

# Hyaluronidase in the Prevention of Sclerotherapy-induced Extravasation Necrosis

*A Dose-Response Study*

*Dermatologic Surgery*, volume 22, 73-77, 1996.

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**BACKGROUND.** *A previous study found hyaluronidase to be effective in the prevention of necrosis following intradermal sodium tetradecyl sulfate and 23.4% sodium chloride. There are no published dose-response studies of hyaluronidase used in this manner.*

**OBJECTIVE.** *To conduct a dose-response study using hyaluronidase in the prevention of necrosis following intradermal 23.4% sodium chloride.*

**METHODS.** *Study I evaluated control vs hyaluronidase groups (150, 300, 450 units; all in volume of 3 mL) in the prevention of necrosis following intradermal 0.25 mL 23.4% sodium chloride. Incidence and size of necrosis were compared between groups. In study II, hyaluronidase was administered in doses ranging from 18.75 to 900 units (all in volume of 3 mL) immediately following the intradermal instillation of 0.25 mL of 23.4% sodium chloride. A control group had no therapy. The incidence of necrosis was compared between groups. A dose-response curve was constructed. Both studies were randomized and blinded and used Sprague-Dawley rats.*

**RESULTS.** *A statistically significant protective effect was found in the treated vs the untreated groups in both studies. Maximal protection was achieved by 75 units of hyaluronidase and was not improved upon by higher doses. CONCLUSION. In the event of extravasation with 23.4% sodium chloride, in the model studied, one can expect maximal protection with a dose of 75 units of hyaluronidase.*

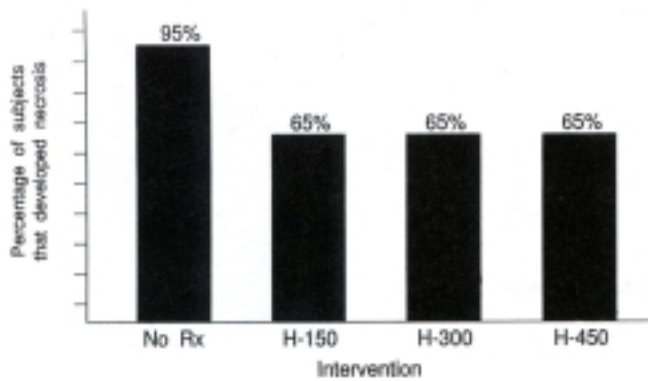
The burgeoning interest in sclerotherapy has given rise to a large number of inexperienced phlebologists. A recent survey conducted by the North American Society of Phlebology found that inexperienced practitioners of sclerotherapy (less than 500 treatments) reported a 5% incidence of postsclero-therapy necrosis.<sup>1</sup> Hypertonic saline (23.4%), widely used in sclerotherapy by US dermatologists, was found in the survey to be the most common sclerosant associated with necrosis. In an attempt to prevent extravasation necrosis most authors have recommended, apparently on an anecdotal basis, infiltrating the extravasated area with 0.9% sodium chloride.<sup>2,3</sup> In the event of extravasation with sodium tetradecyl sulfate some authors have recommended procaine.<sup>4,5</sup> A controlled study in rats regarding the efficacy of various treatments in the prevention of necrosis following extravasation of hypertonic saline and sodium tetradecyl sulfate found hyaluronidase to be the only agent that produced significant protection against necrosis.<sup>6</sup> The dose of hyaluronidase used in that study was 225 units given in a volume of 3 mL. Most authors have recommended using 150-300 units of hyaluronidase given in 1-2 mL following extravasation,<sup>7,8</sup> although some have advocated as little as 15 units given in 1 mL<sup>9</sup> and as high as 500-1000 units.<sup>10</sup> There are no published dose-response studies of hyaluronidase.

Study I compared the efficacy of hyaluronidase (Wydase Lyophilized, 150 USP units/mL; Wyeth-Ayerst Laboratories, Philadelphia, PA) given in 150, 300, and 450 units vs no treatment in the prevention of necrosis following the intradermal administration of 23.4% sodium chloride (Concentrated Sodium Chloride; American Regent, Shirley, NY). In study II, hyaluronidase was administered in doses ranging from 18.75 to 900 units immediately following the intradermal instillation of 0.25 mL of 23.4% sodium chloride. A control group had no therapy. The incidence of necrosis was compared between groups. A dose-response curve was constructed.

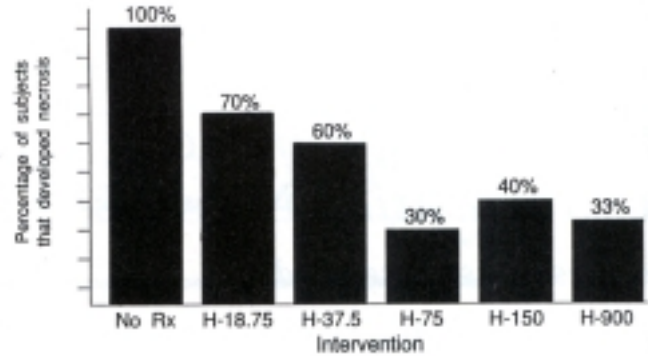
## **Materials and Methods**

Female Sprague-Dawley rats (Harlan Sprague-Dawley, Houston, TX) weighing between 150 and 200 g were utilized as subjects. Animals were housed two or three per cage under identical climate-controlled conditions. The study protocol was approved by the University of Texas Animal Use and Care Committee.

In study I there were 20 animals per group. All subjects had the dorsum of their trunk shaved the day prior to the study. Each subject was given, after induction of halothane anesthesia, an intradermal injection of 0.25 mL of 23.4% hypertonic saline. Immediately following this the subjects were given an injection, about the previous intradermal site, of either 150, 300, or 450 units of hyaluronidase (all in a volume of 3 mL; H-150, H-300, H-450) or no intervention (no Rx). In study II there were 10-15 subjects per group. After shaving and anesthesia as for study I, subjects were given 0.25 mL of 23.4% sodium chloride intradermally. Immediately following this they were given no treatment or 18.75, 37.5, 75, 150, or 900 units of hyaluronidase (all in a volume of 3 mL). Interventions were administered in a blinded and randomized fashion. Subjects were rehoused two or three per cage according to coded groups and placed back in their normal climate-controlled conditions. Three days after the injections all subjects who developed necrosis were noted and, under anesthesia, had their ulcers traced onto tracing paper. The surface area in mm<sup>2</sup> of each ulcer was then determined utilizing a Targa digitizing pad, Javelin camera, and Java software (Jandel Scientific, Corte Madera, CA). At this point the codes were broken and groups identified by their interventions. All injections were done with a 3-mL syringe. A 30-gauge needle was used to inject the 23.4% sodium chloride intradermally while a 27-gauge needle was employed to inject the hyaluronidase. Statistical analysis was accomplished using SPSS software (SPSS Inc., Chicago, IL).



**Figure 1.** Percentage of subjects that developed necrosis in each group following intradermal 23.4% hypertonic saline (study I; No Rx = no treatment). H-150, H-300 & H-450 refer to hyaluronidase group receiving 150, 300, and 450 units hyaluronidase, respectively.



**Figure 2.** Percentage of subjects that developed necrosis in each group following intradermal 23.4% hypertonic saline (study II; no Rx = no treatment). H groups refers to the number of units of hyaluronidase given to each group.

## Results

### Study I

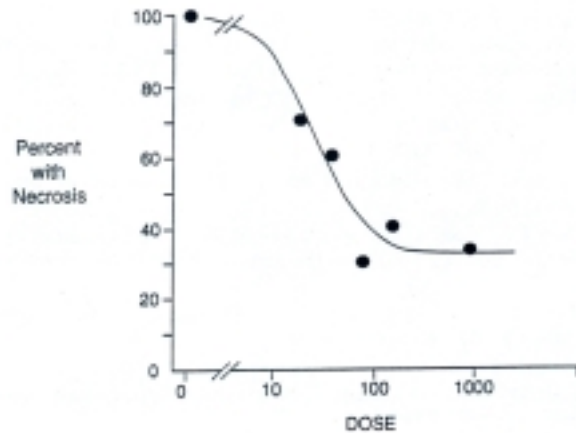
All hyaluronidase groups (150, 300, and 450 units) yielded an equally reduced incidence of necrosis compared with no treatment. The percentage of subjects who developed necrosis for each group is in Figure 1. The mean surface area of necrosis (in subjects that developed necrosis) for the no treatment group was 4.29 mm<sup>2</sup>, while it was 2.35, 3.00, and 1.91 mm<sup>2</sup> for hyaluronidase groups receiving 150, 300, and 450 units, respectively.

Chi square analysis compared the number of subjects that developed necrosis between groups. Since results regarding the incidence of necrosis were the same for all the hyaluronidase groups, these were combined into a single group for testing. Treated groups were found to have a significantly lower incidence of necrosis compared with the no treatment group ( $P < .01$ ). Analysis of variance was used to evaluate the difference between groups regarding mean surface area of necrosis. There was a significant difference between groups ( $P = .0011$ ). A Scheffe comparison found significantly smaller mean surface area of necrosis in comparing the hyaluronidase groups receiving 150 and 450 units with the control group ( $P < .05$ ). The difference between the control group and hyaluronidase 300 unit group was not significant. Scheffe comparison found significantly smaller mean surface area of necrosis in the combined hyaluronidase groups vs the control group ( $P < .01$ ).

### Study II

All hyaluronidase groups had between a 30% and 70% lower incidence of necrosis compared with the control group. The incidence of necrosis for all groups is in Figure 2. Chi square analysis revealed a significantly lower incidence of necrosis in treated vs control groups ( $P < .0005$ ). There was no significant difference between individual treated groups. However, combined higher dose hyaluronidase groups (75, 150, and 900 units) had a significantly lower incidence of necrosis than com-

bined lower dose groups (18.75 and 37.5 units) ( $P < .03$ ). A dose-response curve for the incidence of necrosis across varying doses of hyaluronidase was generated (Figure 3). Parameters of the best fit curve were determined using maximum likelihood criterion with SPSS CNLR nonlinear regression routine.



*Figure 3. Dose-response curve for the incidence of necrosis across varying doses of hyaluronidase (study II) (Maximum protection = 67%; ED<sub>50</sub> = 22.8; logistic slope = 1.77).*

## Discussion

In study I, hyaluronidase given at 150, 300, and 450 units all yielded an identical and significant reduction in the incidence of necrosis compared with the control group. Combined hyaluronidase groups were also found to have a significantly smaller mean surface area of necrosis compared with the control group (comparing only subjects who developed necrosis). All treated groups received their dose of hyaluronidase in the same volume, so the effect cannot be attributed to dilutional differences. In study II, treated groups had a significantly lower incidence of necrosis than the control group. Although incidence of necrosis between treated groups ranged from 30% to 70%, these differences were not significant. This is probably due to the lower number of subjects in the groups. The two lowest dose hyaluronidase groups (18.75 and 37.5 units) had lower levels of protection than the three higher dose hyaluronidase groups (75, 150, and 900 units), and the difference was statistically significant. The dose-response curve demonstrates that maximum protection against necrosis was achieved by 75 units of hyaluronidase, with no further protection being achieved by doses up to 900 units. The ED<sub>50</sub>, the dose at which 50% of the protectable subjects would have been protected, was calculated at 22.8 units. The logistic slope of the dose-response curve was 1.77, indicating a narrow range of response.

Hyaluronidase is an enzyme that breaks down hyaluronic acid in ground substance. This disrupts the normal interstitial fluid barrier and is thought to rapidly diffuse extravasated solutions through tissues, thereby increasing the effective absorption.<sup>9</sup> A pharmacokinetic study found lower peak tissue levels and enhanced egress of extravasated labeled vinblastine following injection of hyaluronidase.<sup>10</sup> It is this increased absorption rather than dilution of the extravasated agent that has been suggested as accounting for the beneficial effect of hyaluronidase.<sup>6,7</sup> Hyaluronidase has been used to treat extravasation of nafcillin,<sup>11</sup> 10% dextrose injection, calcium salts, potassium salts, contrast media, sodium bicarbonate, and aminophylline.<sup>9</sup> It has also been recommended following extravasation of

vinblastine and vincristine.<sup>10,12</sup> Controlled studies have demonstrated the effectiveness of hyaluronidase in reducing necrosis following calcium chloride and hyperalimentation solution.<sup>7,8</sup> There have been conflicting reports about the effectiveness of hyaluronidase in the treatment of doxorubicin extravasation. One study found that hyaluronidase increased doxorubicin-induced necrosis.<sup>13</sup> Another study found hyaluronidase to be significantly protective.<sup>7</sup> This latter study utilized 300 units of hyaluronidase in 2 mL whereas the first study used 7.5 units in an unspecified volume. Hyaluronidase has been shown to increase skin flap survival,<sup>14</sup> and to reduce myocardial necrosis in several animal models of myocardial infarction.<sup>15</sup> It has been suggested that such benefits may be due to enhanced nutritive flow and the reduction of harmful metabolic waste products.<sup>16</sup> Perhaps the protective effect of hyaluronidase following extravasation involves an independent ability to preserve cellular function in addition to enhancing absorption of the extravasated agent.

Side effects due to hyaluronidase are unusual.<sup>17,18</sup> Allergic reactions are rare and generally of the urticarial type. Hyaluronidase should not be injected into infected or cancerous tissues, which might promote the spread of those conditions. Wydase® Lyophilized is reconstituted with 0.9% sodium chloride, usually in the proportion of 1 mL per 150 USP units of hyaluronidase. This contains a small amount of thimerosal as a preservative and is stable for 2 weeks after reconstitution if stored below 30°C (86°F). Wydase Stabilized Solution (Wyeth-Ayerst Laboratories) is an injection solution ready for use. Refrigeration is recommended.

There are some cautions that should be noted in evaluating the results of this study. Rat dermis is thinner than human dermis/ making uniform placement of injections most difficult. Subjects were anesthetized for their injections in order to optimize uniform placement. Also, any variability in placement would be expected to even out over groups. Not all ulcers occurring after sclerotherapy are due to extravasation. Other mechanisms such as microarteriolar injections certainly must cause some ulcerations. Perhaps an etiology other than extravasation is the most common cause of ulceration in the experienced phlebologists hands. The data presented here should not be evaluated for any etiology other than extravasation. Lastly, it is difficult to draw conclusions regarding human beings from a study on rats.

## **Conclusions**

In the event of extravasation with 23.4% sodium chloride, in the model studied, one can expect maximal protection with a dose of 75 units of hyaluronidase, with no further protection being achieved by doses up to 900 units.

***Acknowledgments** Wyeth-Ayerst Laboratories provided a grant for this study. Dr. Pat Randall (Institute for Neuro-science and Division of Pharmacology, University of Texas-Austin) aided in statistical analysis.*

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