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Curcumin as a potential non-steroidal contraceptive with spermicidal and microbicidal properties

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ABSTRACT

Objective: Curcumin, a component of the curry powder turmeric, has immense biological properties, including anticancer effects. The objective of this study was to determine if curcumin can provide a novel non-steroidal contraceptive having both spermicidal and microbicidal properties.
Study design: The effect of curcumin, with and without photosensitization, was examined on human sperm forward motility and growth of several aerobic ($n = 8$) and anaerobic bacteria ($n = 4$) and yeast ($n = 7$) strains implicated in vaginosis, vaginitis, and vaginal infections in women. The effect of various concentrations of curcumin on human sperm and microbes (aerobic and anaerobic bacteria and yeast) was tested. The effect on sperm was examined by counting the sperm forward motility, and on microbes by agar and broth dilutions and colony counting. Each experiment was repeated using different semen specimens, and bacteria and yeast stocks.
Results: Curcumin caused a concentration-dependent inhibition of sperm forward motility with a total block at $\geq$250 μM concentration. After photosensitization, the effective concentration to completely block sperm forward motility decreased 25-fold, now requiring only 10 μM concentration for total inhibition. Curcumin concentrations between 100 and 500 μM completely blocked the growth of all the bacteria and yeast strains tested. After photosensitization, the effective concentration to completely inhibit microbial growth decreased 10-fold for aerobic bacteria and yeast, and 5-fold for anaerobic bacteria.
Conclusions: These findings suggest that curcumin can block sperm function and bacteria/yeast growth. It can potentially provide an ideal non-steroidal contraceptive having both spermicidal and microbicidal properties against vaginal infections.

1. Introduction

The population explosion and increasing rate of unintended pregnancies leading to elective abortions are major public health issues affecting resources worldwide [1]. Other serious health issues faced by sexually active women include sexually transmitted infections (STIs) [2], which increase with the use of hormonal contraceptives [3,4]. Over one-third of women develop vaginal infection(s) during their lifetime, the most common causes being bacterial vaginosis and yeast infections [5–7]. Vaginal infections have been implicated in various etiologies of female infertility, including immunologic infertility and miscarriages [8–10]. There is an urgent need to develop a better method of contraception that is non-steroidal and has antimicrobial properties.

Curcumin, also known as diferulolyl methane, is the yellow pigment and main component of the curry powder turmeric. It is not a "hot" spice, but rather a coloring material. Curcumin has been found to have antimicrobial and anticancer properties without any side effects [11–14]. Our laboratory recently published, for the first time ever, that curcumin affects sperm motility, function, and *in vitro* fertilization (IVF) in human and murine models [15]. Intravaginal administration of curcumin causes reversible contraception in mice [15].

The present study was conducted to determine: (1) if similar concentrations of curcumin are required for spermicidal and microbicidal effects, (2) if curcumin inhibits growth of microbes which cause aerobic vaginitis, bacterial vaginosis, and yeast infection, and (3) if photosensitization increases the spermicidal and microbicidal potency of curcumin, since the photosensitization at the maximum absorbance (A_{max}) of a molecule makes it

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more reactive to the target [16]. We hypothesized that the concentration range of curcumin to have spermicidal effects will be similar to that required for microbicidal activity, and photosensitization of curcumin will significantly enhance its potency. The long term objective is to develop a novel, non-steroidal contraceptive which has both spermicidal and microbicidal properties.

2. Materials and methods

2.1. Materials

Curcumin (>95% pure) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). A stock solution of curcumin (25–50 mM) was prepared in dimethylsulfoxide (DMSO) and used for all experiments. Modified sperm washing medium (SWM) was purchased from Irvine Scientific (Santa Ana, CA, USA). A light emitting diode (LED)-based device was purchased from LED Wholesalers (Hayward, CA, USA). Luria-Bertani (LB) broth/agar was purchased from Thermo Fisher Scientific. Yeast Peptone Dextrose (YPD) broth/agar, thioglycollate broth, and anaerobic blood agar plates were purchased from VWR International (Radnor, PA, USA).

2.2. Collection of sperm cells

Semen was collected from healthy, fertile men attending the West Virginia University Center for Reproductive Medicine. Semen was liquefied and analyzed for volume, sperm concentration, and percent and progressive motility. Only good semen samples were used in the present study [15,17]. This study was approved by the West Virginia University Institutional Review Board (IRB) for human studies and the consent was obtained to participate in the study.

2.3. Determination of effect of curcumin on sperm motility

The effect of curcumin on sperm forward motility was examined by incubating 10–100 μL of sperm suspension (250–500 × 10^6 motile sperm in SWM or seminal plasma) with various concentrations of curcumin (1–1000 μM final concentration) for 20 min. The percentage of forward moving sperm was recorded before and after incubation with curcumin every 5–15 min for 1 h. Controls were treated with an equivalent amount of DMSO. All experiments were repeated at least 3–5 times using sperm from 15 men, tested individually. The viability of sperm was tested by eosin-nigrosin staining [15].

2.4. Microorganisms

The microbicidal activity of curcumin was tested against eight aerobic bacteria strains: six Gram-negative, namely *Alcaligenes faecalis*, *Pseudomonas aerguinosa*, *Escherichia coli*, *Salmonella choleraesuis*, *Salmonella enteritidis*, and *Yersenia enterocolitica*, and two Gram-positive, namely *Bacillus subtilis* and *Staphylococcus aureus*; seven yeast strains, namely *Saccharomyces cerevisiae* YJM975, *Saccharomyces cerevisiae* YJM981, *Saccharomyces cerevisiae* YJM978, *Saccharomyces cerevisiae* 273614N, *Saccharomyces cerevisiae* 378604X, *Saccharomyces cerevisiae* 322134S and *Candida albicans*; and four anaerobic bacteria strains: one Gram-variable, namely *Gardnerella vaginalis*, two Gram-negative, namely *Fusobacterium nucleatum* and *Bacteroides fragilis*, and one Gram-positive, namely *Peptostreptococcus anaerobius*. All bacteria strains (aerobes and anaerobes) were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). Yeast strains were obtained from National Collection of Yeast Cultures (NCYC; Norwich, England). Five of these aerobic bacteria strains, all of these anaerobic bacteria strains, and four of these yeast strains have been shown to be involved in vaginal infections in women (discussed later).

2.5. Culture of bacteria and yeast strains

The concentration of curcumin required to completely block growth of aerobic bacteria and yeast strains was examined by the agar dilution method [18]. Different concentrations of curcumin in agar plates (10–1000 μM) were obtained by adding various amounts of curcumin stock to melted agar. Control plates consisted of standard agar plates without curcumin, and plates containing an equivalent amount of DMSO. Bacterial or yeast cultures (100–300 CFU/mL) were inoculated and the plates were incubated at 37 °C for 24 h for bacteria, or at 30 °C for 48 h for yeast. Bacterial and yeast growth was assessed by the traditional manual colony counts (CFU/mL). The concentration of curcumin required to completely block growth of anaerobic bacteria was examined by the broth dilution method [18]. Bacteria culture (100–300 CFU/mL) was inoculated into thioglycollate broth containing different concentrations of curcumin (10–1000 μM final concentration). Controls included standard thioglycollate broth without curcumin, and broth containing an equivalent amount of DMSO. Bacteria culture was spread onto blood agar plates. Plates were incubated (37 °C, 48 h) in an anaerobic chamber or in a candle jar for *G. vaginalis*. Bacteria growth was assessed by the traditional manual colony counts (CFU/mL).

2.6. Photosensitization of curcumin

For photosensitization experiments using sperm, sperm in SWM or seminal plasma (at the same concentrations and volume as described above) were mixed with various concentrations of curcumin (1–1000 μM final concentration), and the mixture was exposed to the LED device for 20 min. The percentage of forward moving sperm was recorded. The sperm photosensitized without curcumin served as control.

For photosensitization experiments using microbes, various microbes (aerobic bacteria, yeast, and anaerobic bacteria at similar concentrations as described above) were inoculated into growth medium containing various concentrations of curcumin (10–1000 μM final concentration), and the cultures were exposed to the LED device for 20 min. After exposure, microbial cultures were inoculated onto agar plates, incubated, and the microbial growth was assessed as described above. Cultures photosensitized without curcumin served as control.

2.7. Statistical analysis

All experiments were repeated at least 3–5 times using sperm from 15 men and various microbial stocks. The mean and standard deviations (SD) were calculated and statistical significance was examined using the two-way analysis of variance (ANOVA) and Bonferroni post-tests. Statistical analysis was performed using GraphPad Software (San Diego, CA, USA). A P-value of <0.05 was considered significant.

3. Results

3.1. Effect of curcumin on sperm motility

3.1.1. Un-photosensitized curcumin

Un-photosensitized curcumin caused a concentration-dependent decrease in sperm forward motility (Fig. 1, panel A). At lower concentrations (1–10 μM), there was no apparent effect ($P > 0.05$) on sperm forward motility up to 1 h of observation period. At 25