



RISUG[®] based improved intrauterine contraceptive device (IIUCD) could impart protective effects against development of endometrial cancer

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ARTICLE INFO

Keywords:

Uterine carcinoma
Intrauterine contraceptive device
RISUG[®]
Lipid membrane

ABSTRACT

Intrauterine Contraceptive Devices with multifaceted application potential is a need of an hour. Although, copper-based IUDs exert an effective contraceptive as well as anticancer effects in a long-term basis, but also results in multiple complications. In this regard, RISUG[®] a polymer based contraceptive device has been introduced as a suitable alternative. However, its potential to impart protective effects against **development of endometrial cancer still remains unexplored**. This article presents the hypothesis on this unexplored domain and provides scientific facts to support the hypothesis. The mechanism of anticancerous activity is hypothesized that RISUG[®] involves its lipid membrane destabilizing activity. This activity is modulated by both, the cellular microenvironment and lipid bilayer composition. Acidic environment along with the significantly higher fluidic nature of lipid bilayer of the cancerous cells make them more prone to lipid solubilisation effect of RISUG[®]. We here present an in-depth insight into the factors that would favour faster solubilisation of cancer cell membrane, thereby exerting an anticancer effect.

Introduction

Endometrial cancer is considered as fifth most common occurring cancer in women with over 500,000 women diagnosed with it in the year of 2012 worldwide [1]. The mortality rate was 1.7–2.4 per 100,000 women. Women, in their postmenopausal stages, usually are at high risk of endometrial cancers due to excess of endogenous or exogenous estrogen without adequate opposition by progestin. Other risk factors include tamoxifen therapy, obesity, and nulliparity. Notably, the appearance and prognosis of endometrial cancer is not just limited to postmenopausal stages. Around 14% of endometrial cancer causes were determined their identity in premenopausal women and 5% of women less than 40 years old were also diagnosed [4–7]. The clinical treatment for the same includes cyclic gestagen administration, curettage, hysterectomy, lymphadenectomy, radiotherapy, and systemic chemotherapy [2]. However, these procedures are costly, time consuming and often leads to severe side-effects to the patients [3]. Thus, an effective way of cancer management is needed which have effective strategies to prevent cancer during initial stages along with preliminary therapies and diagnosis.

In the recent years, female contraceptives (hormonal or non-hormonal) have emerged as an important component of endometrial cancer management. Continuous use of copper-based IUDs and medroxyprogesterone acetate (DMPA) showed a significant decrease in the endometrial proliferative or cell mitotic activity, thereby imparting protective effect against development of endometrial cancers [4]. IUDs induces morphological and biochemical changes in the endometrium and confers safety against uterine cancer without causing alteration in indigenous estrogen and progesterone levels [5–7]. On the other hand, DMPA confers protective effects via increase in progesterone levels [4]. However, the constant use of copper-based IUDs has also been shown to induce complications such as uterine perforations, pelvic inflammatory diseases, expulsion, improper bleeding, cramping pain, etc. [8]. While long-term use of DMPA could increase the risk of generating cardiovascular diseases due to DMPA associated changes in lipid metabolism [9].

To tackle these complications, the researchers have come up with the use of polymeric biocompatible contraceptive hydrogel named as RISUG[®] (Reversible Inhibition of Sperm Under Guidance). RISUG[®] is composed of Styrene Maleic Anhydride (SMA) polymer dissolved in

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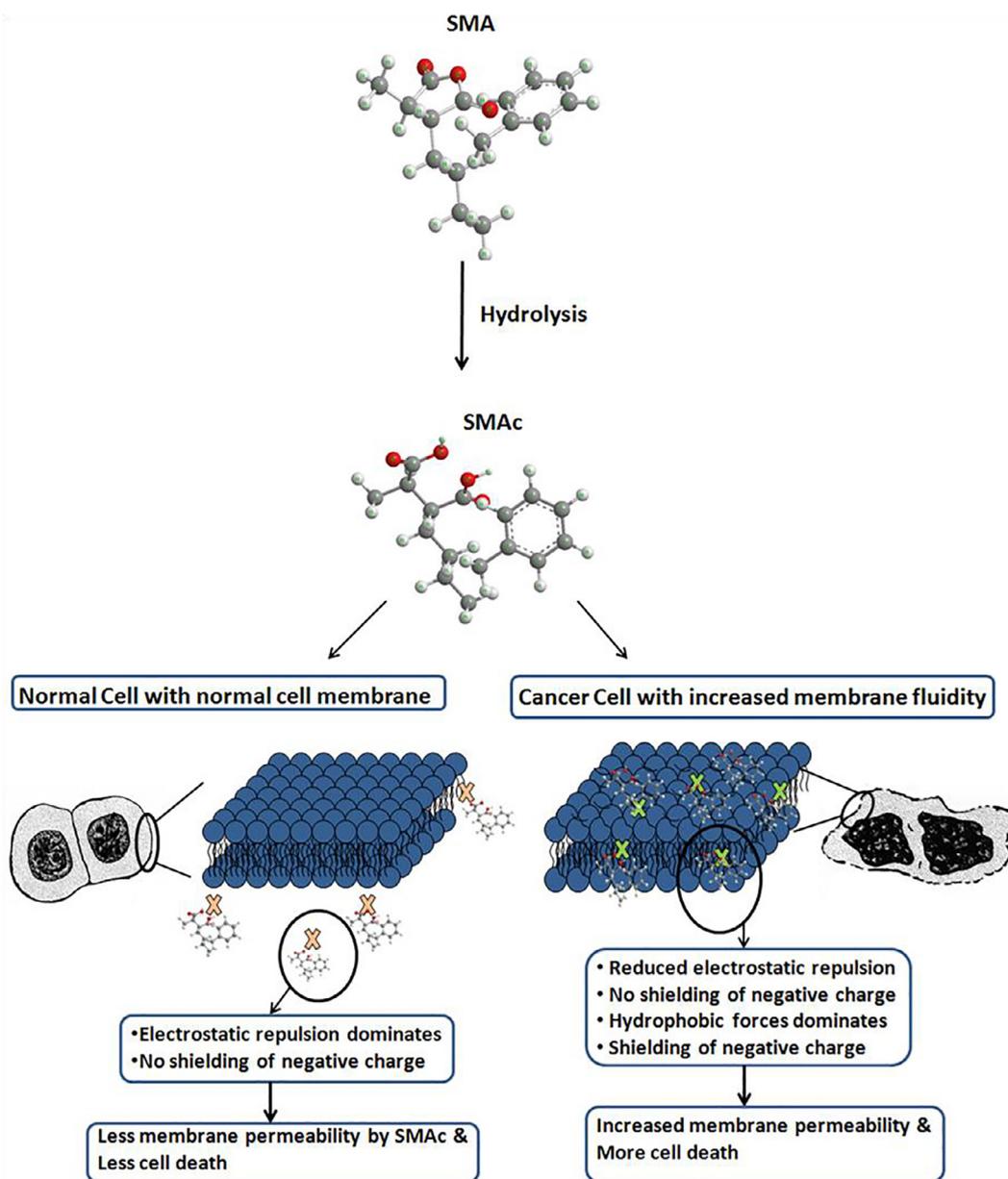


Fig. 1. Membrane destabilization activity of Styrene Maleic Acid (SMAc) derived from Styrene Maleic Anhydride (SMA) in normal and cancer cells.

(DMSO) at a proportion of 1:2. Various short-term and long-term pre-clinical studies conducted in Langur monkeys showed both, the contraceptive potency (99.9 mg–400 mg) and biosafety of RISUG[®] [20–24].

Drug regulatory agency of India approved evaluation of RISUG[®] for phase I and II clinical trials in various centres in India followed by an extended phase III (varied concentration range from 5 mg to 60 mg) clinical trial as a male contraceptive implant [13,16,25,26]. RISUG[®] post implantation inside vas deferens has been reported to provide contraception for more than 10 years without causing any adverse effects [15]. The contraceptive mechanism of RISUG[®] has been explained in various research works. Briefly, SMA of RISUG[®] post implantation inside the vas deferens gets hydrolyzed due to the reaction with water molecules in seminal fluid to produce Styrene Maleic Acid (SMAc). This conversion of SMA to SMAc has the tendency to reduce the pH of area inside the vas deferens which is in immediate vicinity with RISUG[®]. The SMAc mediated pH lowering effect is expected to reduce the motility of the sperm. This process of SMA hydrolysis produces free hydrides inside the vas deferens that helps to generate cationic charge to which anionic molecules will be attracted. Since the membrane of sperm is anionic in

nature, this attraction towards cationic charge exerts a charge imbalance over membrane of sperm that ultimately damages the sperm membrane [10]. At about 0.001 gm in male Albino rats, no tissue damage was observed post infusion of the SMA polymer in vas deferens of test animals [11]. Teratological evaluation done in albino Wister rats (implanted with RISUG[®]) showed that it is safe to use as male contraceptive hydrogel [27]. At a concentration of 0.01 gm of subcutaneous implantation in Albino rats the polymer has not shown excessive incompatibility to the tissues. Furthermore after removal of the implanted polymer post 21 days of vas deferens infusion, the animals had normal morphology of mucosal layer following after 14 days of removal [12]. Implantation of RISUG[®] in the fallopian tube of New Zealand white Rabbits and uterine horns of Sprague-Dawley rats (concentration of 8.03 mg/Kg) has also been reported to show excellent biocompatible property [18,28]. RISUG[®] as a polyelectrolytic hydrogel for the establishment of female contraception is implanted along with the flexible form of Teflon tubelets. These tubelets are designed in order to resist the expulsion post filling it with the RISUG[®]. The soft polymeric RISUG[®] filled tubelets as a form of Improved intrauterine contraceptive device

could able to adopt itself inside uterine cavity which will avoid perforations unlike commercially available copper containing Intrauterine contraceptive device [13]. Moreover, RISUG[®] has also been shown to have selective antimicrobial property to act only against pathogenic bacteria which would prevent reproductive tract infections [18]. However, apart from its contraceptive potential, whether it could exert protective effect against development of endometrial cancers similar to that of copper-based IUDs/DMPA is still not been addressed.

In the present manuscript, we would like to raise the hypothesis that RISUG[®] based IUDs could also exert protective effects against development of endometrial cancer. We also defend our hypothesis on the solid theoretical facts.

Hypothesis and its theoretical validation:

Interaction of RISUG[®] with phospholipid membrane:

The basis of this hypothesis lies in the structure, composition and physiochemical characteristics of RISUG[®] polymer. RISUG[®] mainly consists of Styrene Maleic Anhydride (SMA) [14]. Upon exposure to the biological fluids, it undergoes hydrolysis, thereby, gradually getting converted into Styrene Maleic Acid (SMAc) [15]. This SMAc is an amphipathic molecule consisting of hydrophobic styrene units and hydrophilic carboxyl/carboxylate (COOH/COO⁻) groups [16,17]. The carboxyl groups of the maleic acid unit have 2 pka values; first at pH 6 and other at pH 10 which plays vital role in determining the charge of the SMAc molecules depending on the pH of the surrounding medium [17].

The interaction between phospholipid membrane and SMAc molecule is driven by the hydrophobic interactions between phenyl group of SMAc and lipid fatty acid chains of phospholipid membrane. This interaction is modulated by the electrostatic forces between anionic charges from partially deprotonated maleic acid groups of the SMAc and phosphate head of the phospholipids [18–20]. The binding is followed by insertion of SMAc deeply into the hydrophobic core of the phospholipid membrane. This insertion results in the destabilization of the cellular lipid membrane architecture followed by formation of vesicular intermediates and membrane solubilization to disc-shaped nanostructures. The interaction of RISUG[®] with lipid membrane is an interplay between the composition of both, RISUG[®] polymer (ratio of SMAc units) and lipid membrane (phospholipid type and chemical characteristics of fatty acyl chains) [21]. In addition, the pH of the surrounding medium also plays a crucial role, thereby affecting the interaction of the polymer with the lipid membrane (Fig. 1) [21]. At alkaline pH, the carboxyl groups of SMAc are deprotonated which causes electrostatic forces to dominate over the hydrophobic forces, thereby restricting incorporation of the polymer into the lipid membrane. On the other hand, at acidic pH values, the protonation of the carbonyl groups causes loss of electrostatic repulsion with dominating hydrophobic effect which results in faster incorporation of SMAc molecules with phospholipid membrane.

Interaction of RISUG[®] with normal and cancerous cells:

Several studies have already shown that the SMAc interacts with the mammalian cells and exerts effects based on cellular microenvironment and architecture [21,22]. In this regard, both normal and cancer cells share a number of differences. We made these differences as the base to validate our hypothesis pertaining to the potential anticancer effect of RISUG[®] polymer.

Effect of cellular microenvironment on RISUG[®] incorporation:

Under normal conditions, the ionic and pH homeostasis is maintained with a lower intracellular pH (~7.2) and a higher extracellular pH (~7.4) [23]. However, under cancerous conditions, poor vascular perfusion, regional hypoxia, and fermentative glycolysis results in

reversed pH gradient with higher intracellular (~7.9 to 7.4) and lower extracellular pH (~6.4 to 6.8). Interaction of SMAc with the acidic external environment of cancer cell would result in the shielding of its negative charges. Such decrease in the charge density would cause dominating hydrophobic forces to induce faster incorporation of the SMAc polymer into the lipid bilayer of the cancerous cells which may leads to cytolysis through membrane destabilization (Fig. 1). On the other hand, the external alkaline pH in case of normal cells would resist its incorporation due to the electrostatic repulsion between the negatively charged polymer and negatively charged cell membrane. Previously Scheidelaar et al. has also demonstrated a similar result wherein a decrease in pH of the medium significantly increases the degree of SMAc incorporation into the phosphatidylcholine (PC) lipid membrane [24]. Here, it is important to mention that an optional ratio of SMAc units in the polymer is essential for efficient incorporation into lipid membrane. The variation in the lipid composition significantly alters the physiological behavior of the cells and also alters their response to the chemotherapeutic drugs.

Effect of membrane lipid composition on RISUG[®] incorporation

Apart from the differences in their microenvironment, both the normal and cancer cells also differ in their lipid bilayer composition and also vary significantly between the different cancer types [25]. In this section, we would discuss the efficacy of RISUG[®] as an anticancer agent of different cancer types.

Drug-sensitive cancer cells v/s normal cells. The cell membrane of cancer cells has marked decrease in the molar ratio of cholesterol (C) to phospholipids (PL) [25]. The molar C:PL ratio for healthy normal cells is estimated between 0.6 and 1.0, while for cancer cells, it varies in between 0.31 and 0.46 [25,26]. Such a reduction in the cholesterol levels results in the fluidity of the plasma membrane. These cancer cells also show an increase in short and unsaturated acyl chains (C16:0 and C18:1, n-9) in the bilayer of the membranes which further provide a hint regarding an increase in their fluidity [26–28]. In addition to this, the membranes of the cancerous cells have a disordered symmetrical distribution of lipids [28]. In normal cells, the phosphatidylethanolamine (PE) and Phosphatidylserine (PS) are exclusively localized in the inner leaflet of the membrane, while in the cancerous cells, both, PE and PS also get distributed to the outer leaflet [28]. Such an alteration in the lipid composition and distribution in the bilayer affects its functionality and permeability of the phospholipid membrane. Peetla et al. has stated that the presence of high concentration of PE on the outer leaflet increases the membrane permeability of the cancer cells [28].

Another aspect that needs to be highlighted here is that the lipid membrane of the cancer cells have a significant increase in the negative charge density on their surface due to the localization of PS and increased the concentration of sialic and folic acid [29,30]. Such an increase in the negative charges could slightly alter the interaction of SMAc with the bilayer. But the acidic pH of the microenvironment could also protonate these negatively charged groups and reduce the degree of electrostatic repulsion between polymer and cancer cell membrane.

Previous studies have shown that the presence of short chain fatty acid, lower cholesterol levels in the lipid membrane, and reduced electrostatic repulsion between membrane and SMAc allows faster membrane solubilization [25]. Based on these ground, we could hypothesize that the increase in membrane permeability along with an increase in membrane fluidity would allow faster incorporation of the SMAc in the drug-sensitive cancerous cells in comparison to the normal cells. This would eventually lead to membrane permeabilization, thereby disrupting the ionic balance and allowing unrestrained flow of molecules inside-out of the cell. Such alteration in the cellular environment would ultimately trigger cellular apoptosis/necrosis [31,32]. A similar membrane destabilization and permeabilization has recently

been reported by Tan et. al. as a potential strategy for exerting anticancer effects. The study made use of PE-binding peptides and other small molecules (ophiobolin A) that bind specifically to the ethanolamine head group of PE and facilitate the formation of transmembrane pores and eventually lead to cell death [33].

Multidrug-resistant (MDR) cancer cells v/s normal cells. In case of MDR cancer cells, the fluidity of cell membrane significantly decreases due to marked increase in cholesterol level [34]. The reports have shown up to 50% increase in the cholesterol in case of MDR cells as compared to normal cells [35]. The MDR cells also have high concentration of sphingomyelins, phosphatidylinositol and cholesterol esters [36]. The increased rigidity of the membrane would restrict the incorporation of the SMAc into the membrane of MDR cells [31]. Moreover, higher cholesterol concentrations have been shown to significantly decrease the solubilization of the lipid membranes [24,32,37,38]. Based on these ground, we could hypothesize that in MDR cancerous cells would show greater resistance towards RISUG[®] in comparison to the normal cells.

Conclusion

With the current technological advancements, the market demands for the devices with multi-speciality applications. In this regards, extensively used copper-based IUDs have been shown to exert both contraceptive as well as long-term anticancer effects. However, these copper-based IUDs induce multiple complications including uterine perforations, pelvic inflammatory diseases, expulsion, etc. These disadvantages have lead to the exploration of polymer-based IUDs such as RISUG[®]. Although, contraceptive effect of RISUG[®] is well proven; but no literature is available for its anticancer potential. This article focuses on providing scientific facts in order to validate its anticancer potential post-implantation in the uterine cavity.

Both, the microenvironment and lipid membrane composition affects the incorporation of RISUG[®] into the cell membrane thereby resulting in the cell death. For cancerous cells, the acidic microenvironment and highly fluidic nature of the lipid bilayer results into faster incorporation of RISUG[®]. On the other hand, the normal cells maintain a slight alkaline environment along with higher membrane rigidity which resists easy penetration of RISUG[®]. This provides a hint regarding the specificity of the RISUG[®] polymer towards the cancerous cells. Interestingly, the higher cholesterol concentration of MDR cancer cells, owing to significant rigidity of the lipid bilayer, would reduce the incorporation of RISUG[®] into these cell types.

The proposed hypothesis should further be validated by *in vitro* and *in vivo* studies in order to establish RISUG[®]-based IUDs as an effective anticancerous contraceptive implant. First, a dose dependent anticancer activity of the RISUG[®] polymer needs to be analyzed for both cancerous and normal cell types. The polymeric hydrogel RISUG[®] is a site specific implant in order to provide contraceptive effect. The authors would like to claim that when RISUG[®] is implanted inside uterus as a non-hormonal female contraceptive hydrogel, the sustained exposure of RISUG[®] at uterus could able to prevent the development of uterine cancer in the very early stage. A very minimal concentration of therapeutic drug RISUG[®] could be able to establish anti-cancerous effect due to the early stage of cancer development. The exact concentration at which RISUG[®] could able to establish anti-cancerous effect has to be determined through (i) membrane damage (cell membrane staining by carboxy SMARF[®]-1 dye), (ii) cell morphological and complexity changes (microscopic or flow cytometry-based analysis), (iii) activation of apoptotic/necrotic pathways (western blot based analysis), etc.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2019.02.026>.

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