The Use of Antidotes for Calcium Gluconate Extravasation: An Experimental Study in Mice

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Abstract

Background: Calcium gluconate extravasation is a process that can cause serious lesions, such as necrosis and calcification of the soft tissues. The aim of the present study was to analyze the beneficial effects of four possible local antidotes for calcium gluconate extravasation: hyaluronidase, sodium thiosulfate, triamcinolone acetonide, and physiologic saline solution.

Methods: Seventy-four BALB/c mice were used in the study. The substances selected for use in this study were calcium gluconate (4.6 mEq/ml), hyaluronidase (1500 IU/ml), sodium thiosulfate (25%), triamcinolone acetonide (40 mg/ml 0.5 mg/kg), and saline solution 0.9%. Five minutes were allowed to lapse after the calcium gluconate infiltration, and then an antidote was infiltrated. After 3 weeks, a skin biopsy was performed and a radiographic and histologic study was carried out.

Results: Only in the group infiltrated with sodium thiosulfate did all skin lesions disappear after the 3-week period after infiltration. In the radiographic study, calcium deposits larger than 0.5 mm were observed in 40 percent of cases without an antidote, in 33 percent with triamcinolone acetonide, in 13 percent with a saline solution, and in none with thiosulfate and hyaluronidase. In the histologic study, calcium deposits were found in 53 percent of cases without antidote, 100 percent of cases with triamcinolone acetonide, 33 percent of cases with saline solution, and 13 percent of cases with sodium thiosulfate or hyaluronidase.

Conclusion: Sodium thiosulfate and hyaluronidase prevent the development of calcium deposits after calcium gluconate extravasation.

In scientific literature, numerous studies have been published referring to the effect produced in soft tissues when extravasation of certain drugs takes place. Most of these studies focus on the extravasation of chemotherapy agents and radiocontrast dyes, as these are events that occur relatively frequently.

However, there are other drugs which, because they are less important in this context, have not been the object of so much study (e.g., the extravasation of calcium gluconate). Calcium gluconate is a salt used to correct hypocalcemia, and its use is very common in neonates and, in particular, in infants requiring intensive care. Thus, calcium gluconate extravasation is a process which, although not common, occurs with more frequency in neonatal intensive care units and, above all, in cases of premature birth. The most frequently observed lesions after extravasation are edema and erythema. When some days have passed, papules or whitish or yellowish plaques may appear, with or without associated skin necrosis.[1,2] Finally, insoluble crystalline deposits of calcium phosphate may appear in affected areas, in the form of hydroxyapatite.[1,3]

In 1936, Tumpeer and Denenholz[4] published the first case of calcification in soft tissues after an intramuscular gluteal injection of calcium gluconate in a neonate. In that same year, Von Hofe and Jennings[5] published another case of calcification in soft tissues caused by an intramuscular calcium gluconate infiltration. The route of calcium gluconate administration was changed from intramuscular to intravenous during the 1940s, but it was not until the 1970s that complications began to be reported that were caused by intravenous calcium gluconate extravasation.[6] Goldminz et al.[7] published a study in 1988 in which they reported an 8 percent incidence of complications after calcium gluconate injections in premature babies. Nonetheless, and despite the fact that associated lesions can be serious, very few published studies discuss the use of antidotes in relation to calcium gluconate extravasation. The aim of the present study was to perform an analysis, by means of a controlled prospective study in mice, of the beneficial effects of four possible local antidotes for calcium gluconate extravasation: hyaluronidase, sodium thiosulfate, triamcinolone acetonide, and physiologic saline.

MATERIALS AND METHODS

A study protocol was developed that was approved by the animal experimentation ethics committee in our center. A total of 74 BALB/c mice were included in the final study. All of these were male mice approximately 13 weeks old at the time the study was performed (84 to 97 days).

The substances used in the study were the following: calcium gluconate, 4.6 mEq/10 ml from Ca^{++} Suplecal Mini-Plasco (B Braun Medical SA, Barcelona, Spain); hyaluronidase, 1500 IU/ ml Hyalase (Wockhardt Ltd, Wrexham, United Kingdom); sodium thiosulfate, 25% (magistral formula); triamcinolone acetonide, 40 mg/ml Trigon depot (Bristol-Myers Squibb, S.A, Madrid, Spain); and saline solution, 0.9% (B Braun Medical SA). A pilot study was carried out before the research, which found that the dose required to produce significant calcium deposits is 0.12 cc of calcium gluconate (doses used were 0.05, 0.08, 0.12, and 0.15 cc, and mice were killed after 3 weeks in a group of eight mice, and after 5 weeks in a group of six mice).

Study groups were distributed according to the substances infiltrated in each mouse and the dose used (Table 1), as follows: a control group consisting of 15 mice into which only 0.12 cc of calcium gluconate was injected; a group of 15 mice into which calcium gluconate and 0.1 cc of sodium thiosulfate (25%) was injected; a group of 15 mice into which calcium gluconate and 0.1 cc of hyaluronidase (150 IU) was injected; a group of 15 mice into which calcium gluconate and 0.1 cc of saline solution (0.9%) was injected; and a group of six mice into which calcium gluconate and 0.016 mg of triamcinolone acetonide 40 mg/ml, diluted in 0.1 cc of distilled water (0.5 mg/kg), was injected (the triamcinolone acetonide group was only made up of six mice, as satisfactory results were not obtained during the initial stages of the study).

Four control groups of two mice were also included, into which 0.1 cc of saline solution, 0.1 cc of sodium thiosulfate, 0.1 cc of hyaluronidase, and 0.1 cc of triamcinolone acetonide were injected, with each infiltration into both mice, and in the same concentration as for the previous study groups. All the substances were infiltrated in the subcutaneous plane on the animal's back with a 1-cc syringe and a 29-gauge needle. Under inhalation anesthesia with sevoflurane, the animal's back was shaved with an electric shaver. The calcium gluconate was infiltrated next to the scapular region. In the group with antidote, 5 minutes was allowed to lapse, after which the infiltration of the

antidote was performed in the same area as the calcium gluconate inoculum. The animal was woken after this procedure had finished.

The possible cutaneous lesions present in the area of the infiltrations were noted after 7, 14, and 21 days. Cutaneous lesions were considered to be those cases that presented whitish or yellowish plaques (Fig. 1, left) and those that presented ulcers (Fig. 1, right). Lesions were classified according to their diameter. The mice were killed after 21 days in a carbon dioxide chamber. Afterward, a biopsy of the infiltration area was performed. A radiographic examination was made of each skin biopsy specimen and then the sample was sent for histopathologic analysis. The radiographic examination was performed with a direct digital mammography device. The radiographic findings were presented in four patterns (Fig. 2): without calcium (Fig. 2, above, left); pointed lesion (Fig. 2, above, right); multiple pointed lesions (Fig. 2, below, left); and calcium plaques (Fig. 2, below, right).

To perform statistical analysis, calcium deposits were considered relatively significant using the following measurements: insignificant (lesion <0.1 mm); of little significance (lesion between 0.1 and 0.5 mm); significant (lesion between 0.5 and 1 mm); or very significant (lesion >1 mm). A deferred histopathologic analysis was made of the biopsy specimens. Once the pieces had been cut out, two to four cuts were taken from each biopsy specimen. Cuts were made from the area that presented the most significant macroscopic lesions. If the sample presented no lesions, the cuts were taken dividing the piece into equally sized fragments. All of the samples were dyed using hematoxylin and eosin (Fig. 3, left). Some samples were also dyed with von Kossa stain (Fig. 3, center) and alizarin red solution (Fig. 3, right) to confirm the presence of calcium if there had been any doubt about the initial study. The following findings were evaluated: necrosis, fibrosis, inflammatory infiltrate, and the presence of calcium (Table 2). The presence of calcium was classified according to its location (i.e., epidermis, dermis, subcutaneous, fascia/muscle, or vascular).

Radiologic and histopathologic studies were blind. Data relating to cutaneous lesions, radiographic findings, and the presence of calcium are shown in Table 1. Analysis of results was performed using the software programs Microsoft Excel Version 14.5.3 (Microsoft Corp., Redmond, Wash.) and IBM SPSS Version 21.0 (IBM Corp., Armonk, N.Y.).

RESULTS

We observed that the general trend in all cases is for cutaneous lesions to diminish during the 3 weeks. However, only in the group infiltrated with sodium thiosulfate did all lesions disappear after the 3-week period after infiltration (Fig. 4). There was a statistically significant difference in the number of mice presenting lesions at 3 weeks between the sodium thiosulfate group (zero mice) and the saline group (six mice) (Fisher's exact test, $p = 0.017$). No statistically significant differences were found among the other groups.

Mice infiltrated only with calcium gluconate presented significant or very significant deposits (>0.5 mm) on radiographs in six cases (40 percent). Calcium deposits were larger than 0.5 mm in two of the cases where triamcinolone acetonide was infiltrated (33 percent), in two of the cases where a saline solution was infiltrated (13 percent), and in none of the cases where sodium thiosulfate or hyaluronidase was infiltrated (0 percent). The relationship between infiltration of sodium thiosulfate or hyaluronidase is statistically significant when it comes to preventing calcium deposits larger than 0.5 mm on radiographs (Fisher's exact test, $p = 0.017$), but not in the saline solution group (Fisher's exact test, $p = 0.427$) or in the triamcinolone acetonide group (Fisher's exact test, $p = 1$) (Table 3).

On analyzing the results of the histologic study, we found that calcium deposits were observed in 53 percent of cases without antidote, in 100 percent of cases with triamcinolone acetonide, and in 33 percent of cases with saline solution; whereas in the sodium thiosulfate and hyaluronidase groups, only 13 percent presented calcium. In both the sodium thiosulfate and hyaluronidase groups, calcium deposits were reduced in a statistically significant way in the histologic study compared with the group without antidote (chi-square test, $p = 0.020$). There were no statistically significant differences with respect to the saline infiltration (chi-square test, $p = 0.269$); in the case of the triamcinolone acetonide group, the results were worse than in the group without antidote.

The relationship between the appearance of calcium plaques on radiographs and the presence of lesions at 3 weeks from the infiltration was analyzed. Statistical analysis shows that there exists a statistically significant relationship between the presence of calcium deposits larger than 0.5 mm on radiographs and the appearance of cutaneous lesions (Fisher's exact test, $p < 0.001$), between the presence of calcium in the histologic study and cutaneous lesions (chi-square test, $p = 0.039$), and between the presence of

calcium deposits larger than 0.5 mm on radiographs and calcium in the histologic study (Fisher's exact test, $p \leq 0.001$).

The histologic study also shows that a statistically significant relationship exists between the presence of calcium and the appearance of necrosis (Fisher's exact test; $p < 0.001$), of inflammation (chi-square test, $p < 0.001$), and of fibrosis (chi-square test, $p = 0.021$). Calcium deposits were mainly produced in the muscle [15 cases (23 percent)] and the dermis [11 cases (17 percent)]. Regarding the inflammatory infiltrate, this was positive in 31 cases (47 percent), histocytes were present in 21 cases (32 percent), lymphocytes were present in 19 cases (29 percent), neutrophils were present in 14 cases (21 percent), and eosinophils were present in seven cases (11 percent).

In none of the control cases (in which only the antidote was infiltrated, without calcium gluconate) were cutaneous lesions produced, nor were calcium deposits observed on the radiographic or the histologic study. However, one case of saline solution, two cases of hyaluronidase, and one case of triamcinolone acetonide presented fibrosis. Inflammatory infiltrate was also present in one case of sodium thiosulfate, one case of saline solution, and the two cases of hyaluronidase.

DISCUSSION

In our study, we have observed that local infiltration of sodium thiosulfate or hyaluronidase reduces the presence of calcium in the tissues following calcium gluconate extravasation in mice. In 1972, Pashchuk[8] published the first experimental study in which an attempt was made to find an antidote to prevent calcium chloride extravasation by means of $MgSO_1$ infiltration. In 1974, Berger et al.[9] published a study in rabbits about the effect produced by subcutaneous calcium gluconate infiltration. To this end, they infiltrated 1, 2, or 4 cc of calcium gluconate in the subcutaneous tissue of the thighs of nine rabbits; the rabbits were killed sequentially up to a maximum of 6 weeks to observe their evolution. By the second day, erythema and some induration appeared. By the tenth day, a hard palpable mass was noted. The degree of erythema and induration were dose-dependent, with necrosis appearing in only one case with 4 cc, whereas the rest had healed by the time 6 weeks had passed. The histopathologic study showed calcification of the muscle tissue and of the blood vessels. In the radiographic study, calcium deposits appeared from the fourth day on, reaching their maximum size at 2 weeks. In our case,

neither edema nor erythema was observed, although cutaneous lesions were detected 7 days after infiltration.

In 1976, Heckler and McCraw[10] carried out a study in rats to check on how effective antidotes were in cases of calcium chloride extravasation. They infiltrated 0.2 cc of calcium chloride (10%), giving rise to skin necrosis. They ascertained that necrosis was produced based on the degree of concentration of the calcium extravasation. The histologic findings were similar to those in the study by Berger et al., with the presence of necrosis and calcium deposits in the fascia and underlying muscle being observed, in addition to inflammatory infiltrate. When 150 IU of hyaluronidase was infiltrated, diluted in isotonic saline, 100 percent effectiveness was reached in preventing the appearance of skin necrosis. They posited that the effect of hyaluronidase was combined with the dilution effect of the saline solution when at least 10 times the initial volume of extravasation was administered.

Hyaluronidase is an enzyme that causes the depolymerization of hyaluronic acid and chondroitin sulphate, giving rise to a temporary dissolution of the interstitial barrier. This favors the reabsorption of substances.[11] It has been reported that its infiltration in tissues affected by infection or cancer is contraindicated,[12] as such infiltration could facilitate the propagation of these processes throughout the organism. Hives have also been described as an adverse effect following local infiltration.

In a study published by Laurie et al. in 1984,[13] administering a range of substances and the use of hyaluronidase as an antidote were proven to be effective in the prevention of the appearance of skin necrosis in rabbits. Among these substances was calcium chloride. It was demonstrated that the use of hyaluronidase following subcutaneous calcium chloride infiltration reduced the area of skin necrosis. It was also observed that this effect was significant if antidote infiltration was performed during the first hour after extravasation. It was found, moreover, that when the same volume of saline solution as antidote is infiltrated, no effect at all is produced, so they ruled out the possibility that the benefit of hyaluronidase was attributable solely to the effect of dilution.

In the year 2001, Casanova et al.[14] published an article in which 14 cases of neonates suffering extravasation are reported on, in which hyaluronidase was used as an antidote. Two of these cases were produced by calcium extravasation. In one of these cases,

hyaluronidase infiltration was produced in the first 5 hours, with positive results. The other was produced over 12 hours after extravasation, resulting in necrosis.

With regard to sodium thiosulfate, several articles have been published referring to the beneficial effects of intravenous sodium thiosulfate15 and sodium thiosulfate combined with intralesional thiosulfate,[16,17] in patients suffering from calciphylaxis, above all in patients in need of dialysis because of chronic kidney failure.[18,19] Thiosulfate is combined with calcium salts to produce calcium thiosulfate, a much more soluble molecule, which facilitates its reabsorption.[11] Solubility increases between 250 and 100,000 times more than other calcium salts.[20,21]

In 2008, Pasch et al. published a study in rats concerning the effect of intraperitoneal infiltration of sodium thiosulfate in the prevention of the appearance of calciphylaxis in rats that were induced to have chronic kidney failure.[22] In 2013, Strazzula et al. published a protocol for the treatment of calciphylaxis by means of intralesional sodium thiosulfate (in one case combined with intravenous sodium thiosulfate). They presented four cases in which lesion remission was produced in the space of 5 to 6 weeks.[17] In 2008, Wolf et al. published the clinical case of a patient with calcinosis cutis within the context of systemic lupus erythematosus, in which the topical application of sodium thiosulfate foments (10%) in compresses and perilesional steroids achieve remission of calcium deposits within 6 months.[20]

Recent studies have shown in vitro that sodium thiosulfate cannot reduce the calcium in culture media[23,24] but seems to present cellular effects and acts in the extracellular matrix. However, in another study, it is posited that it reduces calcification of subcutaneous fat.[25]

In 2009, Raffaella et al.[26] published a case of calcium gluconate extravasation in a 5-year-old boy with T-cell acute lymphoblastic leukemia, in whom conservative treatment did not stop the worsening of the calcification of the soft tissues. Following treatment with intravenous sodium thiosulfate, with a 10-g dose (435 mg/kg) three times per week, healing of the lesions was achieved within 3 months. However, we have not been able to find any articles making reference to the effect of intralesional sodium thiosulfate in the prevention of calcium deposits in cases of calcium gluconate extravasation.

We have found no documented cases in the literature about adverse effects related to local sodium thiosulfate infiltration.[17] The adverse effects described associated with intravenous administration of sodium thiosulfate (above all, in dialysis patients) are

nausea, vomiting, and hypernatremia. Cases of acid-base equilibrium alterations have been described with metabolic acidosis, without clinical significance.[20] Likewise, some publications speak of the possible beneficial effects of sodium thiosulfate not provided by the saline solution. Sodium thiosulfate possesses a vasodilator effect by encouraging the generation of endothelial nitric oxide and the production of hydrogen sulfide by the vascular smooth muscle cells. The latter also produces an antiinflammatory and analgesic effect, apart from vasodilation. Besides, sodium thiosulfate has an antioxidant effect, in that it favors the production of glutathione, which reduces the risk of lesions.[26,27] However, the authors agree that it would be interesting to perform further experimental studies to examine the possible adverse effects associated with local infiltration of sodium thiosulfate.

In our case, the beneficial effects of sodium thiosulfate and hyaluronidase were greater than those obtained in the group without antidote. Nevertheless, in the prevention of the appearance of skin lesions, a complete cure was achieved in the space of only 3 weeks in the group in which sodium thiosulfate was infiltrated.

In the study published by Ahn et al. in 1997, the beneficial effects of triamcinolone acetonide in cases of calcium gluconate extravasation were demonstrated in a study in rabbits.[28] The dose administered was 0.5 cc of triamcinolone acetonide, 10 mg/dl, in rabbits with an average weight of 3.2 kg (i.e., a dose of approximately 0.6 mg/ kg). Our results are not meant to contradict this study, as, in our case, the experimental animal chosen (the mouse) may not be appropriate for a study of triamcinolone acetonide, given that the dose (0.5 mg/kg) may not be great enough to offset the effects of the calcium gluconate. Nevertheless, given that triamcinolone acetonide is not indicated for subcutaneous infiltration in humans, given the risk of producing atrophy of body fat, and that it is contraindicated in children younger than 6 years (the group where calcium gluconate extravasation occurs most frequently), we consider that it should not be used in calcium gluconate extravasation in humans.

Limitations

The pilot study showed that, at 5 weeks, the calcium deposits were similar to those at 3 weeks. We were unable to verify whether, given a longer period, the calcium deposits would have disappeared by themselves, without using antidotes. This, however, does not

alter the fact that the use of sodium thiosulfate as an antidote speeds up the healing process, which is beneficial for the neonate.

CONCLUSIONS

Local sodium thiosulfate infiltration in calcium gluconate extravasation in mice reduces the presence of calcium deposits and skin lesions. Local hyaluronidase infiltration in calcium gluconate extravasation in mice reduces the presence of calcium deposits, but not of skin lesions. We have observed, based on the results obtained in the group with saline solution, that the beneficial effects of sodium thiosulfate are not exclusively attributable to the effect of dilution.

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Table 1. Study Groups in Relation to Substances Infiltrated

	Calcium Gluconate (no. of mice)	Nothing (no. of mice)
Hyaluronidase	15	2
Triamcinolone	6	2
Sodium thiosulfate	15	2
Saline solution	15	2
Nothing	15	

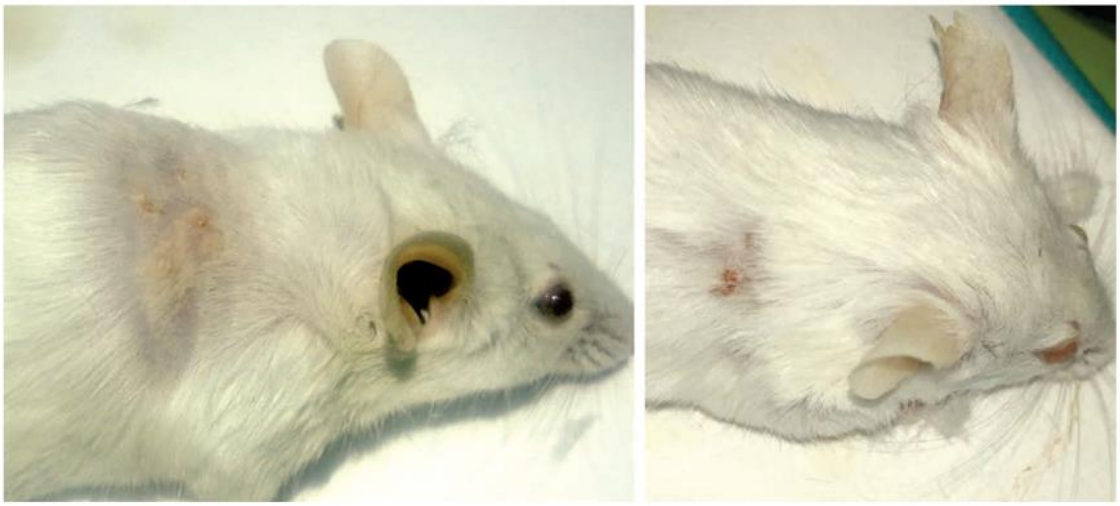

Fig. 1. (Left) Mouse with whitish yellowish plaques on its back. (Right) Mouse with skin ulcer on its back.

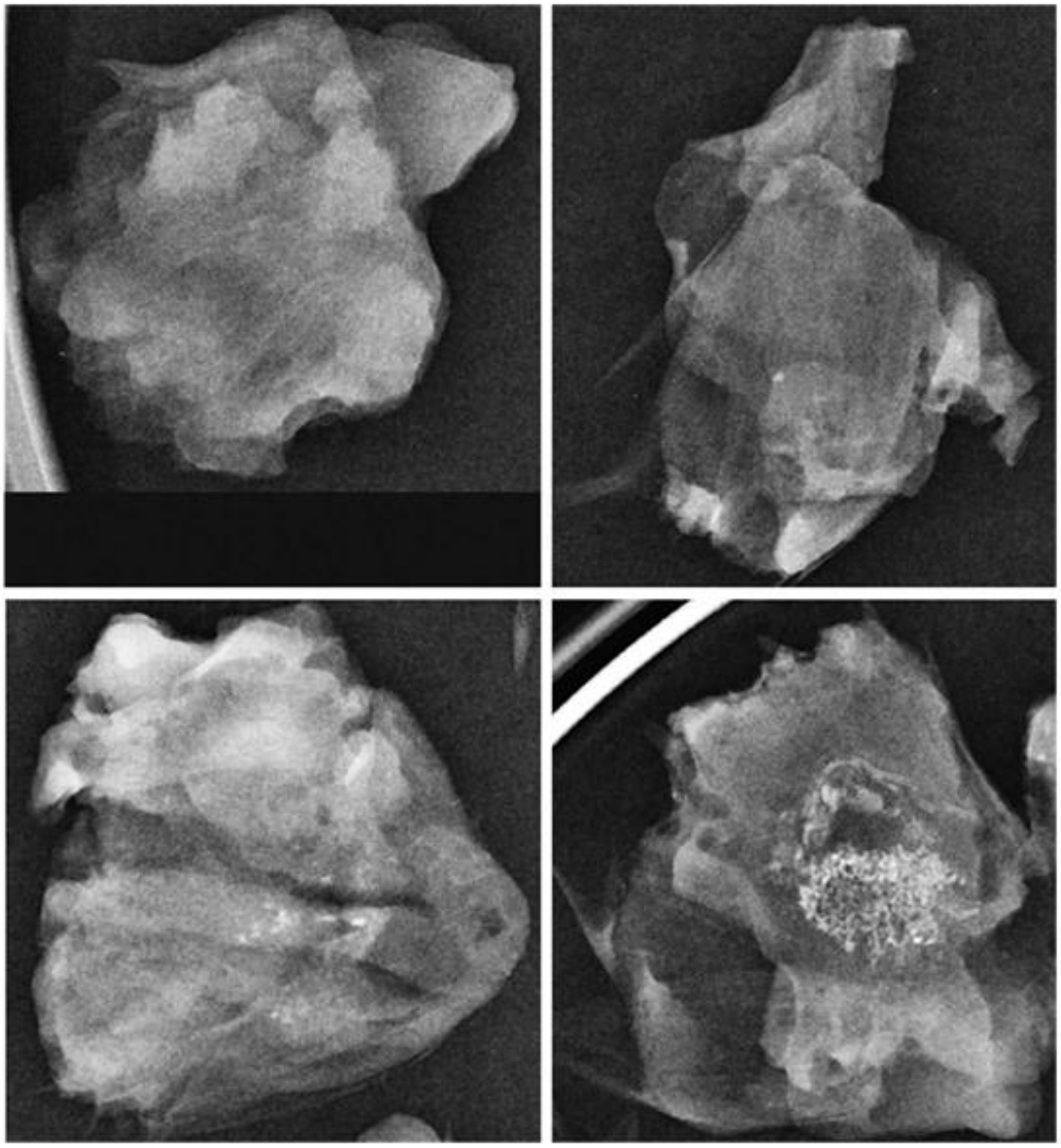

Fig. 2. Radiographic patterns taken by means of mammography (with magnification; focus, 0.1 mm). (Above, left) Calcium deposits not seen. (Above, right) Pointed calcified lesion. (Below, left) Several pointed calcified lesions. (Below, right) Calcium plaque.

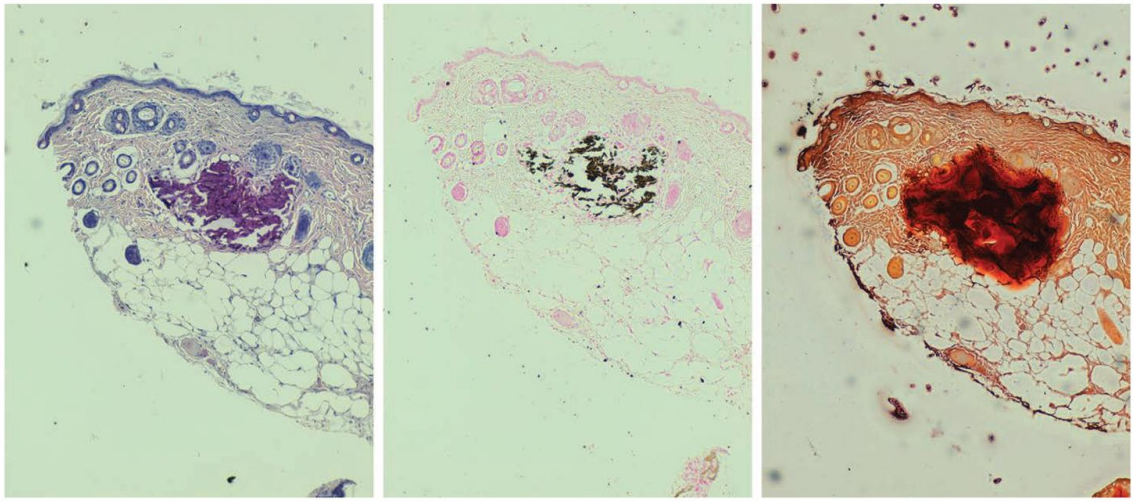

Fig. 3. Histologic study. Detail of calcium deposit on the skin. (Left) Hematoxylin and eosin (original magnification, × 20). (Center) Von Kossa stain (original magnification, × 20). (Right) Alizarin red solution (original magnification, × 20).

Table 2. Presence of Lesions and Radiographic and Histologic Findings in Each Study Group

Findings at 3 Wk	No Antidote (%)	Saline Solution (%)	Triamcinolone Acetonide (%)	Hyaluronidase (%)	Sodium Thiosulfate (%)
Lesions					
No lesions	11 (73)	9 (60)	4 (67)	11 (73)	15 (100)
<2 mm	0 (0)	5 (33)	1 (17)	2 (13)	0 (0)
2–5 mm	4 (27)	0 (0)	1 (17)	2 (13)	0 (0)
>5 mm	0 (0)	1 (7)	0 (0)	0 (0)	0 (0)
Radiography					
No calcium	5 (33)	10 (67)	1 (33)	10 (67)	10 (67)
<0.1 mm	0 (0)	0 (0)	0 (0)	0 (0)	2 (13)
0.1–0.5 mm	4 (27)	3 (20)	2 (33)	5 (33)	3 (20)
0.5–1 mm	1 (7)	1 (7)	0 (0)	0 (0)	0 (0)
>1 mm	5 (33)	1 (7)	2 (33)	0 (0)	0 (0)
Histology					
Calcium	8 (53)	5 (33)	6 (100)	2 (13)	2 (13)
Epidermis	1 (7)	0 (0)	0 (0)	0 (0)	0 (0)
Dermis	4 (27)	2 (13)	3 (50)	2 (13)	0 (0)
Subcutaneous	3 (20)	0 (0)	1 (17)	0 (0)	0 (0)
Fascia/muscle	6 (40)	2 (13)	5 (83)	0 (0)	2 (13)
Vascular	6 (40)	2 (13)	3 (50)	0 (0)	1 (7)
Inflammation	7 (47)	8 (53)	4 (47)	9 (60)	3 (20)
Lymphocytes	5 (33)	3 (20)	2 (33)	8 (53)	1 (7)
Histiocytes	6 (40)	8 (53)	2 (33)	5 (33,3)	0 (0)
Eosinophils	2 (13)	1 (7)	1 (17)	1 (7)	2 (13)
Neutrophils	5 (33)	2 (13)	1 (17)	6 (40)	0 (0)

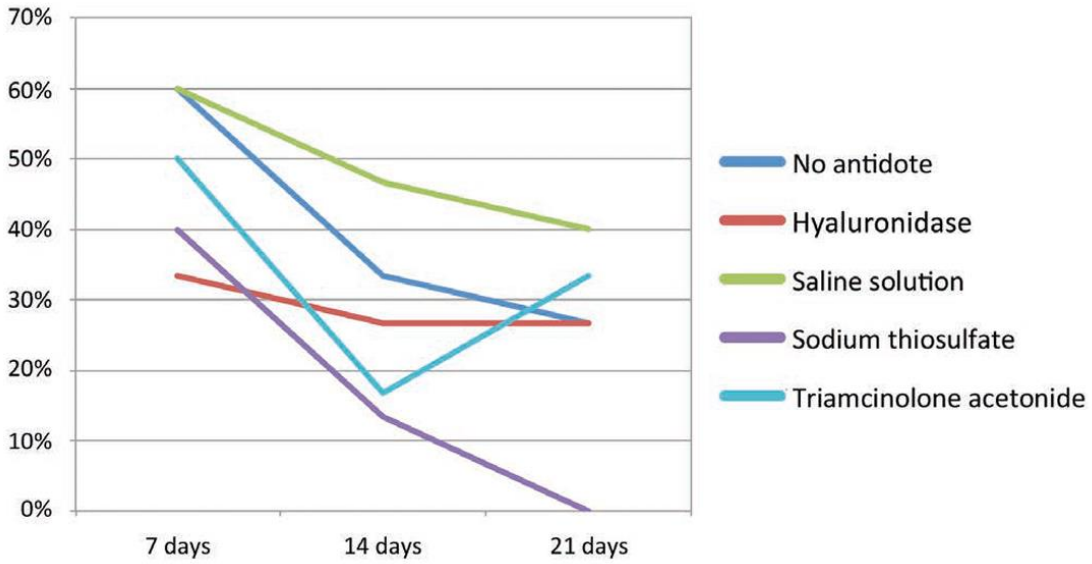

Fig. 4. Percentage of cases with skin lesions in mice infiltrated with different antidotes during the 3-week period.

Table 3. Statistical Significance (p Values) in Relation to Findings in the Radiographic Study

Calcium Deposits	Saline Solution	Triamcinolone Acetonide	Hyaluronidase	Sodium Thiosulfate
>0.1 mm	0.068*	1†	0.068*	0.010*
>0.5 mm	0.427†	1†	0.017†	0.017†
>1 mm	0.169†	1†	0.042†	0.042†

*χ^2 test.

†Fisher's exact test.