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DECREASED GRANULATION TISSUE REACTION AFTER INSTALLMENT OF HYALURONIC ACID

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In 1958 Balazs suggested that hyaluronic acid preparations be used for replacement of the vitreous body of the eye. Widder (1960) and Hruby (1961) used commercially available hyaluronic acid of bovine origin for vitreous replacement in human eyes after animal studies. The hyaluronic acid preparation they used was, however, of low molecular weight. Balazs & Sweeney (1968) pointed out the importance of using a preparation of high molecular weight and of using a method of sterilization that would not cause the hyaluronic acid polymer to degenerate, and reported on a method of preparation and sterilization of hyaluronic acid. This preparation was injected as a replacement for liquid vitreous into the eyes of 50 owl monkeys. No adverse long-term reaction was observed. Hyaluronic acid is now used as a substitute for the vitreous body of the human eye in connection with operations for retinal detachment.

The beneficial effect of installment of hyaluronic acid in certain joint and tendon disorders has recently been described (Rydell et al. 1969, Rydell 1969). These studies were performed on animals, but the results were auspicious and treatment with hyaluronic acid is now being introduced in human patients.

During the studies of intra-articular injections in normal and arthritic horse joint (Rydell et al. 1969), it was observed that injections of hyaluronic acid induce a migration of a huge number of macrophages into the injected area without any clinical sign of inflammation. It was thought that the beneficial effect observed after intra-articular injections of hyaluronic acid was related to the influx of macrophages into the joint. A similar cellular reaction was sometimes found to occur in

the vitreous body of the eye after local hyaluronic acid injection. A further observation during the study of joint and tendon disorders was that the subcutaneous part of surgical incisions healed with less connective tissue reaction and adhesion formation.

To investigate whether granulation tissue formation could be diminished and smoother wound healing achieved by installment of hyaluronic acid, the following study was performed.

METHOD

The hyaluronic acid used in this work was prepared from human umbilical cords or rooster combs and sterilized in our laboratory according to Balazs & Sweeney (1968), and Swann (1968). The protein content varied from 1–2 per cent in the first preparation to 0.2–0.3 per cent in the latest preparation, and the molecular weight was $1-3 \times 10^6$. The sodium salt of hyaluronic acid was dissolved in a concentration of 5–9 mg per ml in physiological saline, 1–2 ml of this solution was used in the experiments.

In order to investigate the effect of the installation of hyaluronic acid on the subcutaneous scar formation after surgical incision, 45 animals were subjected to 4 cm long incisions, symmetrically on both sides of the body, reaching down to the muscular fascia. The fascia was traumatized by 10 strokes of a gauze pad. It was considered to be of great importance that exactly the same damage was performed on both sides of the same animal. Twenty-five rabbits, 10 owl monkeys, and 10 guinea pigs, altogether 45 animals, were used. On one side, hyaluronic acid was installed before the wound was closed; on the other side, the control side, saline was injected in two cases. As the injection of saline alone may be harmful to the connective tissue, this procedure was avoided in most animals. The surgical procedure was identical on both sides. Three to six weeks after the operation the animals were sacrificed and the subcutaneous part of the scar explored. The result was evaluated by comparing the reactions of both sides of the same animal. On each side, however, the amount of reaction was classified in three grades. Grade 1 was used if the adhesions formed were thin and sparse and easily broken. Grade 2 included a more dense adhesion formation which was easily disrupted on dissection. The reaction was classified as Grade 3 if the adhesions formed a dense tissue and a knife was necessary for the dissection. One of the animals died in connection with the operation and 5 developed infection; these were excluded from this study.

RESULTS

Of the 39 animals remaining for evaluation, 32 showed less connective tissue reaction with a smoother subcutaneous scar on the hyaluronic acid treated side, 6 showed no difference between the two sides, and in one case the control side had the smoothest healing (Table 1).

Of the 10 owl monkeys, two were excluded due to infection. In six of the monkeys the hyaluronic acid-treated side was classified as

Table 1. Subcutaneous tissue reaction to surgical skin incision with and without hyaluronic acid installation

Animals	No. evaluated	Less reaction on hyaluronic acid side	Less reaction on control side	No difference between hyaluronic acid and control sides
Owl monkey	8	6	0	2
Rabbit	21	16	1	4
Guinea pig	10	10	0	0
Total	39	32	1	6

Grade 1 and the control side as Grade 2, while in two cases both sides were marked as Grade 2. Of the 25 rabbits, 4 were excluded, 1 of them because of death and 3 because of infection. Of the remaining 21 rabbits, 16 revealed less reaction on the hyaluronic acid-treated side: 12 classified as Grade 1 and 4 as Grade 2, and on the control side all 16 were classified as Grade 3. Four rabbits showed no difference between the two sides: 3 of them were classified as Grade 1 and one as Grade 2 on each side. One rabbit had smoother healing on the control side, marked as Grade 1, while the hyaluronic acid-treated side was marked Grade 2.

None of the 10 guinea pigs was excluded. All were classified as Grade 1 on the hyaluronic acid-treated side and Grade 2 on the control side.

With the encouraging results obtained, it was believed that hyaluronic acid could also be used in decreasing connective tissue reaction around foreign body implants. To obtain information on this, a 3 cm long incision was made in the lower region of the thorax of 5 guinea pigs. The incision was placed on each side 2 cm from the mid-line of the dorsum, with its midpoint over the tip of the tenth rib. On each side, two 1 cm long, hollow polyethylene tubes, with outer diameters of 10 mm and inner diameters of 6 mm, were installed. One of the tubes was placed under the muscle fascia and the other subcutaneously. On one side the tubes were embedded in hyaluronic acid before the installation and on the other side in saline. The fascia and skin were closed by silk sutures. Within the next few days two of the animals showed a marked wound reaction on the saline-embedded side and later rejected the implanted tubes. The wounds then healed within a few days without suppuration. The three animals which did not reject the tubes on the control side showed a marked reaction in the

wound area on this side and a large mass of granulated tissue could be palpated. The tubes embedded in hyaluronic acid did not cause any inflammatory reaction and in all animals the wound healed nicely within a few days. Six weeks after the operation the animals were sacrificed and the subcutaneous wound area explored. On the hyaluronic acid-treated side only minor tissue reaction was observed with a few weak adhesions. The polyethylene tubes were surrounded by a thin soft capsule, which was removed for pathological examination. On the control side a more extensive tissue reaction was observed in all 5 cases, with a more marked adhesion formation. In the 3 animals, which did not reject the tubes, a thick capsule was formed around the tubes. This was removed for pathological examination. The microscopic picture of the capsules showed fewer cells and more fibrous tissue on the control side compared to the hyaluronic acid-treated side.

DISCUSSION

Installation of hyaluronic acid subcutaneously seems to delay and decrease granulation tissue formation and fibrosis. Out of 39 animals, such an effect was observed in 32 cases.

In a recent study of the healing of skin burns, preliminary results show a marked decrease in fibrosis if the wound is treated with hyaluronic acid.

In a study of tendon injuries on rabbits (Rydell 1969), it was shown that installation of hyaluronic acid around the tendon in connection with the injury significantly decreased the formation of adhesions. In a study of hyaluronic acid injections and fracture healing, no change in the healing was observed (Rydell 1969).

The cause of the decrease in connective tissue reaction to injuries and implantation of foreign bodies after installation of hyaluronic acid is not known. Hyaluronic acid seems to have a macrophage-calling effect when injected into a joint or into the vitreous body of the eye. It would probably have the same effect if injected subcutaneously. At the same time a hyaluronic acid preparation has a gel-like composition and could mechanically prevent the ingrowth of tissue. Which of the two factors, the macrophage invasion or the mechanical barrier, is the more important in decreasing the formation of granulation tissue is debatable, but probably the invasion of macrophages is more significant. It should be noted that the influx of macrophages into joints after hyaluronic acid injection occurs after 4 hours and disappears

after about 48 hours and this reaction is not connected with any clinical sign of inflammation (Rydell et al. 1969).

From this work it seems that the installation of hyaluronic acid preparation is important in preventing undesired fibrotic reactions in burns, tendon injuries, and after implantation of foreign bodies, as well as in treatment of certain joint disorders.

SUMMARY

Injections of hyaluronic acid in 39 animals showed decreased fibrotic wound reactions in 32 cases. A decrease in granulation tissue formation was observed as well as diminished formation of adhesions. In 5 animals submitted to implants of foreign bodies, the tissue reaction was significantly less if the implants were embedded in hyaluronic acid. This effect may be explained by the gel-like consistency of hyaluronic acid solutions and by the influx of macrophages which occurs after these injections. The latter mechanism is suggested to be the most important.

Hyaluronic acid application seems to be indicated especially after tendon damage, burns, and implants of foreign material, but also whenever a smooth scar formation is important.

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