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MICROBIAL PRODUCTS: NEW SOURCES FOR A PROMISING CONTRACEPTIVE AGENT

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Abstract: Contraception is a well-known conventional method for stabilizing the world population. Traditional contraceptive techniques that have been used for several years are associated with disadvantages and side effects. There is urgent need of improvements in prevailing birth control methods available to us. Various vaginal contraceptive agents marketed today are chemical or detergent based that are accompanied by numerous negative effects on vaginal flora and epithelium. Therefore, novel mode for birth control that is attaining limelight at the present time is the use of microbial products.

Introduction

Increasing incidences of unplanned pregnancies has put the reproductive health mission of United Nations at risk, which aims at safe child birth (UNFPA, 2013). Globally, unintended pregnancies result in 1 million elective abortions/ annum (Naz and Rowan, 2009). The most susceptible group comprises impoverished women and sexually active adolescents. The requirement of contraceptives for women is getting bigger as the biased status of men and women create a problem for women to reject unwanted sex or to discuss safer sex. To combat these problems, one such field that is being comprehensively exploited is the vaginal contraceptives. Due to nominal systemic involvement, it is considered as one of the harmless means of contraception. However, lack of inquisitiveness and novelty in this significant area of contraception made the existing methods obsolete, consequently reducing acceptability and efficacy. Recently, interest has been reinstated in this area to identify newer and safer birth control agents (Gupta, 2005; Naz, 2005).

At the present time, hundreds of vaginal contraceptives have been marketed but all of them incorporate a non-ionic surfactant nonoxynol-9 (N-9) as an active spermicide (Zaneveld *et al.*, 2002). However, frequent use of N-9 or other surfactants demonstrates detergent-type cytotoxic effect on cervicovaginal epithelium leading to inflammation of vagina and alteration of normal microbial flora of vaginal tract. Vaginal infections resulting from disturbance of the vaginal microbial milieu might increase probability of STI/HIV transmission (Martin *et al.*, 1999). Therefore, identifying new generation of products lacking membrane toxicity that could prevent unintended pregnancies has become the need of the hour.

As a plausible alternative to chemical/ detergent based vaginal contraceptives, innovative and promising candidates appear to be the products of microbial origin that can block or retard the motility of sperm. Contraceptive potential of few microbial peptides such as nisin,

subtilisin, lacticin and fermenticin have already been studied. This review aims at showing that these microbial products are worth pursuing as new and safe candidates for vaginal contraceptives.

Nisin

Nisin, a member of the lantibiotic family containing unusual amino acids produced by *Lactococcus lactis*, is a 34 amino acid cationic peptide with molecular mass of 3.5 kDa. The World Health Organization (WHO) and the US Food and Drug Administration (FDA) have granted GRAS (generally regarded as safe) status on the peptide (Delves-Broughton 1990, Hansen 1997) due to its worldwide use as a food preservative for the past 50 years. Nisin possesses high bactericidal activities and is reported to be nontoxic to humans (Breukink & Kruijff 1994). Although Breukink and Kruijff have extensively evaluated antimicrobial properties of nisin but its contraceptive potential was first reported by Clara *et al* (2004). Nisin was found to be spermicidal under *in vitro* conditions and potent contraceptive agent *in vivo*. *In vitro* studies by Clara *et al* (2004) showed that minimum concentration of nisin requisite to cause complete immobilization of spermatozoa within 20 s was 50 µg (rat), 200 µg (rabbit), and 300-400 µg (monkey and human). No regain in motility was observed when spermatozoa immobilized by nisin were resuspended in buffered glucose-saline indicating its spermicidal nature. When *in vivo* contraceptive efficacy of nisin was assessed by its intravaginal administration in rats and rabbits before mating, it was found to arrest sperm motility and protect against pregnancy at a concentration of 200 µg in rats (Clara *et al.*, 2004) and 1 mg in rabbits (Reddy *et al.*, 2004). For safety evaluation, 14 days subacute toxicity studies were performed which showed no adverse effects on either morphology of vaginal cells or any histopathological lesions, indicating its safety in animals. Thus the peptide with antibacterial activity could also be developed as vaginal contraceptive.

Subtilisin

Subtilisin, a bacteriocin produced by *Bacillus subtilis* ATCC 6633 and *Bacillus amyloliquefaciens*, is a ribosomally synthesized antimicrobial peptide with distinctive post translational configuration. It possesses bactericidal activity against an array of pathogenic organisms, comprising *Gardenerella vaginalis*, *Listeria monocytogenes*, and *Streptococcus agalactiae* (Sutyak *et al.*, 2008) but do not harm normal and healthy *Lactobacillus* vaginal microbiota. Besides having antibacterial activity, subtilisin as reported by Silkin *et al* (2008) also exhibits spermicidal activity against boar, bovine, horse, and rat spermatozoa. It was also shown to diminish the motility and forward progression of human spermatozoa in a dose dependent approach (Sutyak *et al.*, 2008). It also resulted in coiling of sperm tails suggesting plasma membrane damage. Also, human vaginal cell viability assays indicated safety of subtilisin for human use as compared to other available products. Therefore, subtilisin can be established as a valuable component for inclusion in human contraceptive products.

Lacticin 3147

Lacticin 3147, a lantibiotic antimicrobial peptide produced by *Lactococcus lactis*, consists of two components: LtnA1 and LtnA2. It exhibits antimicrobial activity spectrum same as that of nisin A (Martin *et al.*, 2004). Besides lacticin 3147 was capable of immobilizing horse/pony, bovine, and rat sperm at a concentration of 200 µg/mL (100 µg each of LtnA1 and LtnA2 in combination), and as little as 50 µg/mL was able to kill boar sperm within 30 s. Its spermicidal activity decreased considerably when two components, LtnA1 and LtnA2 were tested separately. However when used in synergism, they demonstrated excellent spermicidal activity (Silkin *et al.*, 2008).

Fermenticin Hv6b

Fermenticin HV6b, produced by *Lactobacillus fermentum* HV6b MTCC10770 isolated from vaginal ecosystem of humans, is an antimicrobial peptide belonging to class IIa bacteriocins. It targets wide array of pathogens associated with human bacterial vaginosis including *Bacteroides* species, *Candida albicans*, *Gardnerella vaginalis*, *Listeria monocytogenes*, *Staphylococci*, and *Proteus mirabilis* (Kaur et al., 2012) while leaving normal vaginal flora unaltered (Kaur et al., 2013). Studies by Kaur et al. (2013) also reported it as a spermicidal agent as it was found to halt motility and forward progression of human spermatozoa in a dose dependent way. At higher concentrations, it resulted in coiling of tails and sperm agglutination. These results provided strong evidence suggesting its potential to be used as a contraceptive agent.

Sperm Agglutinating Factors

By the same token, in our laboratory, sperm agglutinating factors (SAF) were isolated and purified from both *Staphylococcus aureus*. It was seen to agglutinate spermatozoa and impede various other sperm parameters *in vitro*. Matrix Assisted Laser Desorption Ionization-Time of flight (MALDI-TOF) and matching with mass spectrum in NCBI database showed sequence homology with hypothetical protein BACPEC_00178 of *Bacteroides pectinophilus* ATCC 43243 (Kaur and Prabha, 2013). When the *in vivo* efficacy of SAF from *S. aureus* was checked using mouse model it rendered female mice infertile at a concentration of 2.5 µg. Moreover no detrimental effects were seen on the vaginal cell morphology and vaginal epithelium (Aggarwal and Prabha, 2006).

Similarly, Spermagglutination factor isolated from *Escherichia coli* showed spermagglutinating and spermicidal properties *in vitro*. It also had negative impact on various other sperm parameters. Characterization of SAF and SAF binding receptor using MALDI-TOF showed its sequence homology to glutamate decarboxylase. Intravaginal administration of SAF at a concentration of 5µg in female Balb/c mouse led to infertility without producing any adverse effects (Kaur and Prabha, 2013).

Sperm Immobilizing Factors

Sperm immobilizing factor (SIF), isolated and purified from *S. aureus* could inhibit sperm motility *in vitro* (Prabha et al., 2009). Characterization of SIF using LC-MS (Liquid chromatography-mass spectrometry) showed that this 20 kDa protein had peptide sequence similarity with hsp-70 protein. Also, it could control pregnancy when intravaginally inoculated in female mice without causing any harmful changes on vaginal epithelium (data not published). Likewise, SIF isolated and purified from *E. coli* also had negative influence on sperm parameters. Characterization of SIF using Liquid chromatography –mass spectrometry (LC-MS) revealed its sequence similarity to chaperone protein HchA of *E. coli* O157:H7 (Vander et al., 2012). The negative influence of this factor could also be exploited to be used as contraceptive

Conclusion

In conclusion, sperm impairing ability of these factors of microbial origin, without disturbing the vaginal flora and morphology offers an advantage over chemical contraceptives to be incorporated in the list of birth control.

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