Review Article

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RISUG: An intravasal injectable male contraceptive

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Over the last two decades RISUG has been drawing attention in the field of male contraception. It promises to sterile men for a period of up to 10-15 years. According to recent studies in animal models, it proves to be completely reversible. Practically, there are no better options available that can assure complete sterility and precise reversibility. Regardless of so much of information available, RISUG is still holding up for many reasons, firstly, the available information engender bewilderment such as what is this copolymer, how does it work and is reversal really possible? Secondly, advancement of this outstanding invention is drastically slow and thirdly, effects of long-term contraception with RISUG and reports on evaluation of anomalies (if any) in F_1, F_2 progenies, are lacking. In this review the lacunae as well as advances in the development of RISUG in the light of published work and available resources are pointed out. Formulation of the RISUG, its mode of action and clinical trials have been addressed with particular emphasis.

Key words Clinical trials - male contraception - RISUG - reversal - sperm - styrene maleic anhydride

Introduction

The burden of population control has been generally borne by women while men fall behind due to lack of efficient and acceptable contraceptive. Available methods are mostly dependent on permanent vasectomy and widely used condoms that are reluctantly accepted in men's world. In early 80s Misro *et al*[1] came with a revolutionary occlusive polymer which was claimed to sterile subjects by single injection of styrene maleic anhydride (SMA) dissolved in dimethyl sulphoxide (DMSO), in both vas deferens; it was named as RISUG (Reversible Inhibition of Sperm Under Guidance) under impression that the contraception with SMA can be reversed at any time following vas occlusion. However, mode of action of SMA and the mechanism of bio-adhesion and bio-sustainability in the vas deferens was poorly explained. Early experiments carried out in rats and monkeys showed loss of fertility by so called pH-lowering effect and convinced to be an effective contraceptive, however, morphological alterations in vas deferens were also documented[2-4]. A study on vas occlusion with RISUG demonstrated 100 per cent sterility within 15 days post injection in rats providing evidence of morphological aberrations in sperms, which included nuclear membrane damage in acrosome, loss of segmented columns and numeric aberration in centriole of the neck, degeneration of mitochondrial sheath and axoneme in the mid-piece and absence of plasma membrane in the mid-piece and tail[5]. This was perhaps the first study to document functional reversal of contraception with RISUG through dimethyl sulphoxide (DMSO)[5]; further, sodium bicarbonate

($NaHCO_3$) was used as another mode of reversal which also showed complete resumption of fertility (unpublished observation). Issues such as mutagenicity, genotoxicity and carcinogenicity are being evaluated at many centres in India. The clinical trials conducted in the last two decades have been drastically slow. Phase I clinical trial reported only the dose regimen and efficacy in terms of sperm count along with behavioural and general observation such as discomfort and scrotal enlargement[6]. The phase II clinical trial also provided limited information such as sperm count, behavioural and general observation, sperm motility, however, was added for the first time, numbers of subjects were also reduced by more than half compared to phase I[7]. Ongoing extended phase III trial results provided some critical information in 25 subjects which included dose of regimen, abstinence from sex, semen volume, sperm count, sperm morphology, germ cell morphology along with biochemistry of fructose, acid phosphatase and α-glucosidase[8]. Phase III clinical trial revealed variation in duration before subjects became azoospermic. Of the 25 subjects, six became azoospermic after 1 month, 15 after 2 months and other 4 in 3-4 months. Aberration in sperm morphology, for the first time was termed as the charge-related effect following the hypothesis given in US patent[8,9]. The term 'partial occlusion' by RISUG was also firstly coined in phase III clinical trial by the same group indicating lower epididymal neutral α-glucosidase and normal fructose level in seminal plasma[8].

Components of the vas-based injectable contraceptive 'RISUG'

RISUG has had a history of names; such as, an injectable non-occlusive chemical[1,2], an injectable non-occlusive contraceptive device[10], *p*H lowering polymer-styrene maleic anhydride (SMA)[11], an anti-fertility agent-SMA[4], RISUG[12], styrene maleic anhydride[13], RISUG hydrogel[14] and finally smart RISUG[15]. Its constituents are as defined 60 mg of SMA dissolved in 120 µl of DMSO (1:2)[4-9]. To understand this contraceptive and its effectiveness, it is important to know the polymer by its components and manufacturing.

Styrene: Styrene ($C_6H_5CH=CH_2$) is a derivative of benzene of molar mass 104.15 g/mol, it evaporates easily and has a sweet smell. The presence of vinyl group allows styrene to be precursor to polystyrene and several other copolymers. It was first isolated from a Turkish tree named oriental sweetgum (*Liquidamber orientalis*). Commercially, it is produced by dehydrogenation of ethylbenzene and oxidation of ethylbenzene hydroperoxide. There is inadequate information available to prove its carcinogenicity in human, however, the US National Toxicology Program (NTP) has described it 'reasonably anticipated to be a human carcinogen'[16]. According to hazardous substances database (HSDB)[17] the oxide form of styrene (Styrene 7,8 oxide) binds to DNA and shows genetic effect. The workers exposed to styrene were detected with presence of styrene 7,8 oxide in their blood (HSDB)[18]. Material safety data sheet (MSDS) shows that exposure to styrene vapour causes irritation in eyes and throat[19]. According to the monograph (monographs.iarc.fr/index.php) on the evaluation of the carcinogenic risk of chemical to man, styrene exposed people have been found with increased chromosomal aberration in their white blood cells. Further studies have shown that the liver, kidney and the haematological abnormalities have neither been linked to styrene toxicity nor does it have any teratogenicity or spontaneous abortion.

Maleic anhydride: Maleic anhydride [$C_2H_2(CO)_2O$] is the acid anhydride of the maleic acid with an acrid odour. It is manufactured by oxidation of benzene or N-butane and has a molar mass of 98.06 g/mol. It generates half easter (*cis*-HOOC–CH=CH–$COOCH_3$) in presence of alcohol and produces maleic acid (*cis*-HOOC–CH=CH–COOH) when hydrolyzed. Upon exposure it causes irritation in skin and eyes. In HSDB[17] maleic anhydride has been classified as non carcinogenic, however, workers exposed in manual processing of polyester lacquers containing maleic anhydride without safety precautions suffered acute poisoning which was manifested as nervous, respiratory, and cardiovascular systems disorders[20]. In another study[21], workers sensitized by one acid anhydride, trimellitic anhydride, could possibly react immunologically to two other acid anhydrides, phthalic anhydride (P) or maleic anhydride (M). In ELISA based cross-inhibition studies, trimellitic anhydride conjugated to human serum albumin (TM-HSA) inhibited IgE binding to TM-HSA, but when 100 times more P-HSA or M-HSA was used, no significant inhibition occurred.

Styrene maleic anhydride (SMA): Styrene maleic anhydride [$(C_8H_8)_n$-$(C_4H_2O_3)_m$] also called Xiran is a crystal clear polymer of variable molar mass and soluble in alkaline solution and polar organic solvents. It is a synthetic polymer built up of perfectly alternating, styrene and maleic anhydride monomers, making it an alternating copolymer. The copolymer is transparent, heat resistant, with high dimensional stability and specific reactivity of the anhydride groups. These

characters allow it to be used in plastic application, manufacturing of poly-methyl methacrylate (PMMA), acrylonitrile butadiene styrene (ABD) and polyvinyl chloride (PVC). When dissolved in dimethyl suphoxide (DMSO) it can penetrate the cells and adhere to it. Based on this property of SMA, RISUG was created. There is no information available about the copolymer in HSDB.

Dimethyl sulphoxide (DMSO): DMSO is organosulphur compound with chemical structure $(CH_3)_2SO$. It dissolves both polar and non-polar compounds and gives garlic like taste. It also has ability to penetrate skin without damaging the cells and is used for administration of many medicinal drugs by clinician. Apart from its ability to restrict DNA to form secondary structure, it is also used as a cryopreservative for stem cells[22]. With its valuable use for its ability to penetrate cells it has also been considered as a potential hazardous chemical, DMSO by itself is considered low toxic but with compounds dissolved in it can cause severe toxicity[23]. DMSO is listed in hazardous substances database, however, no serious toxicity has been reported in human, there are animal data which show varied responses to the DMSO. DMSO produces widespread apoptosis in the developing central nervous system[24], and DMSO exposure to developing mouse brains can produce brain degeneration[24]. Substances dissolved in DMSO may be quickly absorbed; larger amount of DMSO can have adverse effect on relative tissues or skin.

Reversible inhibition of sperm under guidance (RISUG)

There is no published chemical structure of the RISUG available. There have been constant advancements in the development of RISUG during the last two decades after the technology was patented. According to the patent[9], contraceptive was referred to as an injectable fluid of a copolymer of SMA in a solvent DMSO. The copolymer was prepared through cobalt 60 gamma irradiation of the monomers styrene and maleic anhydride in presence of nitrogen in ethyl acetate at a dose of 0.2 to 0.24 megarad for every 40 g of polymer at a dose rate of 30 - 40 rad/sec. The radiation provides a range of polymers of varying molecular weight along with combination of biological effect. For injectable viscosity of 1.5 - 1.9 pa relative to DMSO correspond to a molecular weight ranging from 60,000 - 100,000 g/mol[9]. Inside the vas the anhydride copolymer hydrolyzes in presence of water molecules in the spermatic fluid, this hydrolyzed SMA has a *p*H of 4.0 - 4.5. It is also hypothesized that DMSO helps in the penetration of polymer into the folds of inner wall of vas deferens and provides retention. According to patent information, the injection comprises 40 - 60 per cent (w/v) of SMA providing reason that if less then 40 per cent (w/v), the SMA will freely flow inside the vas, however, if more than 60 per cent (w/v), it will be highly viscous, difficult to inject. Hence, the ratio suggested for styrene and maleic anhydride was 1.2:1 and 1:1. Other ratios were also suggested with different mode of action such as 2:1 which would function mainly as an occlusion device, whereas, 1.5:1 would provide excessive charge but reduced stability[9]. Further details were produced in subsequent research papers such as gamma irradiation of monomers at 0.3 Gy/s at 37°C with a total dose of 2.4 Gy and precipitation of copolymer by petroleum ether and soxhelt distillation using 1,2-dichloroethane[25]. In another study, 50 mg of SMA was dissolved in 100 µl of DMSO (1:2) to obtain RISUG hydrogel and kept for 48 h to dissolve properly and then precipitated with distilled water[14]. Similarly, a study detailed about the time of purging in N_2 after the SMA dissolved in ethyl acetate, which was 5 min[15]. Smart RISUG contained two new components Fe_3O_4 and Cu, by adding 10 per cent (w/v) iron oxide and 5 per cent copper powder in mixture of SMA and DMSO in 1:30 ratio (Fe_3O_4-Cu-SMA-DMSO), in addition, the solution was continuously stirred for 48 h at 35°C, however, molecular weight for this compound was not provided[15]. Another paper in the same year by the same authors indicated molecule bindings as SMA-Fe_3O_4-Cu-DMSO which differed from the binding shown earlier[15,26]. In one study styrene and maleic anhydride mixture in ethyl acetate was shown in 1:1:7 ratio indicating an optimum total dose of gamma irradiation as 2.4 kGy, which was used for RISUG synthesis; this statement differed from the previously reported 2.4 Gy irradiation[27]. Jha *et al*[27] also reported that dose rate and total dose interrelation plays an important role in irradiation based polymeric drug and its acceptability in biological use. Whether RISUG results in any toxicity is not reported extensively, however, genotoxicity, mutagenicity and carcinogenicity studies have been carried out at many centers in India[28]. A recent study on Wistar albino rats for a long-term evaluation of genotoxicity of RISUG has revealed that it is unlikely to produce any DNA damage following injection of RISUG and its reversal[29]. Further studies are required in this area for effective quality control of RISUG.

Mode of action

The US patent reports that the manner by which the contraceptive works is not fully understood, and

it still continues to be ambiguous. However, a few not completely established methods have been reported regarding its mode of action in the following years, such as, partial occlusion, complete occlusion, *p*H lowering, charge effect, sulphur moiety and protein-SMA agglomerate[9].

Partial occlusion: As explained earlier, the ratio of styrene and maleic anhydride and the concentration of SMA in DMSO perform differently in a bio-environment. Higher concentration of styrene and/or higher w/v concentration of SMA will completely occlude the vas deferens whereas lower concentration of styrene alone and SMA as a whole would freely flow inside the vas and could be easily flushed out[9]. This implies that the dose regimen of 1:1 of styrene and maleic anhydride and 40 - 60 per cent (w/v) of SMA in DMSO would not completely block the vas deferens. In phase III clinical trial, neutral α-glucosidase, a biochemical marker for epididymis, was estimated to be gradually decreasing by nearly 8-folds in a period of 6 months post-injection, but not completely absent in the ejaculated seminal plasma, and on the other hand normal range of acid phosphatase and fructose levels were recorded[8]. The authors concluded that the mode of occlusion by 60 mg styrene maleic anhydride dissolved in 120 µl of DMSO (1:2) was 'partial' and not 'complete'[8], however, no further study was published to support this statement.

Complete occlusion: Studies[5,30-34] have been consistently showing evidence of azoospermia in animal models, with doses in exactly same 1:2 ratio. In one study, following vas occlusion by RISUG in rat for short-term complete sterility was noted on 7th day post-injection and azoospermia after 90 days post-injection[5]. Azoospermia can only be achieved if there are no sperms in ejaculates, in case of vas occlusion with RISUG, where all control were proven fertile subjects, can only show azoospermia if the vas is completely occluded. However, before azoospermia the animal models have shown aberration in the ejaculated sperm morphology, which make no sense if it is not passing through the occlusion or came in contact of RISUG[5]. In case of vasectomy, the sperms are still observed in the ejaculates for up to 6 months post-vasectomy and there have been numbers of pregnancies reported even after vasectomy, which entail that there must be a pool of sperm load in distal vas deferens. Hence it is logically admissible that the vas may be completely occluded following injection of RISUG in 1:2 ratio, however, further studies are required. Flickinger[35] provided evidence which was contradicting to the hypothesis of complete occlusion, the study showed that in case of vasectomy in rabbit the sperms accumulated in the male duct system for six months and later large numbers of lysosomes in the epithelium of the caput and cauda epididymis and the proximal vas deferens were perhaps degenerated and engulfed by the corresponding epithelial cells. The study also reported that due to the stress of sperm load epithelial cells of cauda epididymis and proximal vas deferens showed folding of the epithelial and formation of long apical projections which was not observed in case of vas occlusion by RISUG.

pH lowering effect: In 1985, Carr *et al*[36], published a study on effects of *p*H on sperm motility in different species, and provided interesting information with respect to the RISUG. The study showed varied effects on motility at physiological *p*H and non-physiological *p*H. The inhibition of cauda epididymal sperm motility of dog (*p*H 5) was at higher *p*H comparing to that of the bull (*p*H 4), addition of 15mM lactate shifted the *p*H to nearly 2-fold higher, whereas rat, hamster and guinea pig showed inhibition at *p*H 4 with or without lactate. They also reported about human ejaculated sperm which showed gradual increase in motility with increasing *p*H, at *p*H 7.5 it showed optimal motility, addition of lactate inhibited sperm motility completely at *p*H 4, however, without lactate the ejaculated sperm showed motility. The epididymal quiescence factors are important for sperm motility and are greatly influenced by *p*H of semen, weak acids such as lactate mimics the inhibitory effect of cauda epididymis[37]. The hydrolyzed RISUG in the vas deferens is claimed to have a *p*H of 4.0 - 4.5 which is likely to lower the motility but would it completely immotile the sperms is an important question[9]. The damages reported in RISUG treated sperms do not show a *p*H lowering effect, however, whether or not it affected motility is not clear.

Charge effect: 'Stability of the suspension is a function of the charge', this theory has highlighted that the velocity of small particles in a suspension in electric field has a correlation with the phenomenon of flocculation where the velocity is minimum[38]. In 1903, Lillie[39] showed that in a medium, near the neutral point sperms tend to migrate towards the positive pole and has concluded that sperm has a negative charge. Walton's study showed that with increasing *p*H the migration of sperm towards cathode decreases, it also indicate that greater velocity of sperm in alkaline medium will enable a mutual repulsion due to the charge[38]. Sperm surface charge has always given importance assuming

its significance in capacitation and fertilization of egg. Using positively charged ferric oxide it is possible to locate species specific negative charge distribution on different region of sperm surface[40]. It is to be noted that in seminal plasma during capacitation, the process of removal of surface protein occurs which eventually reduces the net negative charge on sperm surface[41]. It is known that RISUG has an acidic *p*H but it should still contain a positive charge to disturb the claimed negative charge of sperm. According to Guha[9], the anhydrons copolymer, when injected in to the vas deferens, hydrolyzes in the presence of water molecules in the spermatic fluid. The formed hydride generate positive change which attract negatively charged sperms and thus results in to membrane charge imbalance. This theory has not been studied very well and requires attention. Another hypothesis given by Guha[9] is that upon conversion to hydride, chloride ions are no longer kept out which allows water to flow in and cause swelling of plasma membrane and rupturing. Guha also reported that the presence of water in the spermatic fluid generated loss of charge; however, more studies are required in this area. Guha[9] also claims that the free flowing charge would not have similar effect on sperm; hence, the RISUG has the property of membrane bound positive charge[9].

Oxidative stress: Damaged acrosome, loss of segmented columns and numeric aberration in centriole of the neck, degeneration of mitochondrial sheath and axoneme in the mid-piece and absence of plasma membrane in the mid-piece and tail are the normal damages found in RISUG treated sperms following vas occlusion[30,42]. These damages are very much similar to that of the damages caused by oxidative stress. The generation of excess of intracellular or extracellular reactive oxygen species (ROS) such as, O_2^-, H_2O_2, $ROO^\bullet$, $OH^\bullet$ is associated with many cell damages, including morphological defects, DNA fragmentation, lipid peroxidation, decrease in acrosome reaction and fusiogenic ability and impaired fertilization[43-46]. Human spermatozoa are specifically vulnerable to oxidative stress due to its unsaturated fatty acid containing plasma membrane, particularly docosahexaenoic acid. Damage in plasma membrane of RISUG treated spermatozoa could represent an oxidative damage[47] (Figure). When ROS attacks the double bonds associated with these unsaturated fatty acid, lipid peroxidation chain reaction becomes operational, which leads to loss of membrane fluidity and loss of sperm function. Lipid peroxidation (LPO) is most common expression of oxygen activation which is catalyzed by Fe^{3+}, Cu^+ and O_2^- [47-49], in general, the most significant effect of LPO in cells is perturbation of membrane structure and function. Besides membrane effects, LPO can also damage DNA and protein through oxidation of DNA bases, however, there is no published report on DNA damage in sperm treated with RISUG[50]. The oxidative damage also occurs in mitochondria and mitochondrial

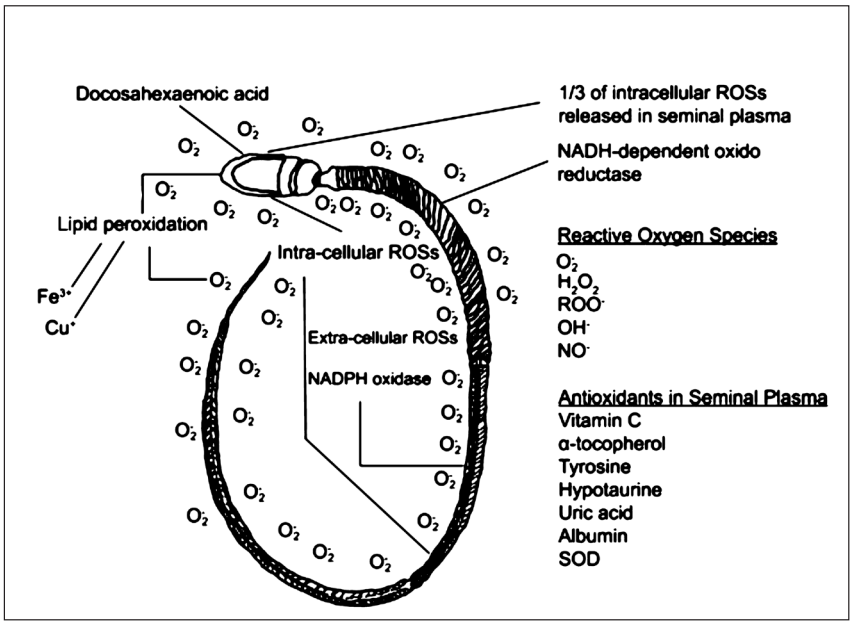

Fig. Diagrammatic representation of reactive oxygen species (ROS) and total antioxidant capacity (TAC) localization in human sperm. SOD, superoxide dismutase.

DNA, in addition, the redox status of spermatozoa is also likely to affect phosphorylation and ATP generation[51]. The stimulation of NADPH-dependent ROS generation in human appears to regulate the acrosome reaction through tyrosin phosphorylation. Two ROS-generating systems have been introduced, an NADPH oxidase-like system at the sperm plasma membrane level, and a sperm diaphorase which is an NADH-dependent oxido reductase located in the mid piece and integrated in to the mitochondrial respiratory system of the sperm[52]. Glutathione peroxidase a selenium-containing antioxidant, has been shown to have a vital role in sperm mitochondria[53]. It is reported by Calvin *et al*[54], that selenium in rat sperm is associated with cysteine-rich structural protein of the mitochondrial capsule. Glutathione plays a likely role in sperm nucleus decondensation and microtubule formation in the ovum, which eventually affects pregnancy[55]. The seminal plasma is a rich source of antioxidants which restrict the oxidative insults, such as, vitamin C, α-tocopherol, tyrosine, hypotaurine, uric acid, albumin, superoxide dismutase (SOD) and glutathione peroxidase 5 (GPx5), research shows that long-term exposure to seminal plasma is detrimental to motility and sperm survival[56,57]. SOD increases DNA fragmentation of sperm, rather than decreasing the oxidative stress[58]. Excess free radicals generated by the spermatozoa of infertile patients reflect underlying defect in Sertoli cells. It is known that a complete healthy morphology of sperm depends on oxidative stress status (OSS) which is between ROS and total antioxidant capacity (TAC). Observed damages in RISUG are similar to that of the damages caused by imbalance of oxidative stress status (OSS), it indicates that there could be a possible crosslink between *p*H lowering, charge distribution and oxidative stress status[42].

Clinical trials

After approval from the drug regulatory agency of India (DCGI), the phase I clinical trial was initiated at a few centers. Male volunteers were enrolled who were injected with RISUG and became sterile for many years. The longest duration of the RISUG bearer was more than 10 years. The phase I and phase II clinical trials were published in 1993 and 1997[6,7]. An extended phase III clinical trial has been launched and a report has been published in 2003[8].

Phase I: Healthy adult male volunteers with normal reproductive system were subjected to RISUG injection. Following medical examination, about 7 mm incision was made in the scrotal skin and the vas was exposed and a 23-gauge needle was inserted pointing distally and different doses of polymeric drug were injected. After one week interval following injection clinical assessment and semenology was periodically performed[6]. The available information about the subjects was for more than two years. Of the 38 subjects, none was undergone semen volume, *p*H, sperm motility, viability, morphology and biochemical analysis. The only information available from phase I clinical trial was sperm count and behavioural study. Injection of 5 - 40 mg SMA did not induce contraception in any subjects, however, from 60 - 140 mg SMA showed varied responses which eventually led to azoospermia. Best results were seen for 70 mg SMA dose which showed azoospermia in nearly three weeks and the subjects stayed azoospermic for 292 days[6] (Table).

Phase II: A total of 12 healthy male volunteers were similarly operated as in phase I and injected with fixed dose of 60 mg of SMA. Pre- and post-treatment semen examinations were carried out which included sperm count, motility and morphology. Individual data for semen analysis were examined for a period of nearly one and half years. The administration of 60 mg of SMA resulted in azoospermia beyond 12 months and an immediate contraceptive effect was seen[7] (Table). A two year clinical efficacy trial with variable doses (40, 50, 60, 65 and 70 mg of SMA) of RISUG was carried out in 20 subjects who were other than those inducted into the formal phase II study[12]. Subjects were monitored for the maximum of 1407 days, among 20 subjects one had a normal child after 145 days post-injection, reason for which was given as slippage during injection. Report suggested that all subjects maintained good health during the course of vas occlusion with RISUG, except one case which showed pelvic inflammatory disease, later treated successfully. It was estimated that dosages ranging from 40 to 70 mg of SMA were effective in giving >2 years of fertility control regardless of their azoospermic or non-azoospermic stage.

Phase III: An extended phase III clinical trial is ongoing. Chaki *et al*[8], showed a short-term evaluation of semen and accessory gland function. The number of subjects was 25 out of 141 enrolled volunteers and the report was limited to a period of 6 months[8,28]. Healthy adult male volunteers were subjected to RISUG administration, a dose of 60 mg SMA dissolved in 120 µl of DMSO (1:2) was administered. Semen and biochemical analysis were done for a period of six months post-injection. The results were predominantly

Table. Clinical trials conducted on RISUG: Summarized information

	No. of Subjects	Dose regimen	Semen volume (ml)	Sperm count (million/ml)	Motility (%)	Sperm morphology	Semen biochemical parameters					
							Fructose µ/Mol/eja. (estimated range)		Acid phosphate U/ml (estimated range)		α-glucosidase mU/ml (estimated range)	
Phase I[6]	38	5 to 140 mg	not reported	For 60-140 mg dose azoospermia was reported during 20-389 days post injection	Not reported	Not reported	Not reported		Not reported		Not reported	
Phase II[7]	12	60 mg	not reported	All subjects were azoospermic within 5-243 days	Duration: 10-193 days; motility: sluggish-zero- azoospermic	Head defect, cytoplasmic droplet and tail defect have been observed	Not reported		Not reported		Not reported	
Extended Phase III[8]	25	60 mg	Period: Pre-treatment; volume (ml): 3.0 ± 0.5	All subjects were azoospermic within 30-120 days	Not reported	Immotile sperms with different abnormal morphology were observed after RISUG injection	Period: Pre-treatment	Concentration: 15 ± 3	Period: Pre treatment	Concentration: 130 ± 50	Period: Pre-treatment	Concentration: 9-80
			Period: Post treatment 60 days; volume (ml): 2.0 ± 0.5				Period: Post treatment 60 days	17 ± 2	Post treatment 60 days	180 ± 10	Post treatment 60 days	0-8
			Period: Post treatment 180 days; volume (ml): 1.75 ± 0.5				Post treatment 180 days	16.8 ± 2.2	Post treatment 180 days	170 ± 10	Post treatment 180 days	0-2.5

Values are mean ± SD

showing immotile and abnormal spermatozoa in all subjects after injection of RISUG. Abnormalities such as bent and coiled tail, amorphous head with large elongated tail, double and tepering head, *etc*. were found. Immature germ cells were reported in all samples including azoospermic samples. Further, the volume of semen was also distinctly declined in all subjects. In addition, the semen biochemistry indicated normal functioning in both prostate and seminal vesicle, however, none of the subjects showed neutral α-glucosidase activity within normal range, it was reported to be significantly lowered. The presence of immature germ cells, occasional sperm or both, was considered as only partial patency. The sperms escaped from the occlusion were found morphologically damaged and without any motility[8] (Table).

Reversibility

At higher *p*H solution RISUG tend to dissolve easily, *p*H ranging from 8-9 in a solution mainly unstable the components of RISUG and allows it to unbound from the wall of vas deferens. DMSO and sodium-bi-carbonate ($NaHCO_3$) are used to dissolve SMA[2,59]. Reversibility of RISUG continued to be an issue, studies carried out in Langur monkeys indicated that complete reversal was achievable by non-invasive reversal technique, the procedure involved percutaneous squeezing of the vas deferens, along with synchronized application of electrical stimulation to the vas segment, supra-pubic percussion and pre-rectal digital massage of the ampullary region of the vas, to loosen the intravasal SMA deposits and push them towards urethera[30-33,60]. However, the same technique is difficult for use in human, as the human vas is difficult to palpate beyond scrotum. Hence, DMSO and $NaHCO_3$ have been used to flush the RISUG out from vas deferens through urethra. The reversal was carried out by flushing the RISUG from urethra by injecting 200 - 500 µl of DMSO or 5 per cent $NaHCO_3$ in to the vas deferens[5,59]. In our earlier study a short-time vas occlusion and reversal was conducted to evaluate the teratological aberration and reversibility of RISUG by DMSO in Wister albino rats. It was reported that following vas occlusion the vaginal smear showed detached heads and tails, acrosomal damage, bent in mid-piece, bent tail and morphological aberrations, which returned to normal within 90 days of reversal with DMSO and 100 per cent fertility was recorded[5]. Larger amount of DMSO can have adverse effect on relative tissues or skin, hence, $NaHCO_3$ could be a better alternative for lessening the RISUG binding to the epithelial cells of vas deferens and to flush out.

Other concerns

RISUG's cell damaging property is not only limited to sperm but it also damages bacteria and possibly viruses. Another component of styrene maleic anhydride has bactericidal property such as Poly(styrene-alt-maleic anhydride)-4-aminophenol. This is being considered as a modified RISUG for female which acts as a contraceptive and bactericidal. RISUG has been reported for exfoliation of the epithelial cells of the vas deferens which recovers in a few months after reversal; however, more attention is required to confirm the time of recovery and extent of exfoliation[13]. Long-term treatment with RISUG and its impact on individual's reproductive health and offspring following reversal along with carcinogenicity and mutagenicity, are major concerns. The teratological evaluation of F_1 and F_2 progenies after reversal must be carefully studied, there are only animal data available showing no physical abnormalities in F_1 progeny[5].

Conclusion

At present, there are limited approaches available for men. The search of an ideal contraceptive for male is still an elusive goal. Among all available approaches for men, the vas based methods are mostly appreciated, vasectomy accounts for more than 20 per cent of the current methods of contraception in male. The RISUG has surely created a new concept of contraception with great feasibility and long lasting sterility. Unfortunately, the advancement of this injectable polymer is slow, the clinical trials are not providing enough robust conclusions. It is, however, understandable that treatment with RISUG and its follow up in human subjects is a difficult task for reasons such as, subjects normally do not want to talk about it after injection unless there are any complications, the follow up must be done for a long time to report polymer's efficacy and side-effects, after reversal the F_1 generation is difficult to track, and overall the clinical trial for RISUG is long run to ascertain its contraceptive and reversal effectiveness. Although many leads have been taken towards making of an effective male contraceptive, many of these failed, many of these succeeded at first and then failed, many are still struggling for recognition, RISUG on the other hand, provides a hope which has a slow pace and drawbacks but it is in a right direction.

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