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Experimental Research on the Effect of Amiodarone on the Healing of Skin Wounds in Rats

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Abstract: At the level of skin wounds, an electrical potential difference develops between the edges of the wound and the center of the wound, which favors the migration of cells in the process of their healing. Cells migrate in an electric field because they have a certain electrical membrane potential. This potential is due to differences in the transmembrane electrochemical gradient. The transmembrane electrochemical gradient is due to the migration of sodium, potassium and calcium ions into the corresponding ion channels. If this is the case, the modification of the functionality of these ion channels should influence the membrane potential and, as a consequence, the wound healing process. In this experiment, we aimed to investigate to what extent amiodarone influences the wound healing process. Amiodarone blocks several types of ion channels but at different concentrations: at low concentrations it blocks only potassium channels, at medium concentrations potassium and calcium channels, and at high concentrations it blocks potassium, calcium and sodium channels. We worked on rats that were given experimental skin lesions and evaluated the influence of the healing of these lesions upon the topical administration of amiodarone in 3 concentrations, 200nM, 2000nM and 200000nM, compared to an untreated group and a group treated with benzyl alcohol, the amiodarone solvent. In our experimental conditions, low concentration amiodarone promoted wound healing both in terms of duration of healing and also in terms of speed of healing. This means that blocking some ions, possibly potassium channels, might promote wound healing.

Keywords: wound healing; potassium channels; amiodarone

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1. Introduction

Potassium channels allow the passage of potassium ions through the membrane, as well as blocking the flow of other ions – particularly, sodium ions. They are composed of two parts: a part that makes the selection and allows the passage of potassium ions, and the gate, which opens and closes the channel based on environmental signals [3,28]. Voltage-gated potassium channels are involved in various physiological processes, from repolarization of neuronal or cardiac action potentials, upregulation of calcium signaling and cell volume, to stimulation of cell proliferation and migration [11,13,26]. It also provides opportunities for the development of new drugs for various diseases and physiological processes such as scarring [2,3,17]. Voltage-gated potassium channels form a large and diverse family that is evolutionarily conserved. There are 40 human voltage-gated potassium channel genes belonging to 12 subfamilies [12,19,27]. These voltage-gated potassium channels show wide distributions in the nervous system and other tissues [10,11,12,13,16]. For excitable cells such as neurons, cardiomyocytes, and muscles, voltage-gated potassium channels regulate the waveform and firing pattern of action potentials. Voltage-gated potassium channels can regulate cell volume, proliferation and migration of a wide range of cell types [13,18,20,25].

Data from published papers suggests that at the level of skin wounds, an electrical potential difference develops between the edges of the wound and the center of the wound, which favors the

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migration of cells in the process of their healing [3,9,15]. In principle, cells migrate in an electric field because they have a certain electrical membrane potential [2,3,9]. This potential is due to differences in the transmembrane electrochemical gradient. In turn, the transmembrane electrochemical gradient is due to the migration of sodium, potassium and calcium ions into the corresponding ion channels. If this is the case, the modification of the functionality of these ion channels should influence the membrane potential and, as a consequence, the wound healing process [3,4,10,15].

In the present experiment, we aimed to investigate to what extent amiodarone influences the wound healing process [1].

Amiodarone is a substance that blocks several types of ion channels but at different concentrations: at low concentrations it blocks only potassium channels, at medium concentrations potassium and calcium channels, and at high concentrations it blocks potassium, calcium and sodium channels [1,7,8,21,24].

We worked on rats that were given experimental skin lesions and evaluated the influence of the healing of these lesions upon the topical administration of amiodarone in 3 concentrations, respectively the concentration of 200nM which blocks only potassium channels, 2000nM which blocks both calcium channels and channels of potassium and the concentration of 200000nM which blocks potassium channels, calcium channels and sodium channels, compared to an untreated group and a group treated with benzyl alcohol, the amiodarone solvent [6,8,14,22].

2. Materials and Methods

All experiments were conducted in accordance with the protocols approved by Carol Davila University of Medicine Bucharest institutional Animal care and use Committee.

A total of 40 albino male Wistar rats were worked on. In each animal, under general anesthesia with Ketamine and Xylazine, a square lesion with a side of 1 cm was performed by skin excision after depilation (Figure 1).

Figure 1. Lesion with a side of 1 cm.

The animals were divided into 5 batches: batch number 1 was untreated, batch number 2 was treated with benzyl alcohol, amiodarone solvent, batch number 3 was treated with amiodarone at a concentration of 200 nM (nanomolar), batch number 4 a was treated with amiodarone at a concentration of 2000 nM, batch number 5 was treated with amiodarone at a concentration of 200 000 nM. Each rat was treated twice daily by topical administration of the substance corresponding to each batch until the lesions were healed.

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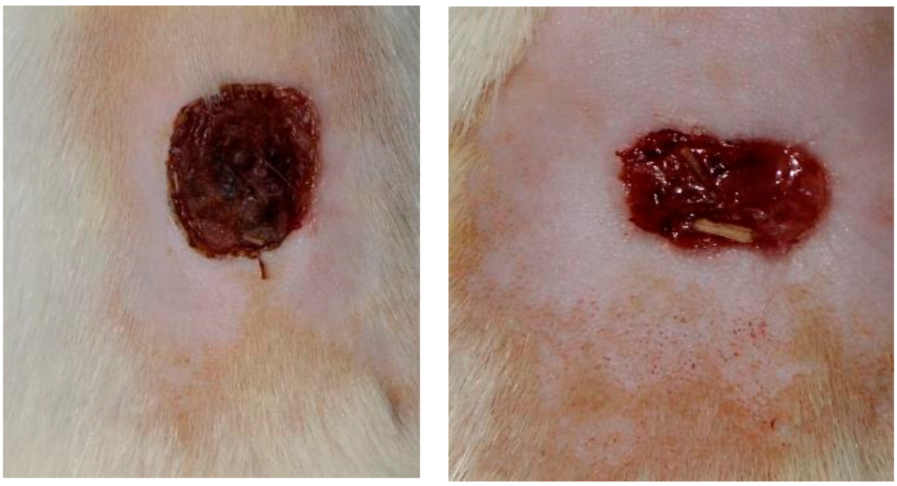
Each lesion was photographed from the same distance and with the same degree of image magnification, every other day for the first nine days and then every three days, respectively at time t_1 - day 1 from the practice of the injury, t_2 - day 3 (Figure 2), t3 - day 5, t_4 - day 7 (Figure 3), t_5 - day 9, t_6 - day 12, t_7 - day 15 (Figure 4) [23]. Using an Image J program, the area of each lesion measured in pixels was calculated at each time of the recording.

(a) (b)

Figure 2. (a) The dorsal and caudal lesion on day 3- natural. (b) The caudal lesion in day 3-. amiodarone 200 nM.



(a) (b)

Figure 3. (a) The dorsal and caudal lesion on day 7—natural. (b) The caudal lesion in day 7—amiodarone 200 nM.

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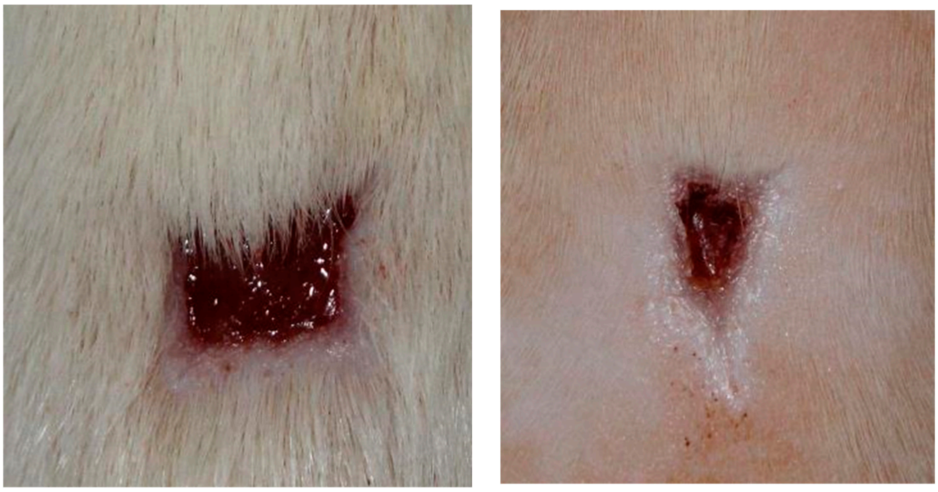

(**a**) (**b**)

Figure 4. (a) The dorsal and caudal lesion on day 15- natural. (b) The caudal lesion in day 15- amiodarone 200 nM.

The main parameter analyzed was the mean duration of wound healing in each group.

In addition to this, secondary parameters were also analyzed, namely the percentage decrease of the lesional surfaces and the average speed percentage per day of the lesional surfaces.

The following parameters were calculated for each rat and time of measurement:

(a) The main parameter - the duration of wound healing measured in days

(b) Secondary parameters

1. The percentage decrease of the area relative to the value of the initial area, according to the formula

$$S = \frac{S_{t1} - S_t}{S_{t1}} x100$$

where S is the percentage decrease in area, S_{t1} is the initial area measured in pixels, and S_t is the area at the time of measurement in pixels.

2. The percentage speed decrease of the lesion surface according to the formula

$$V=\left(\frac{S_t - S_{t+1}}{S_t} x100\right) : [(t+1) - t]$$

where V represents the percentage decrease rate per day of the surface, S_t represents the surface of the lesion at time t, measured in pixels, S_{t+1} represents the surface of the lesion at time t+1 measured in pixels, and t represents the time of surface measurement expressed in days from the beginning of the experiment.

For each batch, the averages and standard deviations of the 3 parameters were calculated at each moment of the measurement, the statistical significance was investigated by the T-Student test. It was considered that the differences between the groups for each moment of the measurement are statistically significant if $p < 0.05$ for the main parameter and for $p < 0.02$ for secondary parameters because the Bonferroni method was applied in order not to produce an alpha risk inflation.

3. Results

3.1. Main parameter: Average duration of wound healing

Average duration of wound healing

The average duration of wound healing was: in the untreated group 27.17±5.19 days, in the group treated with benzyl alcohol 21.83±2.71 days, in the group treated with low concentration

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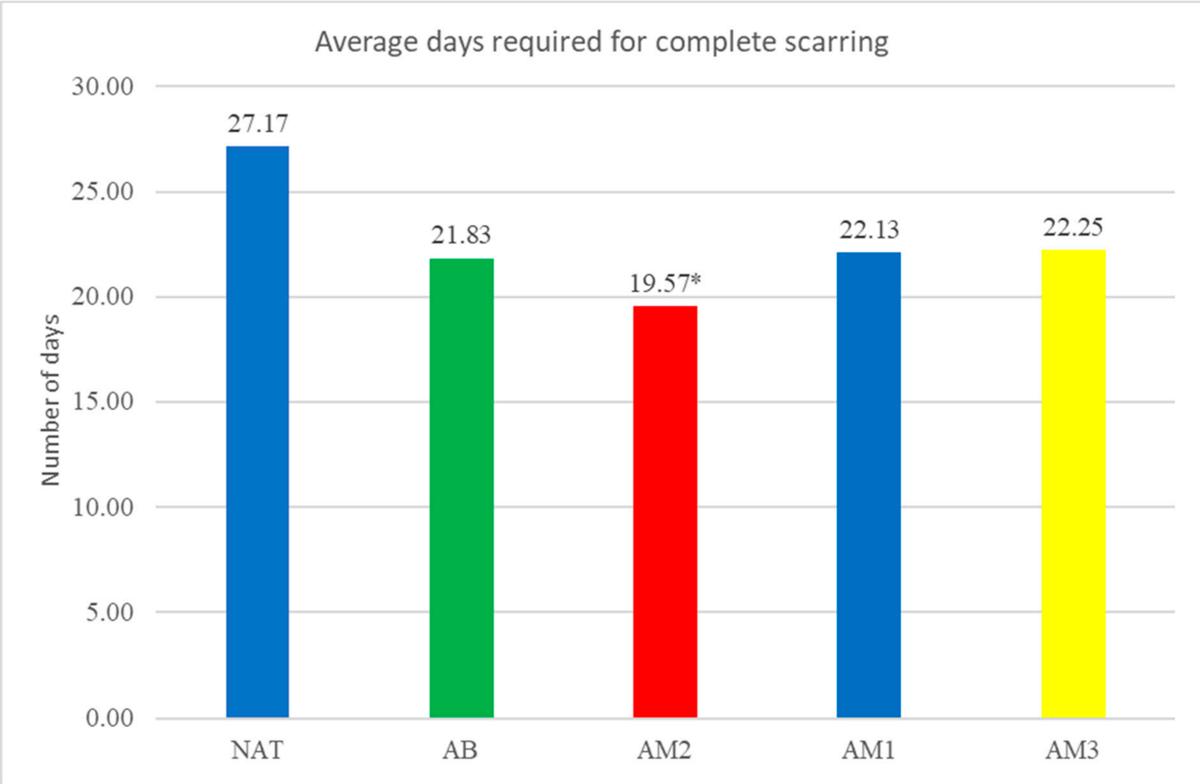
amiodarone 19.57±3.05 days , in the group treated with amiodarone in medium concentration 22.13±5.17 days and in the group treated with amiodarone in high concentration 22.25±4.53 days. There was only one statistically significant difference compared to the natural batch, namely for the group treated with low concentration amiodarone ($p<0.03$).

The results are presented in Table 1 and Graph 1.

Table 1. The average number of days required to achieve complete healing. Each value represents the average healing days and standard deviation for each batch.

Batch	Average scarring days	Statistical significance compared to the natural batch
NAT	27,17±5,19 zile	
AB	21,83±2,71 zile	
AM2	19,57±3,05 zile	p=0,03
AM1	22,13±5,17 zile	
AM3	22,25±4,53 zile	



Graph 1. The time required for complete scarring. Each column represents the average number of days required healing of the wounds. *=p=0,03.

3.2. Secondary parameters

1. The percentage decrease of the lesion surfaces compared to the initial surface

The percentage decrease of the lesion surfaces compared to the initial surface recorded the following values:

- in the untreated batch at time t_1 -5.33% ±3.63, t_2– 10.40%±8.09, t_3– 43.92%±15.81, t_4-58.08%±14.20, t_5- 76,36%±8,20, t_6- 88,48%±5,92
- in the batch treated with benzyl alcohol at time t_1 -7.49% ±11,09, t_2 – 22,41%±13,00, t_3 – 44,35%±18,38, t_4-65,85%±14,13, t_5- 81,06%±4,34, t_6- 92,05%±4,33
- in the batch treated with amiodarone in a concentration of 200 nM at time t_1 -17.03% ±7,38, t_2 – 26,65%±6,20, t_3 – 57,23%±11,82, t_4-71,13%±7,75, t_5- 84,22%±4,18, t_6- 93,50%±4,46

- in the batch treated with amiodarone in a concentration of 2000 nM at time t_1 -9.68% ±7,57, t_2 – 20,78%±10,74, t_3–45,39%±11,48, t_4-64,90%±14,69, t_5- 79,55%±6,09, t_6- 90,51%±5,83
- in the batch treated with amiodarone in a concentration of 200,000 nM at time t_1 -7.05% ±6,98, t_2 – 18,91%±6,96, t_3 – 43,59%±5,59, t_4-61,56%±10,35, t_5- 76,27%±9=6,97, t_6- 86,63%±7,23

Statistical analysis showed that:

1. At time t_2 compared to t_1: the greatest decrease in surface area was in the batch treated with low concentration amiodarone, the difference compared to the untreated batch being statistically significant for a p<0.001
2. At time t_3 compared to t_1: the biggest decrease was in the batch treated with low dose amiodarone, the difference compared to the untreated batch being statistically significant for p<0.0003, but also the group treated with high concentration amiodarone recorded the difference of the untreated batch statistically significant although lower for p<0.02.
3. At time t_4 compared to t_1, amiodarone in low concentration also registered the greatest decrease in surfaces, but the differences compared to the untreated batch are not statistically significant.
4. At times t_5 and t_6 compared to t_1, the only statistically significant difference from the untreated batch was recorded for the low dose of amiodarone (p<0.02 at time t_5, respectively p<0.01 at time t_6).

The results are presented in Table 2 and Graphs 2 and 3.

Table 2. The percentage decrease of the lesion surfaces compared to the initial surface. Each value represents the average of the differences between the initial area and the area at the time of measurement relative to the initial area for each batch, the area being measured in pixels.The significance was calculated compared to the natural batch.

Batch	Z1-Z3	Z1-Z5	Z1-Z7	Z1-Z9	Z1-Z12	Z1-Z15
NAT	5,33%±3,63	10,40%±8,09	43,92%±15,81	58,08%±14,20	76,36%±8,20	88,48%±5,92
AB	7,49%±11,09	22,41%±13,00 (p=0,02)	44,35%±18,38	65,85%±14,13	81,06%±4,34	92,05%±4,33
AM2	17,03%±7,38 (p=0,001)	26,65%±6,20 (p=0,0003)	57,23%±11,82 (p=0,04)	71,13%±7,75 (p=0,02)	84,22%±4,18 (p=0,01)	93,50%±4,46 (p=0,04)
AM1	9,68%±7,57	20,78%±10,74 (p=0,03)	45,39%±11,48	64,90%±14,69	79,55%±6,09	90,51%±5,83
AM3	7,05%±6,98	18,91%±6,96 (p=0,02)	43,59%±5,59	61,56%±10,35	76,27%±6,97	86,63%±7,23

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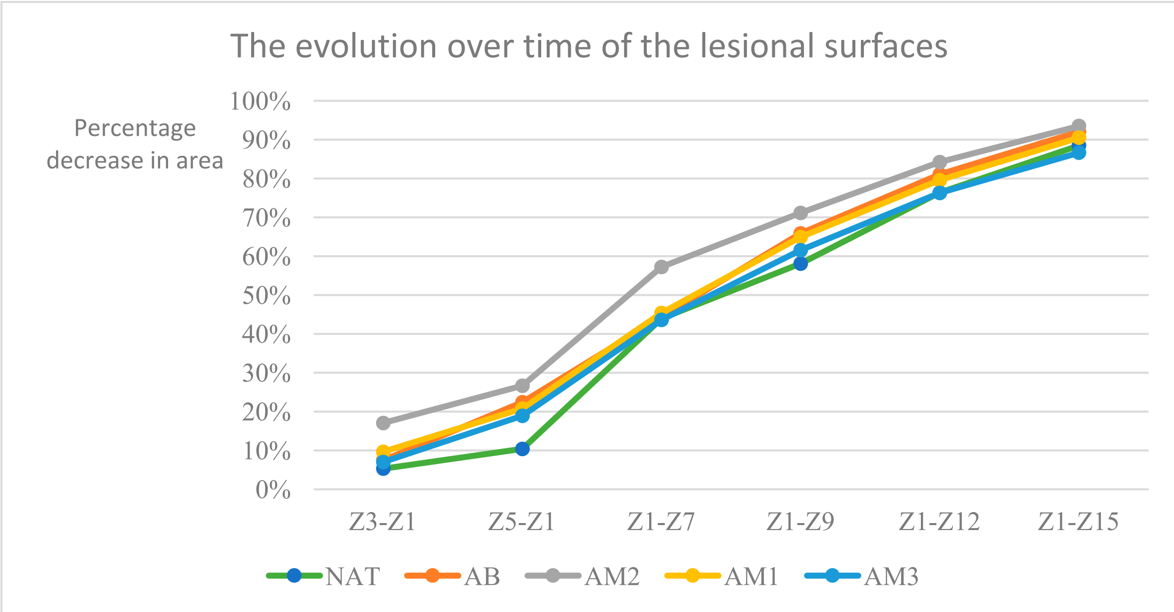
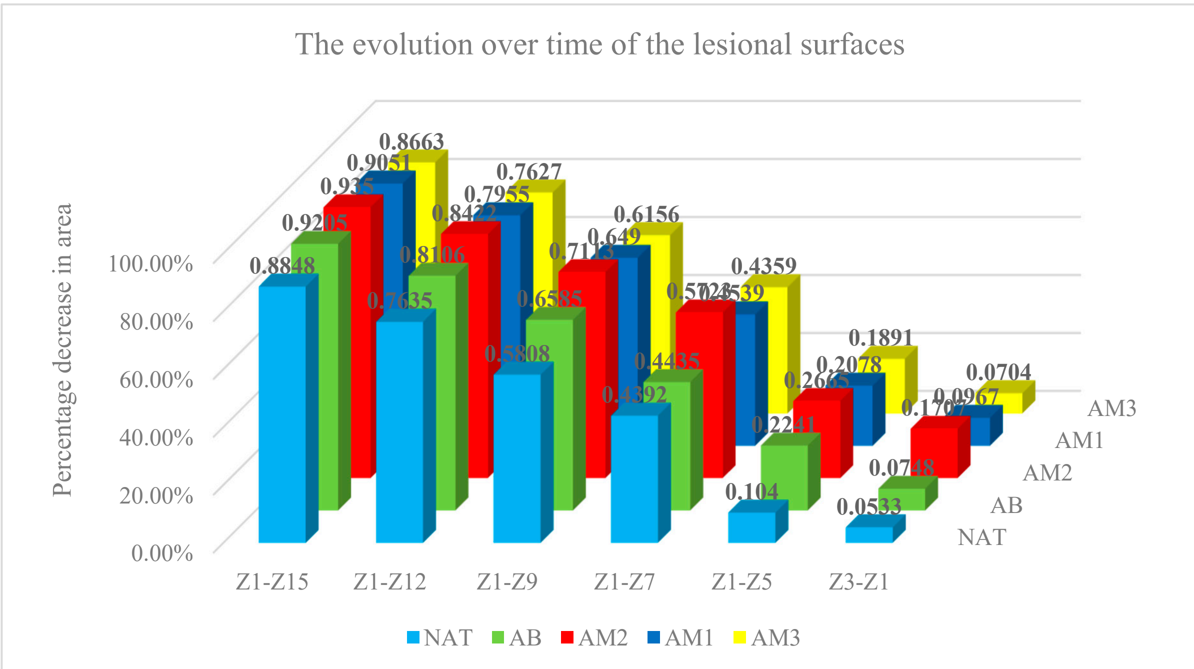


Graph 2. Evolution over time of the lesional surfaces. The percentage difference between the initial surface and the surface at the time of the measurement relative to the initial surface is represented on the vertical axis. The days in which the measurements were taken counted from the start of the experiment are represented on the horizontal axis.



Graph 3. The evolution over time of the lesional surfaces. Each column represents the difference between the initial surface and the surface at the time of measurement relative to the initial surface, all measured in pixels.

2. The rate of daily percentage decrease of the lesion surface

At time t_2, the velocities were: in the untreated batch 2.66±1.81/day, in the batch treated with benzyl alcohol 5.80%±3.71/day, in the batch treated with low concentration amiodarone 8.51%± 3.69/day, in the batch treated with amiodarone in medium concentration 4.79%±3.97/day, and in the batch treated with amiodarone in high concentration 4.96%±3.31/day.

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At time t_3, the velocities were: in the untreated batch 2.72±3.16/day, in the batch treated with benzyl alcohol 8.23%±2.52/day, in the batch treated with low concentration amiodarone 5.70%± 2.89/day, in the batch treated with amiodarone in medium concentration 6.32%±3.08/day, and in the batch treated with amiodarone in high concentration 6.24%±3.93/day.

At time t_4, the velocities were: in the untreated batch 19.06±6.84/day, in the batch treated with benzyl alcohol 15.25%±5.76/day, in the batch treated with low concentration amiodarone 20.94%± 7.11/day, in the batch treated with amiodarone in medium concentration 16.42%±3.66/day, and in the batch treated with amiodarone in high concentration 16.49%±4.33/day.

At time t_5, the velocities were: in the untreated batch 12.61±5.83/day, in the batch treated with benzyl alcohol 18.99%±8.98/day, in the batch treated with low concentration amiodarone 15.68%± 5.87/day, in the batch treated with amiodarone in medium concentration 18.91%±8.31/day, and in the batch treated with amiodarone in high concentration 15.37%±11.41/day.

At time t_6, the velocities were: in the untreated batch 13.41±6.02/day, in the batch treated with benzyl alcohol 13.22%±6.12/day, in the batch treated with low concentration amiodarone 15.04%± 2.52/day, in the batch treated with amiodarone in medium concentration 12.82%±4.18/day, and in the batch treated with amiodarone in high concentration 11.98%±6.10/day.

At time t_7, the velocities were: in the untreated batch 17.60±4.88/day, in the batch treated with benzyl alcohol 18.69%±7.10/day, in the batch treated with low concentration amiodarone 20.73%± 6.74/day, in the batch treated with amiodarone in medium concentration 18.98%±7.46/day, and in the batch treated with amiodarone in high concentration 15.72%±5.30/day.

There were statistically significant differences compared to the untreated batch at time t_2 only in the batch treated with low concentration amiodarone ($p < 0.001$), and at time t_2 the statistically significant difference compared to the untreated batch was for the average dose of amiodarone ($p < 0.02$). The results are presented in Table 3 and Graphs 4 and 5.

Table 3. Each value represents the average of the percentage speed decrease between 2 consecutive measurements, related to the time interval.

Batch	Z1-Z3	Z3-Z5	Z5-Z7	Z7-Z9	Z9-Z12	Z12-Z15
NAT	2.66%±1,81	2,77%±3,168	19,06%±6,85	12,61%±5,83	13,41%±6,02	17,60%±4,88
AB	5.80%±3,71 (p=0,03)	8,24±2,52 (p=0,001)	15,26±5,76	18,99%±8,98	13,22%±6,12	18,69%±7,1
AM2	8.51±8.51 (p=0,001)	5,70%±2,89 (p=0,03)	20,94%±7,11	15,68%±5,87	15,04%±2,52	20,73%±6,74
AM1	4.79%±3.97	6,32%±3,08 (p=0,02)	16,42%±3,66	18,91%±8,31	12,82%±4,18	18,98%±7,46
AM3	2.60±5.99	6,24%±3,93 (p=0,04)	16,49%±4,33	15,37%±11,41	11,98%±6,10	15,72%±5,30

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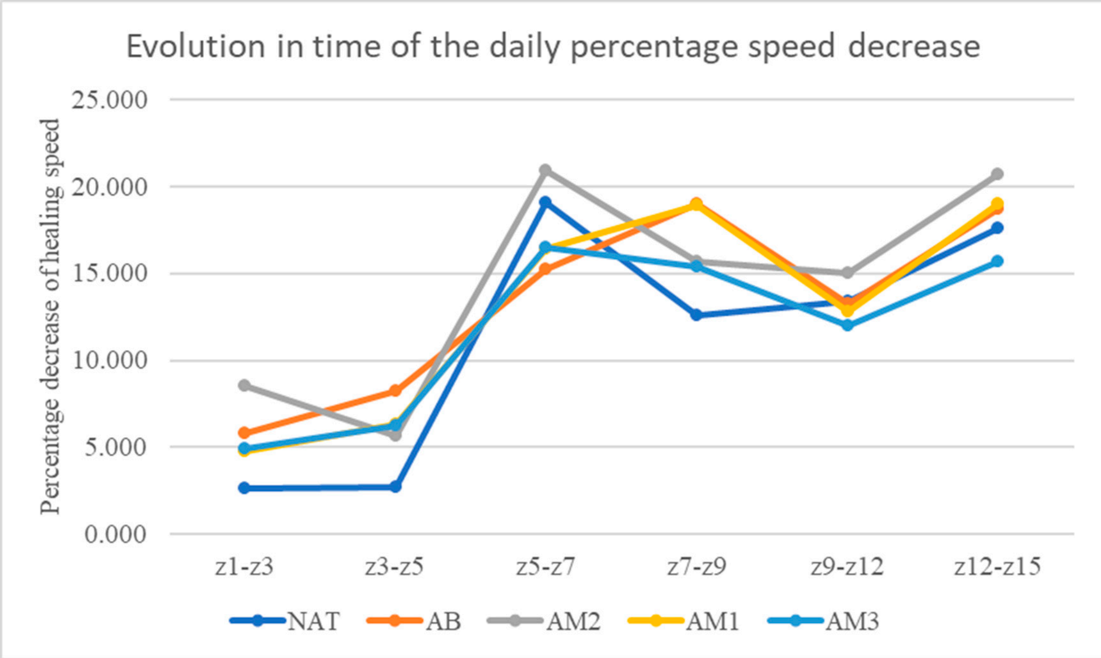
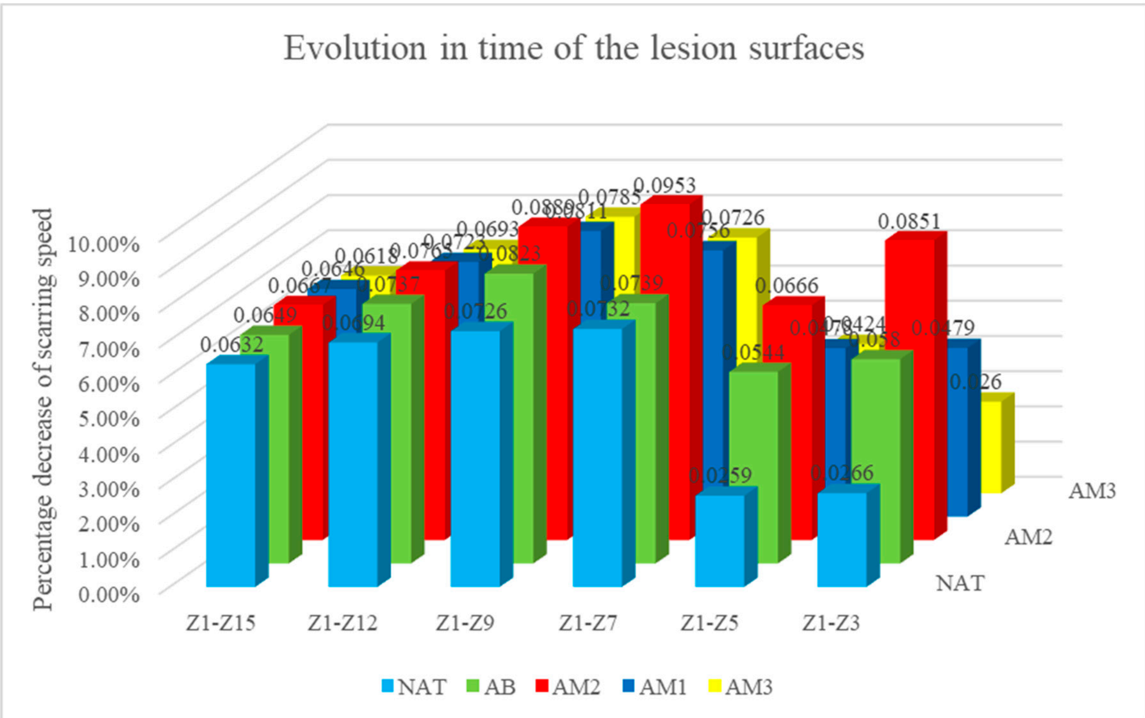


Graph 4. Time evolution of the daily percentage speed decrease. On the vertical axis the percentage speed decrease between 2 consecutive measurements is represented. On the horizontal axis the time interval between the 2 consecutive measurements is represented.



Graph 5. Time evolution of the daily percentage speed decrease. Each column represents the average of the percentage speed decrease between 2 consecutive measurements, related to the time interval.

The results are presented in Table 3. Each value represents the average of the percentage speed decrease between 2 consecutive measurements, related to the time interval.

4. Discussion

As it can be seen from the evaluation of the duration of wound healing, only low-dose amiodarone showed a statistically significant decrease compared to the untreated batch, i.e. 19.57 days for low-dose amiodarone compared to 27.17 days for the untreated batch.

In principle, low-dose amiodarone decreased the time required for wound healing by approximately 8 days, representing 27.97% of the time required for wound healing in the untreated batch.

Considering the working hypothesis, we can assume that blocking of potassium channels accelerates wound healing under our experimental conditions, represented by the decrease in the time required for wound healing. This effect was maintained throughout the entire period of wound healing.

This implies that blocking potassium channels favors wound healing, considering that the medium dose, which in addition to potassium channels also blocks calcium channels, and the high dose, which in addition to potassium channels also blocks calcium and sodium channels, had no statistically significant effect. Furthermore, taking into consideration that medium and high doses of amiodarone had no effect on wound healing, we can presume that both blocking of calcium channels and of sodium channels antagonize the effect of blocking potassium channels.

The hypothesis seems logical because the potassium current is a repolarizing current while the calcium and sodium currents are depolarizing currents. It is very likely that blocking the repolarizing potassium current increased the membrane potential and thereby accelerated cell migration in the electric field. Under these conditions, blocking of depolarizing currents decreased the membrane potential raised by potassium channel blockade and thus antagonized the favorable effect of potassium channel blockade. Indeed, this seems to have been the case in our experiment, because the effect of amiodarone in high concentrations on wound healing was less intense than the effect of amiodarone in medium concentrations.

The secondary parameters used, respectively the percentage decrease of the lesional surfaces compared to the initial surface and the daily percentage speed decrease of the lesional surfaces tried to evaluate by which mechanisms the blocking of the ion channels influenced wound healing.

Amiodarone in high concentration reduced progressively, from one determination to another, the differences between the decrease in lesional surfaces compared to the control group, but only at time t_3 these differences were statistically significant.

Regarding the daily rate of decrease in lesional area, it generally increased during the first 3 days, after which it remained relatively constant. The highest speed of daily decrease in surface area was found in the batch treated with amiodarone in low concentration, the differences being statistically significant compared to the untreated batch at time t_2.

Both the batches treated with medium and high concentration of amiodarone recorded higher rates of daily percentage decrease of the lesion surfaces compared to the untreated batch, but only the average dose of amiodarone was statistically significant at time t_3.

These results show that the shortening of wound healing time is produced by an acceleration of the rate of decrease of the lesional surfaces.

The fact that the differences were statistically significant only at certain times of the measurement, but not at all times, suggests that different mechanisms are involved during healing from one stage to another, and that it is likely that blocking potassium channels promotes wound healing only at certain stages of the healing process, depending on the mechanism involved in the respective stages. The fact that in the analysis of these secondary parameters and amiodarone in medium concentration had statistically significant effects at certain times of the measurement, but amiodarone in high concentration never had statistically significant effects, suggests that blocking calcium channels only partially antagonizes the effect of blocking potassium channels. The effect of blocking potassium channels appears to be completely antagonized only by simultaneously blocking both calcium and sodium channels.

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From a statistical point of view, there is of course the possibility that the small number of lesions per group of animals did not confer enough statistical power to detect differences between the batches treated with different doses of amiodarone.

5. Conclusions

1. Low concentration amiodarone promoted wound healing under our experimental conditions, both in terms of duration of healing and speed of healing.
2. Blocking potassium channels promotes wound healing.
3. Neither medium-dose amiodarone nor high-dose amiodarone had statistically significant effects on wound healing time.
4. The blocking of calcium channels and blocking of sodium channels antagonize the effects of the blocking of potassium channels on wound healing.
5. Given that a potassium current is a depolarizing current, while calcium and sodium currents are repolarizing, it turns out that blocking the potassium current increases the membrane potential, this increase being antagonized by blocking calcium and sodium currents.
6. It is possible that the increase in membrane potential produced by the blocking of potassium channels accelerated the migration of cells into the wound field, which explained the acceleration of healing.

Author Contributions: D.G.A. made the experimental design, conducted the experimental protocol, and analyzed the obtained specimens and data. A.V.B made the experimental design and conducted the statistical and data analysis. S.S, P.E., M.E and A.Z. helped with the experimental protocol and animal housing. O.A.C. analyzed the final version of the manuscript. I.F. analyzed the manuscript and supervised the experiment and analyzed the results. All authors drafted the work or revised it critically for important intellectual content. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania (13310/27 May 2021) for studies involving animals, in conformity with 43/2014 Law regarding animal protection used in scientific purposes, with further completions and 86/609/CEE Directive from 24 November 1986 regarding acts with power of law and administrative acts of member states for animal protection used in experimental purposes and other scientific purposes.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data will be available upon request.

Conflicts of Interest: The authors declare no conflict of interest.

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