/L.

Here, validating data by appropriate controls, we tested the in vitro susceptibility of *E. gingivalis* to metronidazole, a molecule previously demonstrated to be active against anaerobic bacteria and parasites [4]. We observed that growth of *E. gingivalis* was inhibited by metronidazole at concentration ≥ 4 mg/L. More probably, metronidazole was killing *E. gingivalis* cells at concentration as low as 4 mg/L, as revealed by the absence of growth after subculturing. These data therefore support the in vitro effectiveness of metronidazole against *E. gingivalis*. It is not possible to directly extrapolate these in vitro data, to treatment of *E. gingivalis* infections. However, several studies showed that oral metronidazole 750-1500 mg, yielded plasma, saliva and gingival fluid concentration of 8.7-15 ± 7.4 mg/L [5]. Moreover, a unique clinical study found a decreased frequency from 64% to 26% of *E. gingivalis* in periodontal disease after oral metronidazole, 750 mg a day for 7 days [1]. In this study, the number of *E. gingivalis* trophozoites was also significantly reduced from 15.9 ± 4.48 to 6 ± 1.5 per microscopic field, after metronidazole treatment [1].

Combining these clinical data with our in vitro susceptibility data indicates that a standard regimen of oral metronidazole would yield an effective local concentration of metronidazole against *E. gingivalis* cells in periodontal lesions. Therefore, these cumulative data support the use of metronidazole and derivatives in the treatment of periodontal disease.
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References