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The effect of amoxycillin on salivary nitrite concentrations: an important mechanism of adverse reactions?

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Broad spectrum antibiotics are known to predispose towards oral candidiasis and gastroenteritis. Oral nitrite synthesis by commensal bacteria may be important in protecting the mouth and lower intestine from pathogenic organisms, including *Candida albicans*. The effect of 2 days administration of the broad spectrum antibiotic amoxycillin on salivary nitrite concentration, following a 200 mg potassium nitrate oral load, was studied in 10 healthy volunteers. The C_{max} fell by 40% and the AUC was reduced by 1227 μM h (43%, 95% CI 273, 2181, $P < 0.006$) in the antibiotic treated group when compared with control. These findings suggest that destruction of nitrate reductase containing bacteria in the mouth by antibiotics may explain an increased incidence of infection with *Candida* and other pathogens.

Keywords amoxycillin salivary nitrite adverse drug reaction

Introduction

Broad spectrum antibiotics are associated with a number of common adverse drug reactions including oral candidiasis, which can occur in antibiotic treated patients in up to 2% of cases [1] and salmonella gastroenteritis [2]. The way in which commensal flora protect against infection by pathogens is not understood, but we have recently proposed that the conversion of nitrate to nitrite in the mouth by nitrate reductase synthesised by commensal bacteria may, under acid conditions [3], destroy organisms such as *Candida albicans* and *Escherichia coli* [4] to provide host defence against ingested pathogens.

The purpose of this study was to determine whether the broad-spectrum antibiotic amoxycillin was able to alter production of salivary nitrite by inhibition of the bacteria within the mouth which normally convert nitrate to nitrite [5] and so provide a mechanism by which antibiotic therapy may allow the survival of ingested microorganisms in the acidic areas of the mouth and on passage through the stomach.

Methods

Healthy male volunteers (21–37 years) were invited to take part. Those with adverse reactions to antibiotics, therapy with antibiotics within the previous 2 weeks, recent serious illness or treatment with any regular medication were excluded. Local Ethics Committee approval was given for this study. The study was outlined to the volunteers and written consent obtained. Subjects were studied on 2 separate days, at least 2 weeks apart. The protocol was identical apart from treatment with four 250 mg doses of amoxycillin taken over the 48 h period leading up to the second study day. The active treatment was always given before the second study period to avoid any possible carry-over effect. Subjects drank a solution of potassium nitrate BP (200 mg) in 100 ml of diluted orange squash. The same batch of juice was used for each part of the study. Saliva (approximately 1 ml) was sampled at 0, 15, 30, 45, 60, 90, 120, 180 and 240 min, centrifuged and stored at 2–5° C until analysis immediately following the end of the 4 h study period. (Nitrite concentration in samples of saliva

stored in this way were found to reduce by less than 4% over 4 h.) The supernatant of the sample was diluted appropriately and nitrite concentration determined using a standard Griess reaction [6]. Amoxycillin was found not to interfere with this assay in the low concentrations likely to be found in saliva.

The change in salivary nitrite concentration with time was compared by determination of area under the curve (AUC) between the two treatments, analysed using a Wilcoxon signed-rank matched pairs test and results reported with standard error values.

Results

Eleven volunteers were recruited. One was excluded due to a possible penicillin allergy. All other volunteers completed the study.

A graph of salivary nitrite concentration *vs* time can be seen in Figure 1. The t_{max} for both control and treatment groups was at 45 min when the C_{max} values were reduced by 40% from 1132 μM ± 214 to 682 μM ± 160 respectively ($P < 0.037$).

Following antibiotic therapy there was a reduction in salivary nitrite concentration from 2881 ± 528 μM h to 1654 ± 284 μM h (95% confidence interval for difference in AUC: 273, 2181) for the antibiotic treated group. This reduction (43%) was significant ($P < 0.006$).

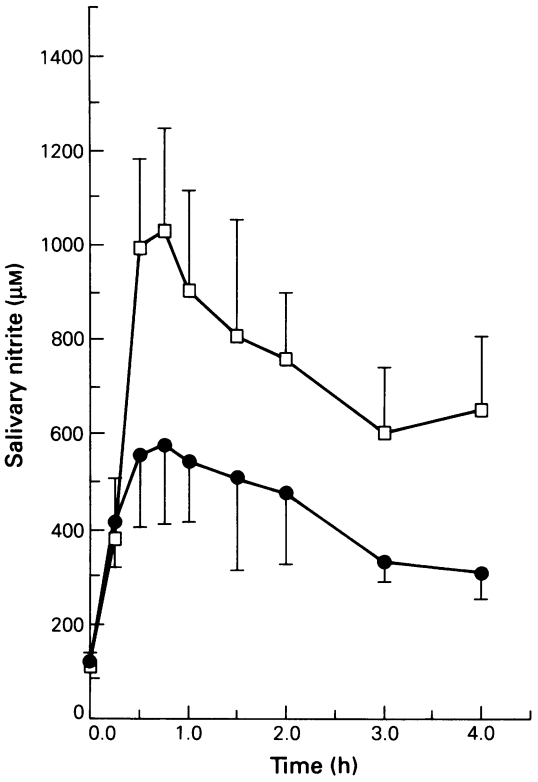

Figure 1 Graph of mean salivary nitrite concentrations (± s.e. mean) after ingestion of potassium nitrate (200 mg) showing the significantly lower levels attained after a 2 day course of amoxycillin (●) when compared with control (□).

Discussion

Candida albicans is commonly commensal in man and in healthy individuals is commonly present on the skin, in the alimentary and upper respiratory tracts, in sputum and in the vagina. It may become pathogenic in certain circumstances including pregnancy, diabetes, malnutrition, general debility and acquired immune deficiency syndrome (AIDS) or following the use of certain antimicrobial agents or immunosuppressive drugs. Oral candidiasis also occurs in the terminally ill and in individuals with hyposalivation [7]. *Candida albicans* and other *Candida* species are opportunistic commensals in the oral cavity of man and are detectable in 30–50% of adults, usually without producing symptoms. Candidal proliferation, and subsequent pathogenic role in the mouth, is strongly correlated with impairment of the immune system along with the inhibition of other oral microflora which normally compete with *Candida*. Several nonimmune molecules in saliva, such as histadine-rich polypeptides and lysozyme [8], are also believed to play a role in the control of *Candida albicans* in the oral cavity.

We have suggested that the formation of nitrite from salivary nitrate may contribute to the host defence against ingested pathogens, including *Candida albicans* and *enterobacteriacea*. As the enzyme nitrate reductase is only present in bacteria, the reduction in salivary nitrite with antibiotics shown in this study is not unexpected and suggests a mechanism for increased susceptibility to pathogenic infection following therapy with these drugs. Consistent with this, Neal *et al.* [2] found a 50% increased risk of developing salmonella infection in individuals who had received recent antibiotic treatment.

It would appear that there is little or no difference in the resting level of salivary nitrite. However, postprandial levels may be the important element in determining the effectiveness of nitrite to act in the role proposed.

The results suggest that increasing dietary nitrate may have a protective function in those who are particularly susceptible to such infections, for instance immunosuppressed or debilitated patients. It will be important to determine whether nitrate therapy may prevent or cure oral candidiasis. The major limiting factor in making clinical use of this finding will be the possible increased formation of nitrosamines which are known carcinogens in mammals.

In conclusion, we have shown that a broad spectrum antibiotic significantly lowers the oral conversion of nitrate to nitrite. This observation, in conjunction with our recent findings that nitrite (at concentrations achieved by the specialised concentrating mechanism in the salivary glands), has antifungal and antibacterial properties, suggests a mechanism for the observed susceptibility to oral and gastrointestinal infection following antibiotic therapy.

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