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Vaginal contraceptive activity of a chelated vanadocene

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Abstract

Bis(cyclopentadienyl) complexes of vanadium (IV) or vanadocenes are rapid and potent inhibitors of human sperm motility with potential as a new class of contraceptive agents. This study sought to determine the vaginal contraceptive activity of vanadocene dithiocarbamate (VDDTC), a stable vanadocene (IV)-chelated complex, using the standard rabbit model as well as the domestic pig as a physiologically relevant animal model for contraception. In experiment I, ovulating New Zealand White does in subgroups of eight were artificially inseminated (AI) with semen mixed with VDDTC (0.01–1 mM) or vehicle. In experiment II, ovulating does in subgroups of 18 were AI at 5 and 60 min after intravaginal application of a gel with and without 0.1% VDDTC or 2% nonoxynol-9 (N-9) (Gynol II, Ortho Pharmaceutical, Raritan, NJ), and allowed to complete term pregnancy. In experiment III, seven sexually mature Duroc gilts in standing estrus were AI with and without intravaginal application of 0.1% VDDTC gel microemulsion. Exposure of rabbit semen to VDDTC at the time of artificial insemination resulted in a dose-dependent reduction in fertility. Exposure of semen to 1 mM VDDTC led to complete inhibition of fertility as assessed by the number of embryos (control 49/94 vs. VDDTC-treated 0/117, $p < .0001$) or the percent embryos (52% vs. 0%, respectively) based on number of embryos to corpora lutea. Intravaginal administration of 0.1% VDDTC gel microemulsion or Gynol II prior to artificial insemination significantly inhibited term pregnancy rates (88% and 62% inhibition, respectively) when compared to control gel alone. Vanadocene dithiocarbamate gel microemulsion provided 80% inhibition of fertility even when insemination was delayed until 60 min after intravaginal application of VDDTC gel microemulsion. Rabbits that delivered litters despite intravaginal exposure of semen to VDDTC via gel microemulsion had healthy offsprings with no apparent perinatal repercussions. In domestic pigs, intravaginal applications of 0.1% VDDTC gel microemulsion prior to artificial insemination led to complete inhibition of fertility as assessed by the number of embryos (control 29/52 vs. VDDTC-treated 0/44, $p < .0001$) or the percent embryos (56% vs. 0%, respectively) based on the number of embryos to corpora lutea. These results suggest that VDDTC is a potent contraceptive agent in vivo. Intravaginal use of VDDTC via a gel microemulsion has clinical potential as a safe alternative to currently used detergent-type contraceptives.

Keywords: Vagina; Contraception; Vanadocene; Vanadium

1. Introduction

There is an urgent need, worldwide, for improved contraceptives, especially spermicidal contraceptives. All currently available spermicidal contraceptives have detergent ingredients that disrupt the cell membrane [1]. These include the neutral surfactants, nonoxynol-9 (N-9), menfegol and octoxynol-9, the cationic surfactant benzalkonium chloride and the anionic detergent sodium docusate. In the United States, this category is restricted to products containing N-9 or octoxynol-9 [2,3]. N-9 has been widely used for more than 30 years in over-the-counter gels, foams, creams and films designed to kill sperm [1–3]. N-9 exhibits both spermicidal and antibacterial/antiviral activities in vitro against pathogens responsible for sexually transmitted infections (STIs) [4–10]. However, several recent large-scale clinical trials have shown that vaginal contraceptives containing N-9 had no effect on the transmission of STIs when provided as part of an overall STI prevention program [11,12]. Furthermore, there is a growing concern that chemical irritation that disrupts the vaginal mucosa from repeated use of N-9 might actually increase the risk of STIs in sexually active women [13,14]. In addition to its membrane disruptive properties [15,16], ability to damage the cervicovaginal epithelium [17,18] and to cause an acute tissue inflammatory response [19], thereby enhancing the

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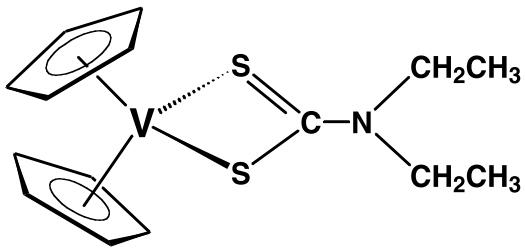
likelihood of STIs [12–14], N-9 also has a high contraceptive failure rate [20]. In several large studies, users of N-9 had first-year pregnancy rates ranging from 11% to 31%, making N-9 approximately 75% effective in preventing pregnancy [20]. Therefore, new, safe, effective and female-controlled topical spermicides lacking detergent-type membrane toxicity should have clinical advantage over the currently available vaginal spermicides.

In a systematic effort to identify nontoxic spermicides potentially capable of performing better and without the drawbacks of detergent-type spermicides, we have rationally designed and synthesized several disubstituted metallocene derivatives, where bis(cyclopentadienyl) moieties are positioned in a tetrahedral symmetry and in a bent conformation with respect to the central transition metal atoms. We discovered bis(cyclopentadienyl) complexes of vanadium (IV) or vanadocenes to have rapid, potent and selective sperm-immobilizing activity (SIA) [21–24]. We have extensively tested how chemical modification of simple inorganic vanadium salts alters the properties of vanadium [22–27]. Whereas non-vanadium-containing metallocenes and inorganic vanadium compounds lacked SIA, vanadocenes elicited potent SIA at nanomolar to micromolar concentrations [22]. The SIA of representative vanadocenes was 400-fold more potent than that of N-9, and unlike N-9, the SIA was not accompanied by membrane disruption at the ultrastructural level [22]. Biochemical studies performed using human, rabbit and boar sperm established that unlike inorganic vanadium compounds, which are only spermicidal in demembranated sperm, vanadocene-induced contact SIA of membrane-intact sperm was not associated with inhibition of dynein adenosine triphosphatase activity or inhibition of protein phosphatase activity [24,28].

We evaluated 45 vanadocene complexes for the effects of their oxidation state, geometry and cyclopentadienyl ring substitution on their SIA [21–27]. Since the stability of vanadocenes in aqueous media is greatly affected by the ancillary groups, we selected different ligands to test their effects on the SIA, lipid peroxidation, stability and solubility. Chelating effects of certain bidentate ligands substantially enhanced the stability of vanadocene complexes with monodentate ligands in aqueous solutions, particularly dithiocarbamate without the loss of SIA [23,27]. The lead vanadocene (IV)-chelated complex, vanadocene dithiocarbamate (VDDTC), was stable at room temperature with good solubility in gel microemulsion. Vanadocene dithiocarbamate is a disubstituted vanadocene with the two cyclopentadienyl rings positioned in a tetrahedral symmetry and in a bent conformation with respect to the central vanadium (IV) atom (Fig. 1, left panel). We have conducted extensive preclinical studies as well as physicochemical characterization of VDDTC by electron paramagnetic resonance spectroscopy, electrochemistry and X-ray crystallography studies [27]. The two sulfur atoms of the dithiocarbamate and centroids of the cyclopentadienyl rings in this vanadocene complex with unique contraceptive potential occupy four tetrahedral-like coordination sites about the central vanadium (IV) atom (Fig. 1, right panel).

Repetitive intravaginal application of VDDTC via a gel microemulsion at 1000- and 2000-fold higher than its spermicidal EC_{50} value did not induce local inflammation, toxicity or retention of vanadium in the rabbit vaginal irritation model [29]. Furthermore, repetitive intravaginal application of VDDTC via a gel microemulsion even at concentrations 5000-fold greater than its in vitro spermicidal EC_{50} value, for up to 13 weeks in mice, had no adverse effects on survival, growth, hematological and clinical chemistries, absolute or relative organ weights and histopathology [30]. Spermicidal vanadocenes did not affect the



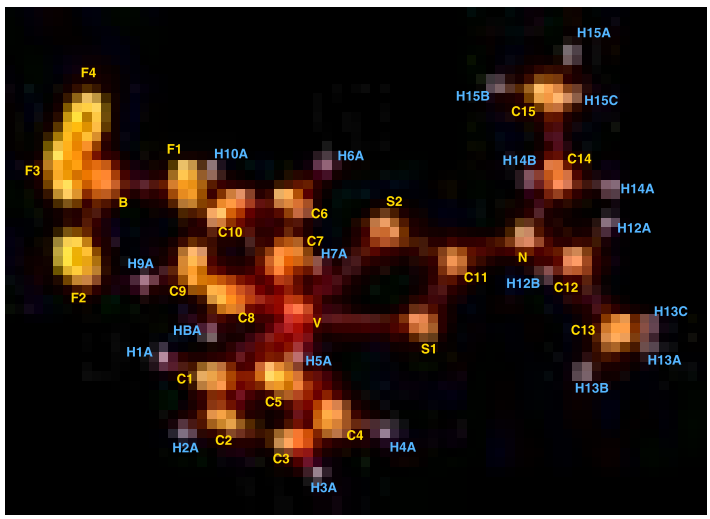


Fig. 1. Left panel, Chemical structure of VDDTC. Right Panel, Oak Ridge Thermal Ellipsoid Plot picture of the crystal structure of VDDTC salt. The three-dimensional solid-state structural characterization of VDDTC was achieved by single crystal X-ray diffraction [27]. Vanadocene dithiocarbamate is a "bent-sandwich" complex where the two cyclopentadienyl rings in a tetrahedral symmetry are positioned in a bent conformation with respect to the central vanadium (IV) linked to the organic ligand, thiocarbamate.