TOPICAL USE OF RECTOGESIC® AND EMLA® TO IMPROVE CUTANEOUS BLOOD PERFUSION FOLLOWING THERMAL INJURY. A COMPARATIVE EXPERIMENTAL STUDY

L'UTILISATION TOPIQUE DE RECTOGESIC® ET EMLA® POUR AMELIORER LA VASCULARISATION CUTANEE APRES 'UNE BRULURE: UNE ETUDE EXPERIMENTALE COMPARATIVE

Tagkalakis P.,[1]✉ Dionyssopoulos A.,[1] Karkavelas G.,[2] Demiri E.[1]

[1] Department of Plastic Surgery, Medical School, Aristotle University of Thessaloniki, General Hospital of Thessaloniki "G. Papageorgiou", Thessaloniki, Greece
[2] Department of Pathology, Medical School, Aristotle University of Thessaloniki, General Hospital of Thessaloniki "G. Papageorgiou", Thessaloniki, Greece

SUMMARY. Early post-burn ischemic necrosis of the skin is of particular interest in modern burn research. The purpose of this study was to test the hypothesis that blood perfusion could be improved by the topical application of vasoactive substances. A sample of 55 wistar rats was used to investigate the effect of 0,4% nitroglycerin ointment (Rectogesic®) comparatively to no application and placebo. The beneficiary action of 5% prilocaine/lidocaine cream (EMLA®) in burn blood perfusion was also tested comparatively to Rectogesic®. Both preparations were tested respectively to non burned controls. Laser Doppler assessment of blood flow at 15, 30, 45, 60, 120 and 180 minutes after preparation application, demonstrated that the use of Rectogesic® improved perfusion at all measurements compared to placebo and to no preparation application ($p<0,05$). There was no statistical significant difference in the effect of the two preparations.

Keywords: burns, Laser Doppler, EMLA®, Rectogesic®, local anesthetics.

***RÉSUMÉ**. La nécrose ischémique précoce de la peau brûlée est d'un grand intérêt dans la recherche actuelle en brûlologie. Le but de cette étude était de vérifier l'hypothèse que la vascularisation peut être améliorée par l'application topique de substances vaso-actives. Un échantillon de 55 rats Wistar a été utilisé pour étudier l'effet de 0,4% onguent à la nitroglycérine (Rectogesic®) comparativement à aucune application d'onguent et un placebo. L'action bénéfique de la crème lidocaïne (EMLA®)(5% prilocaïne) sur la vascularisation cutanée a également été testé comparativement àvec le Rectogesic®. Les deux préparations ont été testées respectivement sur des témoins non brûlés. Une évaluation Laser Doppler du flux sanguin à 15, 30, 45, 60, 120 et 180 minutes après l'application de la préparation, a démontré que l'utilisation de Rectogesic® améliore la circulation à chaque mesure par rapport au placebo et par rapport à la non application d'onguent ($p<0,05$). Il n'y avait aucune différence statistiquement significative dans l'incidence des deux préparations.*

***Mots-clés**: brûlures, Laser Doppler, Emla®, Rectogesic®, anesthésiques locaux*

Introduction

Jackson[1] described three concentric zones of a burn wound: the central zone of coagulation, the intermediate zone of stasis and the outer zone of hyperemia. He also proposed that the tissue between the irreversible necrosis of the zone of coagulation and the edematous red tissue of the zone of hyperemia (zone of stasis), involves a com-

✉ Corresponding author: Mr Pantelis Tagkalakis M.D., M.Sc., 25 Koritsas Street, 141 21, Neo Iraklio, Attica, Greece. Tel./fax: +30210 2849391; email: drpantagalakis@hotmail.com
Manuscript: submitted: 24/7/14, revised: 28/9/14, accepted: 14/2/15.

plete cessation of blood flow within 24h resulting in tissue necrosis, although it is not avascular.[2] Since then a challenging question has been raised: what factors contribute to or ameliorate the progression of tissue destruction in the zone of stasis?

Several experimental studies are focusing to establish adequate perfusion levels within the zone of stasis by reversing vasoconstriction, inflammation, edema-formation and thrombosis of microvessels in order to avoid tissue integrity harm due to hypoxia, lack of nutrients and accumulation of toxic metabolites.[3-4] There are no accepted nor standardized therapeutic approaches in humans today for salvaging the intercalated zone of stasis and preventing secondary burn wound progression.[5]

The hypothesis of this study was founded in previous similar studies attempting to give answers to this question, using topical application of vasoactive substances in order to improve deep partial thickness thermal burns blood perfusion.[6-32]

Burn injury induces a pronounced inflammatory reaction, involving various vasoactive mediators such as histamine,[33-34] serotonin,[35-36] leukotrienes[37-38] and prostaglandins[39-40] with effects on vascular patency and edema formation.[6-32] Local anesthetics have been shown to interfere with inflammation induced, plasma extravasation[41-42] and the release and synthesis of several mediators such as prostaglandins,[43-44] leukotrienes,[45] interleukins[46] and histamine.[47] The mechanism which is responsible for the inhibition of burn edema by local anesthetics is related to the action of local anesthetics on vascular tone. This action could be secondary to inhibition of leukocyte adherence to the endothelium of injured vessels[46-47] and migration into injured tissues,[48-49] as well as the inhibition of the release of tissue toxic lysozymes,[49] oxygen free radicals[43-44, 50] and histamine.[51] All these factors are known to be involved in the control of vascular permeability in burns.[52] The inhibition of burn edema by local anesthetics could, in conjunction with the antithrombotic[53] effects of these agents, account for the prevention of progressive burn ischemia reported following experimental burns.[21]

The vasoactive properties of 5% prilocaine/lidocaine cream (EMLA®) and their effect in burn blood perfusion have already been described.[13, 17, 20-23, 37]

Organic nitrates, including nitroglycerin, are known for their vasodilatory and antithrombotic action. They are used systemically for the prevention and therapy of coronary disease and left heart failure.[54] By topical application, 0,4% nitroglycerine ointment (Rectogesic®) is used for the chronic anal fissure[55] and hemorrhoids[56] treatment. Topical application of nitroglycerine has been used experimentally for the treatment of flap ischemia.[57-63] Very little is known about their effect in burn injury blood perfusion.[24]

In the present study we used the Laser-Doppler, a non invasive method for repetitive measurements of blood flow,[64] to investigate the effects of topically applied 0,4% nitroglycerine ointment (Rectogesic®) in burn blood perfusion. This was the first time that the vasoactive properties of this preparation were investigated in thermal injuries. In addition, the first attempt ever was made to compare its action to the beneficial action of the 5% prilocaine/lidocaine cream (EMLA®) on standardized deep partial thickness burns in rats.

Materials and methods

The study took place at the experimental laboratory of "G. Papageorgiou" General Hospital of Thessaloniki in Greece.

Materials

We used 55 male and female wistar rats because their skin has all the characteristics of mammals. Their weight ranged from 220 to 250 gr. For at least seven days prior to the experiments the animals were housed in special cages in a ventilated and temperature controlled room with food and water provided *ad libidum*. The day and night cycle was available naturally through windows.

The preparations used were the 0,4% (4mg/gr) nitroglycerine ointment (Rectogesic®, ProStrakan Limited, UK) and the 5% (25mg+25mg/ml) prilocaine/lidocaine cream (EMLA®, Astra Zeneca, Recip Karlskoga AB, Sweden).

Method

The sample was based on previous studies. Animals in the study were distributed randomly[65] into 4 groups with burns and 3 control groups without burns. Group A was given Rectogesic®, group B EMLA®, group C was given nothing and group D were given the respective placebo without nitroglycerin. The control group A^c had Rectogesic®, group B^c had EMLA® and group D^c was given the respective placebo without nitroglycerin.

Preparation of the animals

The animals were anesthetized using intramuscular injection of 20-30mg/kg ketamine hydrochloride (Ketalar amp, 50mg/5ml, Warner Lambert, Hellas), in combination with 1-2mg/kg midazolam (Dormicum amp, 15mg/3ml Roche, Hellas). This method gives quick induction and maintenance for at least one hour of anesthesia, as well as analgesia and, if necessary, can be repeated.[66] This was followed by the non traumatic depilation of the dorsal skin.

Experimental burn

A scald was created to the back of the animals.[12] The standardized process was as follows: The boiling water was placed in a 5cm diameter heat resistant container. Following that, the temperature was monitored with an in-

frared thermometer descending down to 75°C. At this temperature the back of the anesthetized animal was kept immersed for 10 seconds in the hot water, creating a standard deep partial thickness burn. This process was followed by the measurements.

Blood flow evaluation

Measurement of the blood flow in ml/min/100gr was performed using Laser–Doppler equipment (PERIFLUX®, PF 4001, PERIMED AB, Sweden). The Laser-Doppler technique allowed non invasive, repetitive measurements as opposed to other described methods requiring removal of experimental tissue.[64] The accuracy of this method as a quantitative tool was evaluated and it was suggested that multiple measurement points be used to increase validity and reliability of measurements.[67] In this study five standard points of the burned area were used for blood flow evaluation and the mean value of these measurements was recorded as the variable for analysis. Six different measurements were performed on a standardized timescale during the early post-burn period, to assess the blood perfusion changes. The preparations were wiped from the experimental area before the measurement process using 95° alcohol wipes and were replaced during the intervals between measurements.

Experimental protocol

The standardized experimental process was performed by the same investigator.

The experimental steps were common for all groups. No burn was performed in controls. The first measurement took place before the burn formation or preparation placement and was used as a reference measurement of the normal skin flow. This was followed by the experimental burn. Five minutes after the burn 0,5gr of the respective preparations were applied in groups A, B and D. In group B the preparation was covered with occlusive dressing (Tegaderm®, 3M, USA). The timescale of blood perfusion measurements was at 15, 30, 45, 60, 120 and 180 minutes after the first placement of preparations. The measurement at 180 minutes was not followed by replacement of the preparation. In group C all measurements were performed without placement of preparation. The experimentation protocol is presented concisely in *Table I*.

Histolopathology

The depth of the experimental burn was confirmed preliminary by the histological examination of burn tissue specimens performed in 3 anesthetized animals.

Ethical considerations

All animals received human care. Experimental protocols were approved by the Thessaloniki District Veterinary Services Direction Ethics committee.

Statistics

All data were stored using MS Excel 2007 for PC. Mean comparison analysis was performed using t-tests. Statistical significance was set at <0.05.

Results

Descriptive statistics

Descriptive statistics for group A (Rectogesic®)

Mean values of blood flow during measurements, presented in *Table II* and *Fig. 1,* ranged from 25,3750 (S.D.=14,05502) to 31,9020 (S.D.=24,92093) ml/min/100gr.

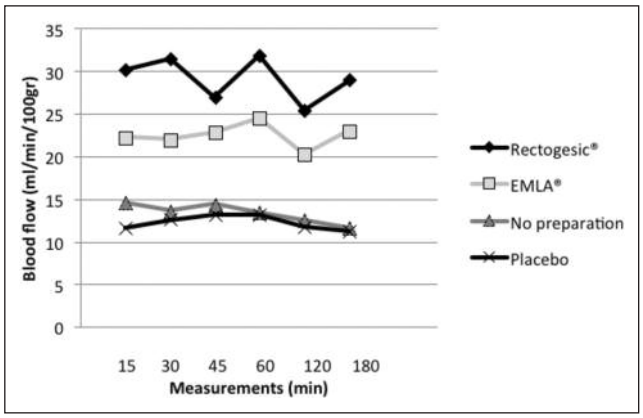

Fig. 1 - Comparative diagram of the measurements for groups A, B, C and D.

Table I - Experimental protocol

Timescale	Action						
	Burn groups				No burn controls		
	A	B	C	D	Ac	Bc	Dc
	n=10	n=10	n=10	n=10	n=5	n=5	n=5
Anesthesia	Intramuscular injection, positioning and depilation						
Normal flow	Measurement						
Burn	Burn formation and positioning				No burn formation		
0 min	Preparation placement						
15 min	Cleaning of preparation, measurement, preparation placement (Only measurement in group C)						
30 min	Cleaning of preparation, measurement, preparation placement (Only measurement in group C)						
45 min	Cleaning of preparation, measurement, preparation placement (Only measurement in group C)						
60 min	Cleaning of preparation, measurement, preparation placement (Only measurement in group C)						
120 min	Cleaning of preparation, measurement, preparation placement (Only measurement in group C)						
180 min	Cleaning of preparation, measurement (Only measurement in group C)						
End of experiment							

Table II - Descriptive statistics for group A (Rectogesic®)

Group A					
Measurement	**N**	**Min**	**Max**	**Mean (ml/min/100gr)**	**S.D.**
15 min	10	9,67	76,83	30,1833	20,00562
30 min	10	9,33	82,50	31,5202	21,22361
45 min	10	6,33	62,50	26,9682	16,41246
60 min	10	10,67	94,17	31,9020	24,92093
120 min	10	10,00	53,41	25,3750	14,05502
180 min	10	11,67	44,83	28,9878	10,64534

Table III - Descriptive statistics for group B **(**EMLA®)

Group B					
Measurement	**N**	**Min**	**Max**	**Mean (ml/min/100gr)**	**S.D.**
15 min	10	10,00	36,83	22,2417	8,68592
30 min	10	8,35	50,64	21,9915	12,59312
45 min	10	9,67	58,00	22,8616	13,63814
60 min	10	12,67	59,44	24,4917	14,25861
120 min	10	11,83	37,67	20,2500	8,39248
180 min	10	12,00	44,99	23,0329	9,28731

Table IV - Descriptive statistics for group c (no prepatation)

Group C					
Measurement	**N**	**Min**	**Max**	**Mean (ml/min/100gr)**	**S.D.**
15 min	10	3,33	27,00	14,5833	7,62478
30 min	10	5,26	29,86	13,6289	7,78955
45 min	10	6,50	25,50	14,4000	6,81828
60 min	10	6,00	24,33	13,4391	5,90400
120 min	10	5,67	21,83	12,5667	5,45984
180 min	10	5,33	20,00	11,6000	4,84271

Table V - Descriptive statistics for group D (placebo)

Group D					
Measurement	**N**	**Min**	**Max**	**Mean (ml/min/100gr)**	**S.D.**
15 min	10	3,33	27,00	11,6341	7,08445
30 min	10	5,26	29,86	12,6060	7,46066
45 min	10	6,33	25,50	13,1333	6,22883
60 min	10	5,67	24,33	13,1924	5,98779
120 min	10	4,55	21,83	11,8185	5,96723
180 min	10	5,00	20,00	11,2877	5,36349

Compared to the intact skin controls, the flow was higher to the injured skin at the 15 minute measurement (t=3,427, p=0.008). It should be mentioned that there was also a trend at the 180 minute measurement (t=1,927, p=0,086).

Descriptive statistics for group B (EMLA®)

Mean values of blood flow during measurements are presented in *Table III* and *Fig. 1* and ranged from 20,2500 (S.D.=8,39248) to 24,4917 (S.D.=14,25861) ml/min/100gr. Compared to the intact skin controls, the flow was higher to the injured skin at the measurements taken at 120 minutes (t=2,823; p=0,02) and at 180 minutes (t=3,166; p=0,011). There was also a trend at the 60 minute measurement (t=1,849; p=0,097).

Descriptive statistics for group C (no preparation)

Mean values of blood flow during measurements are presented in *Table IV* and *Fig. 1* and ranged from 11,6000 (S.D.=4,84271) to 14,5833 (S.D.=7,62478) ml/min/100gr.

Descriptive statistics for group D (placebo)

Mean values of blood flow during measurements are presented in *Table V* and *Fig. 1*. These ranged from 11,2877 (S.D.=5,36349) to 13,1924 (S.D.=5,98779) ml/min/100gr. Compared to the intact skin controls, the flow remained higher to the intact skin in all measurements (p<0.001).

Comparison of blood flow after burn between groups A and C

The results from Restogesic® use compared to no preparation use are presented in *Table VI*. It appears that nitroglycerine improves blood perfusion in all measurements (p<0,05) (at the 180 minute measurement p<0.001). These results are presented schematically in *Fig. 1*.

Comparison of blood flow between groups A and D

The results from Rectogesic® use compared to placebo use are presented in *Table VII.* It appears that nitroglycerine improves blood perfusion in all measurements (p<0,05) (at the 180 minute measurement p=0.001). These results are presented schematically in *Fig. 1*.

Table VI - Comparative results between groups A and C

	Group A		Group C			
Measurement	**Mean (ml/min/100gr)**	**SD**	**Mean (ml/min/100gr)**	**SD**	***t***	***p***
15 min	30,1833	20,00	14,5833	7,62	2,55	,03
30 min	31,5202	21,22	13,6289	7,78	2,75	,02
45 min	26,9682	16,41	14,4000	6,81	2,47	,03
60 min	31,9020	24,92	13,4391	5,90	2,37	,04
120 min	25,3750	14,05	12,5667	5,45	2,98	,01
180 min	28,9878	10,64	11,6000	4,84	6,71	,00

Comparison of blood flow between groups B and C

The results from EMLA® use compared to no preparation use are presented in *Table VIII*. The anesthetic cream improves blood perfusion in most of the measurements (p<0,05) and there was a trend to the rest of them [at 30 minutes (p=0,075) and at 45 minutes (p=0,054)]. These results are presented schematically in *Fig. 1*.

Comparison of blood flow between groups A and B

The comparative results between Rectogesic® and EMLA® use are presented in *Table IX*. The analysis did not demonstrate any significant difference from their use, however there was a trend in favor of nitroglycerine at the measurement taken at 180 minutes (p=0.80). These results are presented schematically in *Fig. 1*.

Comparison of blood flow between groups D and C

The comparative results between these groups are presented in *Table X*. There was no significant difference from the use of placebo compared to no use of

Table VII - Comparative results between groups A and D

	Group A		Group D			
Measurement	**Mean (ml/min/100gr)**	**SD**	**Mean (ml/min/100gr)**	**SD**	***t***	***p***
15 min	30,1833	20,00	11,6341	7,08	2,96	,01
30 min	31,5202	21,22	12,6060	7,46	2,79	,02
45 min	26,9682	16,41	13,1333	6,22	2,56	,03
60 min	31,9020	24,92	13,1924	5,98	2,38	,04
120 min	25,3750	14,05	11,8185	5,96	2,69	,02
180 min	28,9878	10,64	11,2877	5,36	5,13	,001

Table VIII - Comparative results between groups B and C

	Group B		Group C			
Measurement	**Mean (ml/min/100gr)**	**SD**	**Mean (ml/min/100gr)**	**SD**	***t***	***p***
15 min	22,2417	8,68	14,5833	7,62	2,34	,04
30 min	21,9915	12,59	13,6289	7,78	2,01	,07
45 min	22,8616	13,63	14,4000	6,81	2,21	,05
60 min	24,4917	14,25	13,4391	5,90	2,62	,02
120 min	20,2500	8,39	12,5667	5,45	2,58	,03
180 min	23,0329	9,28	11,6000	4,84	3,90	,004

Table IX - Comparative results between groups A and B

	Group A		Group B			
Measurement	**Mean (ml/min/100gr)**	**SD**	**Mean (ml/min/100gr)**	**SD**	***t***	***p***
15 min	30,1833	20,00	22,2417	8,68	1,18	,26
30 min	31,5202	21,22	21,9915	12,59	1,15	,27
45 min	26,9682	16,41	22,8616	13,63	,57	,57
60 min	31,9020	24,92	24,4917	14,25	,79	,44
120 min	25,3750	14,05	20,2500	8,39	,97	,35
180 min	28,9878	10,64	23,0329	9,28	1,97	,08

Table X - Comparative results between groups D and C

	Group D		Group C			
Measurement	**Mean ml/min/100gr**	**SD**	**Mean ml/min/100gr**	**SD**	***t***	***p***
15 min	11,6341	7,08	14,5833	7,62	-,91	,38
30 min	12,6060	7,46	13,6289	7,78	-,29	,77
45 min	13,1333	6,22	14,4000	6,81	-,47	,64
60 min	13,1924	5,98	13,4391	5,90	,09	,92
120 min	11,8185	5,96	12,5667	5,45	,31	,76
180 min	11,2877	5,36	11,6000	4,84	,14	,88

preparation. These results are presented schematically in *Fig. 1*.

Discussion

The main outcomes of the present study confirm the initial hypothesis suggesting that post-burn topical application of Rectogesic® improves blood perfusion after a thermal injury. These results are in agreement with the results of Gorman et al.[24] However it should be mentioned that they used a 2% galenic nitroglycerine ointment. The experimental burn model was also different. They used a heated metallic probe creating a full thickness "comb shape" injury to the back of 4 animals and they used histological examination, assessing at 24 hours post infusion, the degree of Evans Blue pigmented albumin extravasation.

The primary outcomes of this study also confirm that the use of EMLA® may improve blood perfusion after thermal injury. This is in agreement with the findings of Cassuto et al.[13] Although their sample was similar, the experimental full thickness burn model used was different and they used histological examination, assessing the degree of Evans Blue pigmented albumin extravasation at 24 hours post infusion. The results are also in agreement with those of Jonsson et al.[20-21] They used image digital color analysis of Evans Blue pigmented albumin extravasation in 20 rats and Laser Doppler flow measurements in 29 rats respectively, testing the same hypothesis. The experimental full thickness burn model used was different.

Overall, the primary outcomes did not demonstrate any difference in use of the two preparations.

As compared to the intact skin controls, at the 15 minute measurement, the action of Rectogesic® was demonstrated higher in injured skin. The same appeared to happen with EMLA® but at a different time measurement. Initially the blood flow was higher to the intact skin controls ($p<0.001$) but this was later inverted starting with a trend at the 60 minute measurement ($p=0,097$), which became significant at the measurements taken at 120 minutes ($p=0,020$) and at 180 minutes ($p=0,011$). These fluctuations of blood flow by the use of EMLA® have been previously documented.[31]

It is suggested that the transcutaneous absorption of the preparations in injured skin is increased.

At this point it should be useful to briefly mention the limitations of the study, creating a framework that should be considered for future studies.

Because of the complexity of burn injury, it shouldn't be feasible to hypothesize that only one variable, such as blood perfusion, could represent burn wound progression. The zone of stasis pathophysiological events leading to cell death either through apoptosis or necrosis are extremely complex. Several experimental animal studies have already attempted to address neutrophiles aggregation, inflammation mediators production and cell infiltration, infection, alterations to blood flow due to edema formation, vasoconstriction, thrombosis and free radicals formation in a selective way to prevent cell death with variable results.[68] Application of all these selective results to the clinical environment should be made with caution, recognizing the need for integration with the outcomes of future studies, testing the systemic, side and synergy effects, as well as different timings, doses and durations of treatment.

Conclusion

In conclusion, from the assessment of blood flow with the Laser Doppler it appears that Rectogesic® and EMLA® are equally beneficial to the perfusion of deep partial thickness burns.

BIBLIOGRAPHY

1. Jackson DM: The diagnosis of the depth of burning. British Journal of Surgery, 40: 588, 1953.
2. Jackson DM: Second thoughts on the burn wound. Journal of Trauma, 9: 839, 1969.
3. Schmauss D, Rezaeian F, Finck T, Machens HG, Wettstein R, Harder Y: Treatment of secondary burn wound progression in contact burns - a systematic review of experimental approaches. Journal of Burn Care and Research, Aug 4, 2014 [Epub ahead of print]
4. Tobalem M, Harder Y, Tschanz E, Speidel V, Pittet-Cuenod B, Wettstein R: First-aid with warm water delays burn progression and increases skin survival. Journal of Plastic, Reconstructive and Aesthetic Surgery 66: 260-6, 2013.
5. Feck GA, Baptiste MS: Burn injuries: epidemiology and prevention. Accident Analysis and Prevention, 11: 129-36, 1979.
6. Robson MC, Del Beccaro EJ, Heggers JP: The effect of prostaglandins on the dermal microcirculation after burning and the inhibition of the effect by specific pharmacological agents. Plastic and Reconstructive Surgery, 63: 781-7, 1979.
7. Ehrlich HP: Promotion of vascular patency in dermal burns with ibuprofen. American Journal of Medicine, 77: 107-13, 1984.
8. Del Beccaro EJ, Robson MC, Heggers JP, Swamminthan R: The use of specific thromboxane inhibithors to preserve the dermal microcirculation after burning. Surgery, 87: 137-41, 1980.
9. Heggers JP, Robson MC, Zachary LS: Thromboxane inhibithors for the prevention of progressive dermal ischemia due to the thermal injury. Journal of Burn Care and Rehabilitation, 6: 466-8, 1985.
10. Eriksson E, Robson MC: New pathophysiologic mechanism explaining post-burn edema. Burns, 4: 153, 1978.

11. Robson MC et al.: Prevention of dermal ischemia following thermal injury. Archives of Surgery, 113: 621, 1978.
12. Zawacki BE: Reversal of capillary stasis and prevention of necrosis in burns. Ann Surgery, 180: 98, 1974.
13. Cassuto J, Nellgard P, Stage L, Jonsson A: Amide local anesthetics reduce albumin extravasation in burn injuries. Anesthesiology, 72: 302-7, 1990.
14. Choi M, Ehrlich P: U75412E a lazaroid prevents progressive burn ischemia in a rat burn model. American Journal of Pathology, 142: 519-27, 1993.
15. Tarnow P, Jonsson A, Rimback G, Cassuto J: Increased dermal perfusion after skin burn injury by D-myo-inositol-1,2,6-triphosphate. Burns, 22: 363-8, 1996.
16. Battal MN, Hata H, Matsuka K, Ito Matsuda H, Yoshida Y, Kawazoe T: Reduction of progressive burn injury by a stable prostaglandin I2 analogue, beraprost sodium (Procyclin): An experimental study in rats. Burns, 22: 531-8, 1996.
17. Pedersen JL, Callesen T, Moiniche S, Kehlet H: Analgesic and anti-inflammatory effects of lignocaine-prilocaine (EMLA) cream in human burn injury. British Journal of Anaesthesia, 76: 806-10, 1996.
18. Cetinkale O, Demir M, Sayman HB, Ayan F, Onsel C: Effects of Allopurinol, Ibuprofen and Cyclosporin A on local microcirculatory disturbances due to burn injuries. Burns, 23: 43-9, 1997.
19. Tan Q, Ma W, Wang L, Chen HR: Can superoxide dismutase prevent post burn dermal ischaemia? Burns, 23: 228-31, 1997.
20. Jonsson A, Mattsson U, Tarnow P, Nellgard P, Cassuto J: Topical local anesthetics (EMLA) inhibit burn-induced plasma extravasation as measured by digital colour analysis. Burns, 24: 313-8, 1998.
21. Jonsson A, Brofeldt BT, Nellgard P, Tarnow P, Cassuto J: Local anesthetics improve perfusion after burn injury. Journal of Burn Care and Rehabilitation, 19: 50-6, 1998.
22. Mattsson U, Cassuto J, Jontell M, Jonsson A, Sinclair R, Tarnow P: Digital image analysis of erythema development after experimental thermal injury to human skin: Effect of postburn topical local anesthetics (EMLA). Anesthesia and Analgesia, 88: 1131-6, 1999.
23. Jonsson A, Cassuto J, Tarnow P, Sinclair R, Bennett A, Tavares IA: Effects of amide local anaesthetics on eicosanoid formation in burned skin. Acta Anaesthesiologica Scandinavica, 43: 618-22, 1999.
24. Gorman PJ, Saggers G, Ehrlich P, Mackay DR, Graham WP: Effects of topical nitroglycerin and flurbiprofen in the rat comb burn model. Annals of Plastic Surgery, 42: 529-53, 1999.
25. Arildsson M, Nilsson GE, Stromberg T: Effects on skin blood flow by provocation during local analgesia. Microvascular Research, 59: 122-30, 2000.
26. Mattsson U, Cassuto J, Tarnowc P, Jonsson A, Jontell M: Intravenous lidocaine infusion in the treatment of experimental human skin burns digital colour image analysis of erythema development. Burns, 26: 710-15, 2000.
27. Arildsson M, Asker CL, Salerud G, Strombeg T: Skin capillary appearance and skin microvascular perfusion due to topical application of analgesia cream. Microvascular Research, 59: 14-23, 2000.
28. Lindblom L, Cassuto J, Yregård L, Tarnow P, Räntfors J, Löwhagen HP: Importance of vasoactive intestinal polypeptide in the regulation of burn perfusion. Burns, 26: 435-42, 2000.
29. Barrow R, Ramirez R, Zhang XJ: Ibuprofen modulates tissue perfusion in partial thickness burns. Burns, 26: 341-6, 2000.
30. Tan Q, Lin Z, Ma W, Chen H, Wang L, Ning G, Zhou X: Failure of Ibuprofen to prevent progressive dermal ischemia after burning in guinea pigs. Burns, 28: 443-8, 2002.
31. Yregard L, Cassuto J, Tarnow P, Nilsson U: Influence of local anaesthetics on inflammatory activity postburn. Burns, 29: 335-41, 2003.
32. Singer J, McClain SA, Romanov A, Rooney J, Zimmerman T: Curcumin reduces burn progression in rats. Academic Emergency Medicine, 14: 1125-9, 2007.
33. Friedl HP, Till, GO, Trentz O, Ward PA: Roles of histamine, complement and xanthine oxidase in thermal injury of skin. American Journal of Pathology, 135: 203-17, 1989.
34. Boykin JV, Eriksson E, Sholley MM, Pittman RN: Histamine-mediated delayed permeability response after scald burn inhibited by cimetidine or cold water treatment. Science, 209: 815-7, 1980.
35. Holliman CJ, Meuleman TR, Larsen KR: The effect of ketaserin a specific serotonin antagonist, on burn shock hemodynamic parametersin a porcine model. Journal of Trauma, 23: 867-71, 1983.
36. Carvajal HF, Brouhard BH, Linares HA: Effect of antihistamine-antiserotonin and ganglionic blocking agents upon increased capillary permeability following burn trauma. Journal of Trauma, 15: 969-75, 1975.
37. Alexander F, Mathieson M, Teoh K: Arachidonic acid metabolites mediated early burn edema. Journal of Trauma, 24: 709-712, 1984.
38. Denzlinger C, Rapp S, Hagmann W, Keppler D: Leukotrienes as mediators in tissue trauma. Science, 230: 330-2, 1985.
39. Westcott JY, Thomas RB, Voelkel NF: Elevated urinary leukotriene E excretion in patients with ARDS and severe burns. Prostaglandins Leykotrienes and Essential Fatty Acids, 43: 151-8, 1991.
40. Arturson G, Jonsson CE: Effects of 0-(β-xydroxyethyl) rutosides (HR) and Indomethacin on transcapillary macromolecular transport and prosdtaglandin following scalding injury. Bibliog of Anatomy, 12: 456-70, 1973.
41. Heggers JP, Loy GL, Robson MC, Robson JDE: Histological demonstrations of prostaglandins and thromboxanes in burned tissues. Journal of Surgical Research, 28: 110-7, 1980.
42. Ehrlich HP, Needle AL, Rajaratnam J, White ME, White BS: The role of prostaglandin and thromboxane in rat burn and freeze injuries. Experimental Molecular Pathology, 38: 357-67, 1983.
43. Rimback G, Cassuto J, Wallin G, Westlander G: Inhibition of peritonitis by amide local anesthetics. Anesthesiology, 69: 881-6, 1988.
44. Nellgard P, Jonssson A, Bojo L, Tarnow P, Cassuto J: Small bowell obstruction and effects of lidocaine, atropine and hexamethonium on inflammation and fluid losses. Acta Anesthesiologica Scandinavica, 40: 287-92, 1996.
45. Horrobin DF, Mankau MS: Roles of prostaglandins suggested by the prostaglandin agonist/antagonist actions of local anesthetic, antiarrhythmic, anti malarial tricyclic antidepressant and methyl xanthine compounds, effects on membranes and on nucleic acid funtion. Medical Hypotheses, 2: 71-86, 1977.
46. Goel RK, Tavares IA, Nellgard P, Jonsson A, Cassuto J, Bennett A: Effect of of lignocaine on eicosanoid synthesis by pieces of human gastric mucosa. Journal of Pharmacy and Pharmacology 46: 319-20, 1994.
47. Kunze H, Bohn E, Vogt W: Effects of local anesthetics on prostaglandin biosynthesis in vitro. Biochemica et Biophysica Acta, 360: 260-9, 1974.

48. Sinclair R, Eriksson AS, Gretzer C, Cassuto J, Thomsen P: Inhibitory effects of amide local anesthetics on stimulus induced human leukocyte metabolic activation, LTB4 release and IL-1 secretion in vitro. Acta Anesthesiologia Scandinavica, 37: 159-65, 1993.
49. Giddon DB, Lindhe J: In vivo quantitation of local anesthetic suppression of leukocyte adherence. American Journal of Pathology, 68: 327-38, 1972.
50. Eriksson AS, Sinclair R, Cassuto J, Thompsen P: Influence of lidocaine on leukocyte function in the surgical wound. Anesthesiology, 77: 74-8, 1992.
51. Sasagawa S: Inhibitory effects of local anesthetics on migration extracellular release of lysosomial enzyme and superoxide anion production in human polymorfonuclear leukocytes. Immunopharmacology and Immunotoxicology, 13: 607-22, 1991.
52. Goldstein IM, Lind S, Hoffstein S, Weissmann G: Influence of local anesthetics upon human polymorphonuclear leukocyte function in vitro. Journal of Experimental Medicine, 146: 483-94, 1977.
53. Kazimierezak W, Peter M, Maslinski C: The action of local anesthetics on histamine release. Biochemistry and Pharmacology, 25: 1747-50, 1976.
54. Gibran NS, Heimbach DM: Mediators in thermal injury. Seminars in Nephrology, 13: 344-58, 1993.
55. Luostarinen V, Evers H, Lyytikainen M.T. Scheinin A, Wahlen A: Antithrombotic effects of lidocaine and related compounds on laser induced microvascular injury. Acta Anesthesiologia Scandinavica, 25: 9-11, 1981.
56. Marmo E: Farmacologia Generale e Speciale. Unione Tipografico-Editrice Torinese, Torino: UTET, 1991.
57. Fenton C, Wellington K, Easthope SE: 0.4% nitroglycerin ointment: In the treatment of chronic anal fissure pain. Drugs, 66: 343-9, 2006.
58. Tjandra JJ, Tan JJ, Lim JF, Murray-Green C, Kennedy ML, Lubowski DZ: Rectogesic (glyceryl trinitrate 0.2%) ointment relieves symptoms of hemorrhoids associated with high resting anal canal pressures. Colorectal Disease, 9: 457-63, 2007.
59. Atalay, C, Kockaya EA, Cetin B, Kismet K, Akay MT: Efficacy of topical nitroglycerin and transcutaneous electrical nerve stimulation on survival of random-pattern skin flaps in rats. Scandinavian Journal of Plastic Reconstructive Surgery and Hand Surgery, 37: 10-3, 2003.
60. Rohrich RJ, Cherry GW, Spira M: Enhancement of skin-flap survival using nitroglycerin ointment. Plastic and Reconstructive Surgery, 73: 943-8, 1984.
61. Dunn CL et al.: A single postoperative application of nitroglycerin ointment does not increase survival of cutaneous flaps and grafts. Dermatologic Surgery, 26: 425-7, 2000.
62. Davis RE, Wachholz JH, Jassir D, Perlyn CA, Agrama MH: Comparison of topical anti-ischemic agents in the salvage of failing random-pattern skin flaps in rats. Archives of Facial Plastic Surgery, 1: 27-32, 1999.
63. Karacaoglan N, Akbas H: Effect of parenteral pentoxifylline and topical nitroglycerin on skin flap survival. Otolaryngol Head Neck Surg, 120: 272-4, 1999.
64. Watson RC, Lewis DR, Watson HJ: Instruments for motion measurement using Laser Doppler heterodyning techniques. ISA Trans, 8: 20-8, 1969.
65. Moeschberger M, Williams J, Brown C: Methods of randomization in controlled clinical trials. The American Journal of Emergency Medicine, 3:467-73, 1985.
66. Chen G: The pharmacology of ketamine. Anesthetist, 18: 24, 1969.
67. Smits GJ, Roman RJ, Lombard JH: Evaluation of laser-Doppler flowmetry as a measure of tissue blood flow. Journal of Applied Physiology, 61: 666-72, 1986.
68. Shupp JW, Nasabzadech TJ, Rosenthal DS, Jordan MH, Fidler P, Jeng JC: A review of the local pathophysiologic bases of burn wound progression. Journal of Burn Care and Research, 31: 849-73, 2010.