ANTIBACTERIAL TOOTHPASTE:

DO NOT SWALLOW

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BACKGROUND, PURPOSE, HYPOTHESES

Market statistics indicate a significant increase in the use of antibacterial products in North American households. The market has expanded from a few dozen products in the early 1990s to more than seven hundred at present[i]. The most common antibacterial agent in these products is triclosan ($C_{12}H_7Cl_3O_2$)[ii], which was initially introduced to the market as a surgical scrub in 1972 and has since been utilized as a preservative, fungicide, and biocide. Triclosan is a chlorinated aromatic compound, which inhibits the process of fatty acid synthesis through blocking the active site of the enoyl-acyl carrier protein reductase. Thus, this organic chemical possesses distinct antibiotic qualities. It is also very similar in chemical structure to dioxins, a few of which are deemed to be the most toxic chemicals on the planet. Increasingly, humans are being exposed to this chemical in small amounts and it is questionable whether this should be of concern.

Antibacterial agents such as triclosan are now being used in toothpastes and if ingested, may affect the probiotic intestinal microflora of the human gastrointestinal tract. These probiotic bacteria, serve as a defence system against pathogenic bacteria by attaching themselves to the cells lining the intestines at “enteric sites”, thus inhibiting the penetration of pathogens into the intestinal cells. Prevention of invasion by disease-causing microbes is maintained through an ecological microflora balance, where the probiotic bacteria are able to proliferate and become predominant over the pathogenic bacteria. Lactobacillus (gram-positive facultative bacteria) is the predominant probiotic bacteria in the upper intestinal tract. Their high hydrogen peroxide

tolerance, ability to survive without iron, and production of lactic acid, creates a hostile environment that inhibits the growth of some harmful bacteria, moulds, spores, and yeasts. Lactobacilli also produce an enzyme known as lactase, which is required in the digestion of milk. A disruption to the balance of the lactobacillus with other pathogenic bacteria may result in increased symptoms of irritable bowel syndrome, Crohn's disease, and some forms of colitis. One may become more susceptible to infectious diseases such as rotavirus, often resulting in diarrhoea. A person with an extremely low lactobacillus balance may even develop lactose intolerance due to an inability to digest lactose. Therefore, I investigated the amount of inhibition of lactobacillus growth by antibacterial toothpaste containing triclosan to determine if there could be a disruption in the ecological intestinal balance significant enough to increase susceptibility to illness.

My initial hypothesis was that ingestion of antibacterial toothpaste containing 0.3% triclosan could decrease the amount of the probiotic lactobacillus microflora of the human gastrointestinal tract. My second hypothesis was that the 0.3% triclosan used in the antibacterial toothpaste would not remain effective for 12 hours, as claimed on the label, due to the low concentration of the chemical.

PROCEDURE

The contents of a capsule of probiotic supplement containing 80% lactobacillus (HA-111) and 20% bifidus (HA-135)[iii] were grown in a nutrient broth[iv] for 24 hours in a incubator environment (37°C) and subsequently streaked in one direction on a blood agar plate (Columbia/ 5% sheep)[v] (BAP). This plate was incubated for 36 hours and lactobacillus colonies were identified based upon the double-ring beta-hemolysis observed, the colour, and the texture. Three lactobacillus colonies were then isolated and implanted onto a second BAP and incubated for 36 hours. A suspension was created with one colony of lactobacillus isolate and sterile water. A

bacterial lawn (0.08CFU/cm^2) was then produced with the suspension on a third BAP and incubated for 24 hours. Nine filter paper disks (0.6 cm in diameter) were inoculated with three of each; 0.1mL sterile water (control I), 0.1mL diluted (0.01mL) regular fluoride toothpaste (0.76% sodium monofluorophosphate) (control II), and 0.1mL diluted (0.01mL) Colgate Total antibacterial toothpaste (containing 0.3% triclosan and 0.243% sodium fluoride). The inoculated disks were then placed on the bacterial lawn. Zones of inhibition were measured at specified intervals and the experiment was repeated, in its entirety, twice more, to ensure repeatability.

RESULTS, OBSERVATIONS, AND CONCLUSIONS

Distinct zones of inhibition, containing no bacterial growth, were observed around the circumference of the filter paper disks inoculated with the Colgate Total antibacterial toothpaste containing 0.3% triclosan, subsequent to the creation of a pure lactobacillus field. No inhibition was observed about the two controls, thus indicating that the antibacterial product in the toothpaste may be the inhibitor. Measurements of the zones of inhibition surrounding each of the nine disks varied slightly, but were compared statistically using an ANOVA test, which demonstrated a 0.94051 probability of population relationship. This observation supports my first hypothesis that the antibacterial toothpaste inhibits the growth of the probiotic lactobacillus found in our gastrointestinal tract. This could cause an alteration in the fine ecological balance of probiotic and pathogenic microorganisms in the gastrointestinal tract, which could make one more susceptible to illness.[vi]

However, measurements of the mean zones of inhibition after 24 hours in comparison to the colony forming unit (CFU) content on the agar plate revealed that the actual amount of lactobacillus destroyed by the Colgate Total was very minute. A mean 15.33mm diameter zone of inhibition was present around disks inoculated with 0.01mL of toothpaste after 24 hours, on plates with a CFU content of 0.08CFU/cm^2. This indicates that with the average area of

inhibition approximately $2cm^2$, 0.16CFU of lactobacillus were killed. When converted to the average toothpaste serving size of 1mL**,** which is one hundred times the amount tested and probably more than would be ingested, only approximately 16CFU would be inhibited. This is a very miniscule amount, which can be easily replenished through taking supplements containing up to $16x10^9$ viable lactobacillus cells[vii], or through eating yogurt with active bacterial cultures, which is reported to contain a mean of $3x10^9$CFU/mL of probiotic bacteria at the time of manufacture.

A third observation might indicate that complete benefit from foods or supplements containing lactobacillus may not be attained for 32 hours subsequent to ingesting even 0.001mL of antibacterial toothpaste. The zones of inhibition continued to grow until approximately 24 hours of incubation. Visible colonies did not begin to grow in the inhibited regions until approximately 32 hours. These results were a rejection to my second hypothesis that the antibacterial agent would be effective for less than the claimed 12 hours and further enhances the common recommendation to have a diet containing yoghurts, cheeses, and other fermented milk products. The reasoning for this understatement of effectiveness by Colgate may lie in the discrepancy between a BAP and human mouth environment. In the mouth, constant salivation and swallowing is likely to decrease the time period of effectiveness of the triclosan as it is flushed from the mouth.

Although antibacterial toothpaste may not have a dramatic effect on the ecological balance of the gastrointestinal tract, it indeed inhibits bacterial growth in small amounts. Therefore I raise the question of whether, through this constant, small amount of exposure to the anti-bacterial agent triclosan, bacterial resistance may develop rendering the toothpaste inadequate in inhibiting the growth of any bacteria, probiotic or pathogenic.

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Endnotes (references cited)

[i] Levy, Stuart. "Antibacterial Household Products: Cause For Concern" December 2005, Centres for Disease Control and Prevention, www.cdc.gov .
[ii] American Medical Association. 2000. "Use of Antimicrobials in Consumer Products." Report 2 of the Council on Scientific Affairs
[iii] Swiss Natural Sources Probiotics purchased at Pharma Plus
[iv] Supplied by Grand River Regional Hosptial Pediatric Labs
[v] Supplied by Grand River Regional Hospital Pediatric Labs
[vi] Sullivan A, Edlund C, Nord CE, "Effect of antimicrobial agents on the ecological balance of human microflora"PubMed, September 2002. National Library of Medicine www.pubmed.gov.
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