Reversibility With Sodium Bicarbonate of Styrene Maleic Anhydride, an Intravasal Injectable Contraceptive, in Male Rats

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A copolymer of styrene and maleic anhydride (SMA) was dissolved in dimethyl sulfoxide (DMSO) and injected into the lumen of the vas deferens of rats. The polymer was retained in the lumen for a period of more than 90 days. Sodium bicarbonate (10%), pH 8.9, was used to flush the polymeric material from the vas deferens lumen. The reversibility and potency of the vas deferens was thus obtained. Sodium bicarbonate proved to be an effective alternative to dimethyl sulfoxide for reversal.

**KEY WORDS:** contraceptive, reversibility, styrene maleic anhydride (SMA), dimethyl sulfoxide, sodium bicarbonate

**Introduction**

Styrene maleic anhydride (SMA) has been used as a male intravasal contraceptive agent both in animals and in humans. The polymer, once injected into the lumen of the vas deferens, has long term effectiveness. In rats, it has been shown to be effective for >6 months, in monkeys, for >1 year, and in humans, >1 year. Evidently, the compound is retained even though it is not sclerosing or tissue adhesive. Therefore, removing the compound and restoring fertility is possible.

Injecting the solvent dimethyl sulfoxide (DMSO) has effected earlier removal of SMA. Although this approach to removal has been found to be effective, an exploration of other reversal possibilities, using compounds that are more routinely used in general clinical medicine than DMSO, is important. Clinical acceptability of any new methodology is higher if it employs drugs with which the clinical community is already familiar. It is with this objective that the chemical properties of SMA were first investigated.

Theoretically as well as experimentally, it was seen that SMA is unstable in the basic pH environment. Scanning the medical pharmacopoeia, sodium bicarbonate was identified as a suitable candidate compound to wash out the SMA. It is commonly used and is of a basic pH in the range 8–9 in a solution concentration of 10%. The present study ascertained whether injection of 10% sodium bicarbonate solution into the lumen of the vas deferens removed SMA from the lumen, allowing unaffected spermatozoa passage and restoring fertility.

The reversal procedure was carried out early in the post-SMA injection period before any loss of polymer occurred due to chemical and biological breakdown processes.

The reversal study in rats was conducted 2 months after the SMA injection to establish fertility control.

Earlier reports on this subject indicates that the dose of 1 mg of SMA injected into the lumen of the vas deferens is effective as a contraceptive agent for 6 months. When the SMA methodology is finally clinically used, the duration of contraceptive action desired may vary over a wide range depending upon the individual user demand. Sometimes a dose higher than the minimum effective dose may be delivered on this account. The reversal technique should be such as to restore fertility even when higher than the minimal dose has been injected. To generate information on this point, the present study investigated sodium bicarbonate mediated reversal not only with the standard 1 mg SMA treatment but also twice the standard dose, i.e., 2 mg of SMA.

**Materials and Methods**

**Animals**

A total of 40 adult Wistar rats, weighing approximately 150 ± 10 g and of proven fertility, were
obtained from the animal house of the All India Institute of Medical Sciences for experimentation. They were divided into two groups: 1) group A, 20 rats injected with 0.001 g of SMA, and 2) group B, 20 rats injected with 0.002 g of SMA. They were then caged and kept under a controlled photoperiod of 14 h light and 10 h dark. Food and water were allowed ad libitum.

Infusion of the Polymer
The rats were anesthetized by inhalation of ether (supplied by Krishna chemicals, Bombay). In all cases, a single cut was made just above the urethral opening to expose the vas deferens. The vas deferens was accessed in the inguinal region, as the lumen of the vas deferens in the scrotal region is too small for convenient injection. Using a microsyringe of 250 μL and 23-gauge needle, the polymer dissolved in DMSO was injected toward the distal direction of the vas deferens while maintaining a slight compression on the proximal side. After an appropriate amount of the drug had been injected, the needle was withdrawn and, with the help of fingers, compression was maintained on the vas deferens at a site proximal to the testis from the injection point to prevent any retrograde flow to the drug.

Postcontraceptive Assessment
Allowing for a 2-week postoperative recovery period, each injected male rat was then caged with two female rats. Vaginal smears of the females were taken daily for 2 weeks and examined microscopically for spermatozoa. Thereafter, the females were caged separately and kept under observation for determination of pregnancy. After confirming that vaginal smears did not have spermatozoa and also that the females did not show evidence of pregnancy over a period of 1 month after mating, the males were used for other investigations.

Five rats each from groups A and B were killed and their reproductive system, particularly the vas deferens, was sectioned for histological examination and stained with hematoxylin and eosin. The remaining 15 rats from the respective groups were subjected to the following reversal procedure.

Reversal Procedure With Sodium Bicarbonate
The vas deferens of all male rats were exposed near the site of original contraceptive intervention. At the contraceptive injection site, there is generally a small tissue nodule. Any further manipulation at this point is not convenient. Instead, a location on the vas deferens about 5 mm proximally toward the testis was selected. With a 23-gauge injection needle and 1 mL tuberculin syringe, 0.5 mL of a 10% solution of sodium bicarbonate was injected slowly into the lumen of the vas deferens with the needle pointing distally. The slow infusion is necessary to ensure that the intraluminal drug dissolves.

Follow-Up to Reversal Procedure
After 2 weeks, each male rat of groups A and B was caged with two female rats. Vaginal smears of the females were taken and examined microscopically for sperm as well as for any identifiable breakdown products of sperm.

Within 2 weeks, intact spermatozoa were observed in the vaginal smears of most of the mated females. From each group, five of the sperm-producing male rats were killed and the vas deferens prepared for histological examinations. The remaining males were allowed to continue the mating program.

After 2 more weeks, five males each from group A and B were killed and the vas deferens prepared for histological examination. Mating of the five remaining males was carried on for 2 more months.

Results
After the contraceptive treatment, no intact spermatozoa were seen in the vaginal smears of any of the females. A few deformed spherical structures, which appeared to be tailless sperm heads, were observed, especially in the early periods after the contraceptive treatment. Figure 1 is the histological section of the vas deferens of a rat treated with 1 mg of SMA in group A. Figure 2 is the histological section of the vas deferens of rats treated with 2 mg of SMA in group B.

The lumen was distended with luminal folds almost absent. The mucosa was considerably attenuated with pseudostratified epithelial character and barely discernible. In the vas deferens of group B animals, the lumen was seen to be almost fully occupied by the polymer, which appeared glassy pink. The mucosa was virtually absent. The surrounding muscular layers appeared compressed and a more significant infiltration was visible.

After the reversal procedure of flushing out SMA, the lumen was distended, initially, with only a vestige of mucosa in the group B animals (Figure 3). With additional time, the mucosa re-established itself, with the folds restored and the normal pseudostratified ciliated columnar epithelial character seen once again (Figure 4). Of the 15 rats of group A, 13 rats had spermatozoa after reversal and one animal died. In group B rats, 14 of 15 animals had spermatozoa.

After flushing of the polymer from the lumen of the
vas deferens, rats from groups A and B became pregnant after mating and delivered healthy offspring.

**Discussion**

Observations demonstrate that SMA dissolved in DMSO produces a depot in the vas deferens. A partial vas blockage as well as active biophysical mechanisms of the depot lead to breakdown of spermatozoa. No intact spermatozoa can be found in vaginal swabs of the females mated with the males. Behavioral observations have also been conducted; mounting takes place even after the contraceptive treatment. Therefore, the absence of sperm in the vaginal swabs is not due to physical discomfort after contraceptive

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**Figure 1.** Transverse section of vas deferens, 1 mg SMA injected into the lumen [hematoxylin and eosin, ×200].

**Figure 2.** Transverse section of vas deferens, 2 mg SMA injected into the lumen [hematoxylin and eosin, ×200].
Figure 3. Transverse section of vas deferens just after flushing with 10% sodium bicarbonate [hematoxylin and eosin, x200].

treatment that prohibits mounting. Clearly the polymer depot has a direct contraceptive effect.

Histological examination of the treated vas deferens indicates that the polymer has more effect on the epithelial cells than on the muscle cells. Even in regions in which the epithelium is totally denuded, there is no significant degradation in the muscular structures. Appearance of spermatozoa on vaginal swabs and pregnancy show that flushing with sodium bicarbonate is an effective reversal procedure.

Figure 4. Transverse section of vas deferens three weeks after flushing with 10% sodium bicarbonate [hematoxylin and eosin, x200].
The question arises as to whether the removal of the polymer is a purely mechanical phenomenon caused by the pressure exerted by the injected sodium bicarbonate solution or whether there are chemical actions involved. When sodium bicarbonate is injected, there is always a transient ballooning out of the vas deferens, followed by the vas deferens collapsing to its original form. In vitro, experiments give the explanation that when sodium bicarbonate reacts with SMA, it leads to the formation of carbon dioxide gas. The bicarbonate ion gets reduced by the acidic nature of the polymeric material. Consequently, we conclude that sodium bicarbonate has a chemical action on the intravas SMA depot that leads to the dissolution of the depot, hence infusion helps in the evacuation.

Progressive recovery of the mucosa and the normal character of the muscular layer suggest that the sodium bicarbonate as well as the breakdown product have no adverse effects. The choice of 10% sodium bicarbonate solution instead of the more commonly used 7.5% concentration has helped in obtaining near total removal of SMA depot without any adverse biological effects. Carbon dioxide gas being a tissue absorbable gas, there is no risk of gas gangrene formation, making the whole reversal procedure safe and bioacceptable. Spermatozoa flow restoration and fertility recovery is comparable to that reported earlier with flushing using DMSO.

Based on these findings we suggest that 10% sodium bicarbonate solution may be effective for reversal in subhuman primates. These studies should be followed up with clinical trials.

References