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Short Communication Open Access

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®, 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 by using an optical 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 above 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.

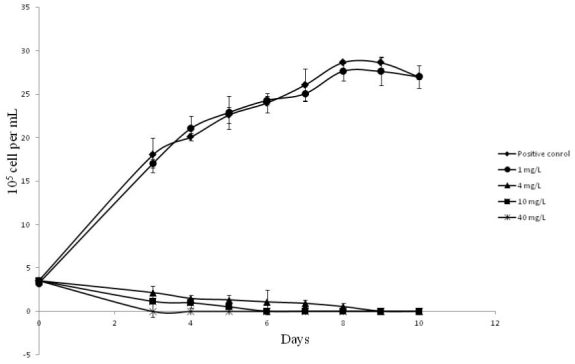

Figure 1: Growth monitoring of *Entamoeba gingivalis* strain ATCC 30927 with metronidazole. The data result from triplicate experiment. Positive control (no metranidazole); •1 mg/L of metronidazole; ▲ 4 mg/L; ▪ 10 mg/L; ∗ 40 mg/L.

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 750-1.500 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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