Implantation and permanency of *Candida albicans* in the oral cavity of normal and sialoadenectomized mice after a single inoculation of yeast

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Abstract

Candidosis is a common fungal infection in the oral cavity of humans. *Candida albicans* is the specie most frequently isolated from this infection. *C. albicans* ability to adhere to host tissue is considered an important step in the development of candidosis. In order to verify the role of saliva on the implantation and permanency of *C. albicans* in the oral cavity, 52 mice, 26 with normal salivary flow and 26 xerostomic, received a single inoculum with 0.1 mg of the sediment of *C. albicans* (ATCC 36801) suspension containing 10⁶ viable cells/mL. The permanency of *C. albicans* in the oral cavity of mice was verified 1, 2, 3, 5, 8 days after inoculation and then, in 15 days intervals until negativity of the test. Saliva sample was collected with a sterile “cotton ball” kept in the oral cavity of the animals for 5 minutes. The data obtained showed that xerostomic mice harbored *C. albicans* in the oral cavity until 75 days while the normal group was positive until 30 days after inoculation. The number of xerostomic and normal mice positive to *C. albicans* was the same until 48 h, and greater in the xerostomic group until the end of experiment. No statistically significant differences were observed between the cfu/mL of *C. albicans* recovered from the oral cavity of xerostomic and control mice. These data showed that although xerostomia did not have any influence on the number of candidal particles in the oral cavity, it favored the permanence of the yeast in the mouth of the animals for a longer period.

Key Words:

Experimental candidosis, *C. albicans*, xerostomia, sialoadenectomy.
**Introduction**

The incidence of yeast infections increased in the last years, mainly related to immunosuppressive conditions, particularly in AIDS patients\(^2\). In the oral cavity, candidosis is the most common fungal infection and *C. albicans* the specie most frequently related to these infections\(^5\). As an opportunistic infection, the development of candidosis is related to the virulence of the yeast and also to the presence of predisposing factors in the host. Among the virulence factors, the yeast ability to adhere to oral tissues is considered the first step to the development of candidosis. In the host, saliva is responsible for maintaining the integrity of the oral cavity, by its mechanical action and antimicrobial properties. Salivary flow is an important modulator of Candida population in the mouth and its reduction may facilitate the installation of candidosis\(^6,7,8\). There are few data in the literature on the mouth and its reduction may facilitate the installation of Candida in the oral cavity of animals\(^5\). The use of mice as experimental animals allows the study of the permanency of Candida in the oral cavity, once these animals do not harbor Candida in the mouth as an indigenous microorganism. The aim of this study was to verify the implantation and permanence of *C. albicans* in the oral cavity of normal and xerostomic mice after a single inoculation of the yeast.

**Material and Methods**

Fifty-two male mice (*Mus musculus*, albinos, Swiss) weighing 25 to 30g were studied; 26 with normal salivary flow (control) and 26 with xerostomia induced by sialoadenectomy of the major salivary glands, based on CHEINE (1939)\(^9\) technique, with modifications. The presence of *Candida* in the oral cavity of the mice was verified before the beginning of the experiment and also after sialoadenectomy, by collecting material from the oral cavity of mice with a sterile swab. The material was plated on Sabouraud Dextrose agar (Difco, Detroit, USA) supplemented with chloramphenicol (Quemicentina Succinato – Carlo Erba – 0.1mg/mL of media) and incubated for 48h at 37°C and 5 days at room temperature. All animals were negative to *Candida* in the oral cavity of animals\(^5\). The use of mice as experimental animals allows the study of the permanency of *Candida* in the oral cavity of normal and xerostomic mice\(^5\). The inoculation was performed with the aid of a sterile swab. The inoculation was performed with the aid of a sterile swab. Two groups of 6 animals, one xerostomic and one normal, were inoculated with 0.1 mg of the sediment of a *C. albicans* (ATCC 36801) suspension with 10\(^8\) viable cells/mL. The inoculation was performed with the aid of a sterile swab. Two groups of 6 animals, one xerostomic and one normal, were inoculated with *C. albicans* (control groups). The permanence of the yeast in the oral cavity of the animals was verified in the periods of 1, 2, 3, 5, 8 days after inoculation and than in 15 days intervals until the negativity in two consecutive samples. The quantity of *C. albicans* was determined by collecting saliva with a “small cotton ball” during 5 minutes. The material was transferred to a tube and the volume completed to 2 mL with sterile physiological saline solution (NaCl 0.89%). After decimal dilutions up to 10\(^{-3}\), 0.1 mL of each dilution was plated in duplicate onto Sabouraud dextrose agar.
C. albicans was recovered from the oral cavity of all the animals 1 and 2 days after inoculation. In normal group, a decrease in the number of positive mice was observed, from 20 to 17 mice, after 3 days, 14 after 5 days, 3 after 8 days, 5 after 15 days and 1 after 30 days. In the xerostomic group, 15 animals were positive after 3 days, 16 after 5 days, 7 after 8 days, 12 after 15 days, 6 after 30 days, 2 after 45 and 60 days and 1 after 75 days from inoculation. Statistical analysis by non-parametric Mann-Whitney test revealed statistical significant difference at the level of significance of 5% in the periods from 1 to 30 days in inoculation. The comparison between the number of positive mice is presented in Table 3. Similar behavior in both groups can be observed until the 5th day. After the 5th, the number of xerostomic mice positive to C. albicans was higher. The means of cfu/mL recovered from xerostomic mice were higher, but the difference was not statistically significant. All the recovered samples were C. albicans Killer biotype 111, the same specie and genus can be found in the oral cavity of normal mice. After one inoculation of 10^8 cells suspension of C. albicans in the oral cavity of mice, the yeasts were recovered from all the animals in the periods of 24 and 48h after inoculation, and then gradually decreased in both groups. In all studied periods, the number of xerostomic mice harboring C. albicans in the mouth was higher than in normal group, from 5th to 75th day after inoculation. The quantity of C. albicans recovered was higher in xerostomic mice, although not statistically significant. Statistical analysis corroborates that the two groups behavior was different after inoculation (normal and xerostomic). Their behavior was different in the periods from 1 to 30 days after inoculation. This implies that xerostomy, induced by sialoadenectomy, influenced the presence and colonization of the oral cavity by C. albicans. These results are in accordance to the findings of JORGE et al. (1993)\(^7\) in sialoadenectomized rats. The use of mice as experimental animals allows the study of the permanency of Candida in the oral cavity, once these animals do not harbor Candida in the mouth as an indigenous microorganism. Although xerostomia had not significant influence on the number of Candida in the oral cavities of the studied animals, it favored the permanence of C. albicans for a longer period.

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<th>Table 3- Number of normal and xerostomic that harbored C. albicans in the oral cavity after a single inoculation of a yeast suspension of 10^8 cells</th>
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<td>normal mice (n=20)</td>
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Discussion

Surgical removing of the major salivary glands produced intense xerostomia in mice, reducing in 75% the volume of saliva. In human beings, the symptoms of xerostomia occur when the salivary flow is reduced in 40 to 50%\(^6,11\). After the surgery, the oral cavity of the mice became dry, with only residual saliva and food adhered on mucous tissue and teeth surface. Yeasts from Candida genus can be found in the oral cavity of human beings and some predisposing factors, including xerostomia, may change its commensal condition to a pathogenic one, favoring the development of candidosis\(^15,16\). BROWN et al (1975)\(^7\) observed an increase of the prevalence of Candida, mainly C. albicans, in xerostomic patients undergoing head and neck radiation. Only sialoadenectomy, in this study, did not favored the implantation of C. albicans in the oral cavity of mice. This may be related to the fact that this fungus is not a normal habitant of the oral cavity of mice. After one inoculation of 10^8 cells suspension of C. albicans in the oral cavity of mice, the yeasts were recovered from all the animals in the periods of 24 and 48h after inoculation, and then gradually decreased in both groups. In all studied periods, the number of xerostomic mice harboring C. albicans in the mouth was higher than in normal group, from 5th to 75th day after inoculation. The quantity of C. albicans recovered was higher in xerostomic mice, although not statistically significant. Statistical analysis corroborates that the two groups behavior was different after inoculation (normal and xerostomic). Their behavior was different in the periods from 1 to 30 days after inoculation.

References