Contraception xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Contraception

journal homepage: www.elsevier.com/locate/con

Original research article

Effect of a novel copper-containing intrauterine device material on the endometrial environment in rabbits

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ARTICLE INFO

Article history:
Received 2 February 2018
Received in revised form 5 June 2018
Accepted 11 June 2018
Available online xxxx

Keywords:
Cu/LDPE/MVQ microcomposite
IUD
Contraceptive effectiveness
Adverse effects
Endometrium
Rabbits

ABSTRACT

Objective(s): This study aimed to determine whether intrauterine placement of a novel composite material [copper (Cu) microparticles, low-density polyethylene, and methyl vinyl silicone rubber (Cu/LDPE/MVQ)] could prevent pregnancy in rabbits, and to evaluate the effects of Cu/LDPE/MVQ on the endometrial environment.
Study Design: Eighty sexually mature female rabbits were randomly divided into four groups (n=20 each group): control (sham-operated), LDPE/MVQ, Cu/LDPE/MVQ microcomposite, and bare Cu. Ten rabbits from each implant-bearing group were randomly selected for a mating experiment beginning 30 days after insertion. Pregnancy outcomes were observed 15 days after mating. Factors associated with endometrial bleeding and inflammation in the remaining rabbits in each group, and the surface conditions of the implants, were investigated 90 days post-insertion.
Results: The Cu (0 embryo) and Cu/LDPE/MVQ (0 embryo) groups had significantly fewer embryos than the LDPE/MVQ (1.0±0.6 embryos, $p<.05$) and sham-operated groups (4.1±1.3 embryos, $p<.05$). Compared with bare Cu, the Cu/LDPE/MVQ composite material was associated with considerable reductions in injuries and factors associated with abnormal endometrial bleeding and inflammation, such as matrix metalloproteinase 9 (MMP9) and prostaglandin E2 (PGE_2). Additionally, the surface of implanted Cu/LDPE/MVQ remained much smoother than that of implanted bare Cu.
Conclusion(s): This novel Cu-containing intrauterine device material exhibits a similar effect in prevention of pregnancy with bare copper, and lower levels of inflammatory markers.
Implications: This study demonstrates the potential of the novel Cu/LDPE/MVQ microcomposite material as a future substitute for conventional intrauterine device materials.

1. Introduction

The copper intrauterine device (Cu-IUD) is the most extensively used long-acting reversible contraceptive worldwide [1, 2]. A growing body of evidence suggests the appearance of a Cu ion "burst release" within the first few months after Cu-IUD insertion, followed by a gradual decrease and stabilization of Cu ion release [3, 4]. This large release of Cu ions into the uterine cavity is thought to correlate with the adverse effects of Cu-IUDs, such as menorrhagia, intermenstrual bleeding and spotting. Furthermore, constant corrosion leads to the deposit of various products on the surfaces of Cu-IUDs, which may also contribute to side effects [5, 6]. Therefore, although the conventional Cu-IUD achieves a desirable contraceptive efficacy among women of reproductive age, continued use of the device is complicated by numerous side effects, including intermenstrual bleeding, pelvic pain, menorrhagia, and uterine perforation.

Polymer matrix composites with superior abilities to control the release of copper ions have been developed in an attempt to eliminate the disadvantages of the conventional Cu-IUD [7, 8]. Low-density polyethylene (LDPE) and methyl vinyl silicone rubber (MVQ) have been extensively applied in various biomedical contexts because of their excellent biocompatibilities and mechanical properties [9–11]. MVQ is widely used in orthopedic and cardiovascular devices [12], while LDPE has been included in polymer matrix composites, such as the Cu/LDPE nanocomposite IUDs described in our previous studies [13–15]. However, these earlier IUDs did not resolve the aforementioned disadvantages of Cu-IUDs [16]. Therefore, to overcome the disadvantages of conventional Cu-IUD materials, in this study we evaluated the usefulness of a Cu/LDPE/MVQ microcomposite developed in our laboratory as a novel IUD material [17]. Despite previous analyses, however, it

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https://doi.org/10.1016/j.contraception.2018.06.002

remained unclear whether this novel Cu/LDPE/MVQ microcomposite meets the requirements for a contraceptive device and whether this material adversely affects the endometrial environment.

Therefore, the aims of this study were to determine whether intrauterine placement of the novel composite material Cu/LDPE/MVQ could prevent pregnancy in rabbits, and to evaluate the effects of Cu/LDPE/MVQ on the endometrial environment by measuring the expression of inflammatory markers, such as matrix metalloproteinase 9 (MMP9) and prostaglandin E2 (PGE_2), and factors associated with endometrial bleeding, such as angiopoietin-2 (ANG2), tissue plasminogen activator (t-PA), and CD34.

2. Materials and methods

2.1. Materials

The IUD component materials were formed by the Department of Materials Science and Engineering of Huazhong University of Science and Technology. Briefly, a Cu microparticle (micro-Cu)/LDPE/MVQ composite was constructed using physicochemical methods. Using a melt-blending process, the MVQ and LDPE powders were combined with high-quality micro-Cu (15% weight) in a single-screw extruder at a screw speed of 20–25 rpm. The extruder was maintained at temperatures of 115°C, 140°C, and 155°C from the hopper to the die, respectively. The composite material contained uniformly distributed micro-Cu within a framework comprising the LDPE/MVQ matrix. The spacing within the matrix provides osmotic pathways for Cu ions and corrosion mediation. Accordingly, the corrosion rate and Cu ion release velocity are effectively controlled by the separation of micro-Cu within the matrix. Three different materials were formulated: micro-Cu/LDPE/MVQ, LDPE/MVQ, and bare copper (Cu).

2.2. Animals and treatment

Sexually mature female and male rabbits weighing 2.5–3.0 and 3.0–4.0 kg, respectively, were purchased from the experimental animal center of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. The rabbits were maintained under controlled conditions (12-h light/dark photoperiod; temperature: 23±2°C, 50±10% relative humidity) and allowed access to food and tap water ad libitum. The rabbits were acclimated to this environment for 5 days prior to the experiments. All of the animal experiments in this study were performed according to the National Institutes of Health Guiding Principles in the Care and Use of Animals, and the protocols were approved by the Reproductive Medicine Review Board of Tongji Medical College, Huazhong University of Science and Technology.

Eighty sexually mature female rabbits were randomly assigned to four experimental groups (n=20/group): sham-operated (SO) control, bare Cu (Cu), LDPE/MVQ, and Cu/LDPE/MVQ; the latter three groups were used to evaluate the contraceptive effects and safety of the implanted bare Cu and microcomposites, respectively. Before insertion, rabbits in the Cu, LDPE/MVQ, and Cu/LDPE/MVQ groups were anesthetized, and then the designated material was inserted into the caudal portion of the right uterine horn and secured to the uterine wall with sutures via laparotomy and uterotomy. The contralateral horn was left untreated. Rabbits in the SO group underwent the same surgical procedures without the implantation of material into the uterine horn. All implanted materials were approximately 1 mm in diameter and 5.9 mm in length, with an approximate surface area of 20 mm^2.

The rabbits' estrous cycles were monitored daily by vaginal lavage, and a microscopic cellular stage assessment was used to calculate cycle length. Ten rabbits from each material-bearing group were randomly selected for a mating experiment beginning 30 days after insertion of the implant. The selected female rabbits were allowed to cohabit with male rabbits, and successful mating was assessed by a light microscopic examination of vaginal swabs during the mating period. Subsequently, the animals were sacrificed and pregnancy outcomes were observed via uterotomy on day 15.

The remaining rabbits in each material-bearing group were killed via laparotomy on day 90 post-insertion. The uterine tissues were quickly removed, placed on ice, dissected, and frozen at -80°C. The remaining tissues were fixed for histopathological examination.

2.3. Immunohistochemistry (IHC) and Western blotting

The effects of the different implanted materials on MMP9, CD34, and ANG2 proteins in endometrium were analyzed using immunohistochemistry (IHC) and western blotting. The methods are detailed in the supplementary material.

2.4. ELISA

The concentrations of PGE_2 and t-PA in the endometrial tissues of the rabbits were determined as described in a previous report [14]. Briefly, tissue samples were homogenized on ice, centrifuged for 15 min at 4°C (3500 rpm), and examined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) according to the manufacturer's instructions. The absorbances in the ELISA plate wells at 450 nm were measured using a microplate reader. Data were expressed as the means±S.D. of three independent experiments.

2.5. Determination of the surface characteristics of the implanted materials using scanning electron microscopy

Following removal from the rabbits, the surface conditions of the Cu/LDPE/MVQ microcomposite and Cu implants were characterized via scanning electron microscopy (SEM). All samples were gilded before examination. For comparison, the surface features of original (i.e., non-corroded) Cu/LDPE/MVQ microcomposite and Cu materials were also examined using the same process and instrument. At least three replicates of each type of material were evaluated.

2.6. Statistical analysis

Data from repeated experiments were presented as means±S.D.. SPSS (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. A one-way analysis of variance or chi-square test was used for the statistical evaluation, as appropriate. A $p<.05$ was considered statistically significant.

3. Results

3.1. Fertility trial

Table 1 presents the results of the fertility trial. We detected no embryos in the uterine horns of rabbits fitted with Cu alone or Cu/LDPE/

Table 1
Fertility test of different materials

Group (n=10 each)	No. of embryos in material-bearing uterine horn (mean±S.D.)	No. of embryos in contralateral uterine horn (mean±S.D.)	No. of pregnant animals	Fertility rate (%)
SO	4.1±1.3*	3.8±0.9	10	100
LDPE/MVQ	1.0±0.6#*	4.6±1.3	7	70
Cu/LDPE/MVQ	0#	4.2±1.4	0	0
Cu	0#	3.4±0.8	0	0

SO, sham-operated; LDPE/MVQ, low-density polyethylene/methyl vinyl silicone rubber; Cu/LDPE/MVQ, copper microparticle/low-density polyethylene/methyl vinyl silicone rubber; Cu, copper.
* $p<.05$ when compared to the Cu group.
# $p<.05$ when compared to the SO group.

Please cite this article as: Hu S, et al, Effect of a novel copper-containing intrauterine device material on the endometrial environment in rabbits, Contraception (2018), https://doi.org/10.1016/j.contraception.2018.06.002