

EFFECT OF TERBUTALINE ON PERIPHERAL LYMPH FLOW, PROTEIN CONCENTRATION AND TRANSPORT, AND EDEMA FORMATION AFTER THERMAL INJURY IN RABBITS

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ABSTRACT

In the rabbit, intraarterial infusion of 5 µg/min of terbutaline within the first two hours after moderate thermal injury prevents edema and reduces augmented lymph flow, lymphatic protein transport, and tissue fluid protein concentration. Terbutaline, however, fails to prevent edema 4 hours after thermal injury although the increase of leg volume (24%) is less than in the untreated, burned control leg (56%). A higher dose of terbutaline (15 µg/min) also fails to block edema 4 hours after burning and its effect on leg volume, lymph flow, and lymphatic protein concentration is similar to that of 5 µg/min infusion. Terbutaline does not alter the extent of tissue injury after burning as the increase in tissue fluid lactic dehydrogenase and potassium are similar with "treated" and "untreated" burns.

Histamine and bradykinin are considered important mediators for increased microvascular permeability in various inflammatory conditions. In the hamster cheek pouch increased microvascular permeability induced by these agents is blocked by topical application of the β_2 agonist, terbutaline (16). Infusion of terbutaline also prevents increases in lymph flow and lymphatic protein transport induced by histamine and bradykinin in the dog hind limb (2, 16). In the present study, we examined the effect of terbutaline on edema formation, regional lymph flow and lymphatic and tissue fluid protein concentrations after thermal injury in the rabbit hind limb.

MATERIALS AND METHODS

Experiments were carried out using rabbits of both sexes of grey Hungarian stock with body weights of 3.2-4.0kg under pentobarbitone anesthesia. In 16 rabbits a hind limb was immersed for 20 seconds up to the knee joint in a water bath of 80°C. Immediately after scalding in 8 rabbits terbutaline (Bricanyl-Geigy) was infused into the femoral artery of the burned leg at a rate of 5 µg/min. The other 8 rabbits served as untreated controls. In each rabbit a prenodal popliteal lymphatic vessel was cannulated in both hind limbs. Lymph was collected 30 minutes after thermal injury and was continued in 30 minute intervals for 90 minutes. The volume of the burned limb was measured before and 2 hours after scalding by water displacement after limb immersion in a graduated cylinder. Popliteal arterial blood was sampled before and 2 or 4 hours after scalding.

In a further group of 20 rabbits one hind limb was burned as described above. In 10 of these rabbits terbutaline was infused intraarterially at a rate of 5 µg/min with the other 10 rabbits receiving no infusion and serving as "untreated" controls. Tissue fluid was sampled from both legs by the cotton wick method. Three-four cotton threads 4-6cm long were sewn one hour after burning into the subcutaneous tissue of the shank. The threads were withdrawn after one hour and the fluid expressed.

Four hours after burning the volume of each hind leg was measured as previously described.

In six other rabbits popliteal lymph was collected for four hours after burn and in three of these rabbits intraarterial terbutaline was infused at 15 $\mu\text{g}/\text{min}$ while the other three were used as controls.

In blood plasma, popliteal lymph and hind limb tissue fluid total protein was measured according to Lowry et al(8) and in tissue fluid lactic dehydrogenase (LDH) activity was determined by method of Wroblevsky and La Due (19).

RESULTS

The data shown in the text, tables, and figures represent means \pm S.E.M.

In control ("untreated") rabbits the volume of the burned leg increased 35% in two hours from 105 to 142 ml, whereas in rabbits receiving 5 $\mu\text{g}/\text{min}$ terbutaline there was no volume change. After four hours, however, in the "treated" rabbits, the leg volume increased by 24% (from 116 to 144 ml) but in control rabbits the burned leg volume increased by 56% (108 to 169 ml) (Table 1). Terbutaline also reduced lymph flow in the burned extremity. Thus in the burned leg of controls, lymph flow varied in the three collection periods between 22.7 ± 4.2 and 31.4 ± 5.5 $\mu\text{l}/\text{min}/\text{g}$ body weight whereas in the terbutaline infused rabbits lymph flow was between 6.2 ± 2 and 9.2 ± 5.3 $\mu\text{l}/\text{min}$ ($p < 0.05$). Terbutaline also reduced popliteal lymph flow in the non-

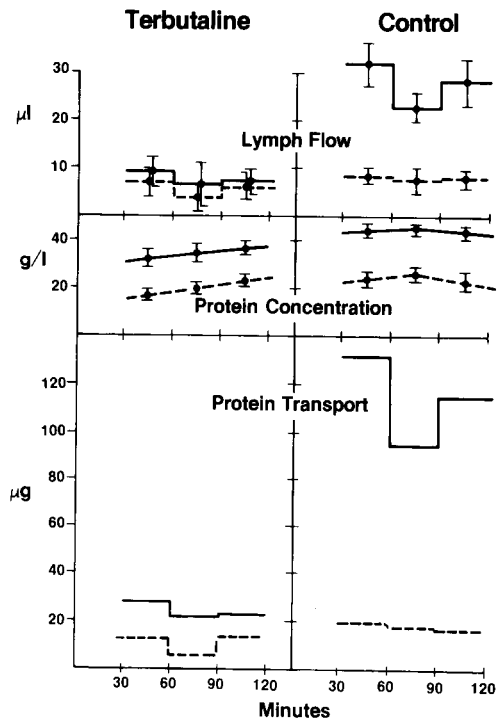


Fig. 1: Effect of terbutaline on lymph flow, lymph protein concentration and lymphatic protein transport in the burned leg of rabbits. Full lines: burned leg; broken lines: intact leg.

burned leg. Thus mean lymph flow in unburned legs without terbutaline was 7.8 ± 0.2 $\mu\text{l}/\text{min}$ whereas in rabbits receiving terbutaline the flow was 5.4 ± 0.1 $\mu\text{l}/\text{min}$ ($p < 0.01$) (Table 2). Less marked changes were also observed in the protein concentration of popliteal lymph (Fig. 1). The

Table 1.
Effect of Terbutaline on the Volume of Rabbit Hindlimbs/ml after Burning

Time (Hours)	n	Dose $\mu\text{g}/\text{ml}$	CONTROLS			TERBUTALINE		
			Burning Before	Burning After	Diff %	Burning Before	Burning After	Diff %
2	8	5	105 ± 8	142 ± 8	35	112 ± 5	111 ± 3	0
4	10	5	108 ± 3	169 ± 6	56	116 ± 5	144 ± 4	24
4	3	15	97 ± 1	148 ± 10	52	113 ± 8	145 ± 10	28

average protein concentration in the burned leg lymph of untreated rabbits was 42.1g/l whereas in rabbits receiving terbutaline it was 33.8g/l ($p < 0.01$). In the unburned leg the protein concentrations were 24.8 and 20.5g/l without and with terbutaline respectively. Protein transport in the burned extremity of untreated controls was 114.5 ± 10.8 or approximately 6x the unburned leg ($19.2 \pm 5.2 \mu\text{g}/\text{min}/\text{kg}$) and approximately 5x higher than rabbits with burned legs receiving terbutaline (24.7 ± 2.1 and approximately 11x greater than non-burned legs with terbutaline ($10.8 \pm 2.1 \mu\text{g}/\text{min}/\text{kg}$) (Table 2). Terbutaline also influenced the protein content of tissue fluid. Thus, total protein in tissue fluid of the burned leg of untreated controls was $31.6 \pm 4.0 \text{ g/l}$ and in the normal leg $14.8 \pm 1.6 \text{ g/l}$. The same values in "treated" rabbits were 23.1 ± 1.4 and $11.9 \pm 1.4 \text{ g/l}$ respectively. For the burned legs the difference was significant ($p < 0.05$).

The difference in protein escape into the extravascular space is reflected also in loss of protein from blood plasma. With burned rabbits without terbutaline, plasma total protein concentration decreased in two hours by about 10% whereas in terbutaline treated rabbits by 3.7% ($p < 0.05$).

Terbutaline did not influence the degree of tissue injury by burning. In untreated rabbits scalding increased tissue fluid LDH activity from 473 ± 66 (normal leg) to $10829 \pm 1279 \text{ mU/ml}$. In "treated" rabbits the change was from 498 ± 130 to $12776 \pm 2355 \text{ mU/ml}$. Tissue injury was also associated with a higher potassium concentration in lymph from the burned limb ($4.6 \pm 0.15 \text{ mmol/l}$) compared to the unburned limb ($3.33 \pm 0.25 \text{ mmol/l}$); these values were unaffected by terbutaline.

A higher dose of terbutaline ($15 \mu\text{g}/\text{min}/\text{kg}$) did not prevent edema formation four hours after burning and its effect on leg volume, lymph flow, and lymphatic

Table 2
Mean Lymph Flow Rate, Protein Concentration and Transport
in the Rabbit Hindlimb after Burn

Terbutaline dose $\mu\text{g}/\text{min}$	n	Collection period min	Lymph flow $\mu\text{l}/\text{min}/\text{kg}$			
			Controls		Terbutaline	
			intact leg	burned leg	intact leg	burned leg
5	8	30-120	7.8 ± 0.2	27.3 ± 2.5	5.4 ± 1.0	7.4 ± 0.9
15	3	30-120	6.1 ± 0.4	23.4 ± 1.4	4.5 ± 0.2	8.0 ± 0.6
15	3	120-240	6.1 ± 0.3	19.9 ± 2.1	4.2 ± 0.1	10.0 ± 0.9
			Lymph Total Protein g/l			
5	8	30-120	24.8 ± 0.7	42.1 ± 0.4	20.5 ± 1.4	33.6 ± 1.4
15	3	30-120	22.4 ± 1.8	40.3 ± 0.5	21.6 ± 1.5	34.1 ± 1.4
15	3	120-240	20.8 ± 1.1	39.7 ± 1.5	26.3 ± 1.0	34.0 ± 0.9
			Protein Transport $\mu\text{g}/\text{min}$			
5	8	30-120	19.2 ± 5.2	114.5 ± 10.8	10.8 ± 2.1	24.7 ± 4.2
15	3	30-120	13.2 ± 2.9	94.5 ± 5.3	7.9 ± 1.0	27.2 ± 2.1
15	3	120-240	12.7 ± 3.4	76.6 ± 6.2	9.4 ± 3.1	34.0 ± 4.2

protein transport was similar to that after infusion at 5 $\mu\text{g}/\text{min}/\text{kg}$ (Table 1,2).

During 210 minutes of lymph collection, mean lymph flow in the burned leg without terbutaline was $21.0 \pm 1.4 \mu\text{l}/\text{min}/\text{kg}$ compared with the unburned leg of $6.1 \pm 0.4 \mu\text{l}$. In rabbits receiving terbutaline lymph flow was 9.6 ± 0.6 (burned) and $4.3 \pm 0.2 \mu\text{l}/\text{min}$ (nonburned). Protein concentration in the lymph of the burned extremity was slightly higher in "untreated" controls than in "treated" rabbit (39.9 ± 1.2 versus $34.0 \pm 1.0 \text{g/l}$; $p < 0.05$). Mean lymphatic protein transport was, however, substantially greater in the burned legs of "untreated" controls than in rabbits receiving terbutaline but the effect of 15 $\mu\text{g}/\text{min}$ infusion on lymph protein concentration and transport was similar to terbutaline infusions of 5 $\mu\text{g}/\text{min}$ (Table 2).

DISCUSSION

Histamine-type mediators may be important factors in protein leakage and edema formation after moderate thermal injury. Until recently it has generally been assumed that increased microvascular permeability after scalding is secondary to physical forces (15). Contraction of large veins increases venular pressure with disruption of the vein wall and development of submicroscopic gaps between endothelial cells (10). The effect of histamine and bradykinin on microvascular permeability, however, is independent of increased venular pressure (7,9). Rather, the increased protein flux signaled by increased lymph flow and total protein concentration is attributable to a direct effect on the microvascular membrane. Bradykinin generates, for example, greater permeability to fluorescent macromolecules and electron microscopy of leaking venules reveals formation of large gaps as endothelial cells separate (11, 12, 17). In these experiments (7, 9) simultaneously administered catecholamines not only prevents protein leakage but also decreases development of venular gaps.

Experimental second degree burns produced by brief immersion in hot water increase lymph flow and lymphatic protein

transport (3,4,6,14) changes similar to that seen after administration of histamine and bradykinin. Indeed, Cope et al (4) recognized that these physiological effects of thermal trauma may be ameliorated by adrenal cortical and posterior pituitary extracts. In the present study terbutaline, a β_2 agonist, markedly reduced edema formation and protein efflux after a scalding burn, but this effect was most noticeable in the first two hours after burn. After two hours edema formation was not blocked even when the dose of terbutaline was increased three-fold and plasma protein extravasation, although reduced, was not eliminated.

Edema formation and capillary leakage in second degree burns is not simply a consequence of derangements in blood flow and microvascular pressure. With thermal injury, as with administration of histamine or bradykinin there is regional arterial vasodilatation but postcapillary resistance is not increased and microvascular filtration pressure rises only slightly (1,13,17). These hemodynamic effects are transient and limited primarily to the first hour after injury (1). Edema accumulation continues, however, long after. Thus terbutaline's beneficial effect during the first two hours is likely other than its β agonist vasoactive action and its inability to block edema formation thereafter signifies some other ongoing process.

Second degree burns locally injure cells. The concentration of cytoplasmic enzymes, especially LDH in the tissue fluid is a sensitive indicator of tissue injury (18). Because terbutaline does not alter the rise in tissue fluid LDH after burns, this agent's activity is not directed at altering the degree of tissue injury sustained.

Lack of protection against cell injury is probably the main reason for failure of terbutaline to prevent edema formation after two hours. In the early stages after burn injury, plasma osmolality in venous blood draining the burned area and tissue fluid osmolality increase. This derangement establishes a transcapillary osmotic gradient which favors fluid extravasation into the burned region (1). The local increase of osmolality results from cell destruction or

injured cell membranes which facilitates escape of small molecules from cells into the adjacent extracellular space. However, these postulated disturbances of osmotic equilibrium probably occur early post burn (within 10 minutes) and accordingly it seems unlikely that this explanation accounts for late formation of edema. During the first two hours in rabbits receiving terbutaline, protein and fluid efflux into the extravascular space is lower than in burned controls, and it is likely that functioning lymphatics adequately cope with modest increases in tissue protein and fluid extravasation when limited by infusion of this β agonist. After this time period, however, edema results from several other factors. Progressive microvascular permeability seems unlikely because already augmented lymphatic protein transport increases no further two to four hours after burn (Table 2). On the other hand, a limited lymphatic drainage capacity is only partly removing the ever-rising tissue protein load and accordingly plasma protein progressively accumulates in the extravascular space with greater osmotic imbalance between filtering and reabsorbing forces. Edema is also favored by extra- and intravascular clotting of protein-rich fluid with obstruction of regional lymphatics (6), a phenomenon seen in our study as hypercoagulable lymph occasionally blocked the cannulated lymphatics with fibrin 2-3 hours post burn.

After thermal trauma, besides early microcirculatory derangements somewhat amenable to treatment with vasoactive agents such as terbutaline, there occurs a more lasting direct injury to the endothelium. This explanation is consistent with observations about changes of transcapillary leakage during healing of experimental burns (2). In second degree burns, greater microvascular permeability is maximal about one to three hours after injury and thereafter gradually decreases. One week post-burn, however, permeability is still increased and a normal state is not restored until three weeks. Protracted altered microvascular permeability undoubtedly relates to direct anatomic injury

to the endothelium, a derangement not readily responsive to infusion of vasoactive agents.

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REFERENCES

1. Arturson, G, S Mellander: Acute changes in capillary filtration and diffusion in experimental burn injury. *Acta physiol Scand* 62: 457-463, 1964.
2. Arturson, G, S Doeds: Changes in transcapillary leakage during healing of experimental burns. *Acta Chir Scand* 133: 609-614, 1967.
3. Cope, O, FD Moore: A study of capillary permeability in experimental burns and burn shock using radioactive dyes in blood and lymph. *J Clin Invest* 23: 241-257, 1944.
4. Cope, O, JB Graham, G Mister Jr, MR Ball: Threshold of thermal trauma and influence of adrenal cortical and posterior pituitary extracts on the capillary and chemical changes. *Arch Surg* 59: 1015-1030, 1949.
5. Dobbins, DE, CY Soike, AJ Premen, GJ Grega, JM Debney: Blockade of histamine and bradykinin induced increases in lymph flow, protein concentration, and protein transport by terbutaline in vivo. *Microcirculation* 2: 127-150, 1982.
6. Glenn, WWL, DK Peterson, CK Drinker: Flow of lymph from burned tissues with particular reference to effects of fibrin formation upon lymph formation and composition. *Surgery* 12: 685-693, 1942.
7. Kline, RL, JB Scott, FJ Haddy, GJ Grega: Mechanisms of edema formation in canine forelimbs by locally administered bradykinin. *Amer J Physiol* 225: 1051-1056, 1973.
8. Lowry, OH, NJ Rosenbrough, AL Farr, RS Randall: Protein measurement with the folin-phenol reagents. *J Biol Chem* 193: 265-276, 1951.
9. Maciejko, JJ, DL Marcinisk, EF Gersabeck, GJ Grega: Effects of locally and systematically infused bradykinin on transvascular fluid and protein transfer in the canine forelimb. *J Pharmacol Exp Ther* 205: 221-235, 1978.
10. Majno, G, GE Palade: Studies on inflammation I Effect of histamine and serotonin on vascular permeability. An electron microscopic study. *J Biophys Biochem Cytol* 11: 571-591, 1961.
11. Majno, G, V Gilmore, M Leventhel: On the mechanism of vascular leakage caused by histamine-type mediators. *Circ Res* 21: 833-847, 1967.

12. Majno, G, SM Shaa, M Leventhal: Endothelial contraction induced by histamine type mediators. *J Cell Biol* 42: 647-642, 1969.
13. Mellander, S: Transcapillary fluid exchange in experimental burn and after administration of 48/80. *Acta Physiol Scand* 59: suppl 213,101-102, 1963.
14. Perlmsn, GE, WWL Glenn, D Kaufman: Changes in the electrophoretic pattern in lymph and serum in experimental burns. *J Clin Invest* 22: 627-633, 1943.
15. Rowley, DA: Venous constriction as the cause of increased permeability produced by 5-hydroxytryptamine, histamine, bradykinin and 48/80 in the rat. *Brit J Exptl Path* 45: 56-61, 1964.
16. Svensjo, E, CCA Persson, G Rutli: Inhibition of bradykinin induced macromolecular leakage from post-capillary venules by a 2-adrenoreceptor stimulant, terbutaline. *Acta Physiol Scand* 101: 504-506, 1977.
17. Svensjo, E, KE Arfors, RM Raymond, GJ Graga: Morphological and physiological correlation of bradykinin induced macromolecular efflux. *Amer J Physiol* 236: H600-H606, 1979.
18. Szabo, G, Z Magyar: The relationship between tissue fluid and lymph II. Enzymes in tissue fluid and lymph. *Lymphology* 11: 101-105, 1978.
19. Wroblewsky, F, JS Ladue: Lactic dehydrogenase activity in blood. *Proc Soc Exp Biol Med* 90:2130-2136, 1955.