Edema-preventing mechanisms in rat gingiva

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The long-term effect of increased local venous pressure (P_v) on interstitial fluid pressure (P_i), colloid osmotic pressure (COP_i), and fractional removal rate of ^{125}I-labeled human serum albumin (k_{Alb}) was studied in rat gingiva. Measurements were performed on experimental animals and sham-operated controls up to 4 days after ligation of jugular veins. On the day of ligation P_v in the facial veins rose from 2.5 ± 0.3 (SD) to 15.8 ± 2.8 mm Hg and stayed at about this level for 2 days before a decrease to 7.4 ± 0.9 mm Hg on day 4. In free gingiva P_i rose from an average of 3.5 ± 0.4 to a maximum of 6.3 ± 0.7 mm Hg, whereas in attached gingiva the corresponding increase in P_i was from 6.0 ± 0.7 to 11.1 ± 2.1 mm Hg. One day after the ligation COP_i in wick fluid from gingiva was reduced from the control level of 10.6 ± 1.4 to 4.5 ± 0.9 mm Hg. COP in plasma and COP_i in subcutaneous tissue on the back were unaffected. The removal rate of ^{125}I-labeled albumin (k_{Alb}) from the gingiva showed a nearly threefold increase after venous ligation, from 0.073 ± 0.01 to 0.211 ± 0.06 h^{-1}. It is concluded that in free and attached gingiva, both a rise in P_i and a decrease in COP_i will counteract the increased filtration pressure and thus prevent edema formation during venous stasis. The fall in COP_i is most likely due to increased lymph flow and not dilution, as venous stasis significantly increased k_{Alb} without any visible increase in gingival volume. □ *Capillary filtration; colloid osmotic pressure; edema; lymph flow; microcirculation; tissue pressure*

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As already realized by Starling (1), the net filtration of fluid across the capillary wall (J_c) is determined by the capillary blood pressure (P_c), the interstitial fluid pressure (P_i), and the colloid osmotic pressure of plasma (COP_p) and interstitium (COP_i), as shown in the following modification of the Starling equation:

$$J_c = K_f(P_c - P_i - \sigma(COP_p - COP_i)),$$

where K_f is the filtration coefficient and σ is the reflection coefficient for proteins across the capillary wall. According to the equation, any rise in capillary pressure will increase fluid filtration across the capillary wall. Depending on the tissue compliance, this may raise P_i, whereas interstitial colloid osmotic pressure (COP_i) may fall because of dilution and/or increased washout of interstitial proteins. These changes will counteract the increased filtration pressure and thereby prevent or limit edema formation.

Interstitial compliance (C) is defined as the change in interstitial fluid volume ($\triangle$IFV) per change in interstitial fluid pressure ($\triangle$IFP)—that is, $C = \triangle IFV/\triangle IFP$. In low-compliant tissues, with limited possibilities to expand, venous stasis leading to increased capillary pressure and filtration may thus cause a rise in P_i to rather high levels (2). The major counterpressure, or edema-preventing mechanism, in low-compliant tissues surrounded by rigid structures—such as brain (3), dental pulp (4), and rat tail (2, 5)—seems to be an increase in P_i. On the other hand, it has been found that a fall in COP_i provides two to four times more edema prevention than hydrostatic counterpressure in highly compliant tissues such as the skin of rats (6) and the skin and skeletal muscle of cats (7) and dogs (8). Nevertheless, an increased protein washout and subsequent fall in COP_i may also be of considerable importance as edema-preventing mechanism in low-compliant tissues, as shown in a recent study of the rat tail (5). The purpose of these experiments was to study the long-term effect of elevated local venous pressure on P_i, COP_i, and albumin

removal in free and attached gingiva. The rate of removal of isotope-labeled albumin (k_{Alb}) was used as an indicator of lymph flow.

Materials and methods

All experiments were performed on female Wistar rats with body weights (BW) of 200–300 g, purchased from K.E. Møllegaard, Ejby, Denmark. The rats were studied while under anesthesia with pentobarbital, 9.7 mg; chloral hydrate, 42.5 mg; magnesium sulfate, 21 mg; propylene glycol, 428 mg; ethanol, 76 mg; and distilled water up to 1 ml (Equisthesine), 0.35 ml/100 g BW, given intraperitoneally. Additional anesthesia was supplied when needed. During measurements the rectal temperature was kept at 37.5°C by means of a servo-controlled heating pad. Experiments were performed on 20 rats with experimentally induced venous stasis and on 19 sham-operated controls (SOC), divided into 3 separate series:

Series I

Five experimental and six SOC were used for measurements of P_v in the facial vein and P_i in free and attached gingiva on days 0, 1, 2, 3, and 4.

Series II

Removal rate of albumin (k_{Alb}) was measured in a local depot in gingiva along with P_v and P_i in free and attached gingiva in nine experimental rats and seven SOC 1 day after venous ligation (day 1).

Series III

For measurements of COP_i, interstitial fluid was collected from gingiva and the back by a dry wick method in six rats with venous stasis and six SOC on day 1.

Preoperative measurements

On the day of operation (day 0) the rats were anesthetized and weighed, and 0.5 ml penicillin (Ditardopen®, Leo) was injected subcutaneously. A blood sample was taken from the great saphenous vein for measurement of hematocrit (Cellkrit AB, L. Ljungberg & Co., Stockholm, Sweden) and plasma COP with a colloid osmometer for small fluid samples (9), using an Amicon PM 30 membrane and 0.9% saline in the reference chamber.

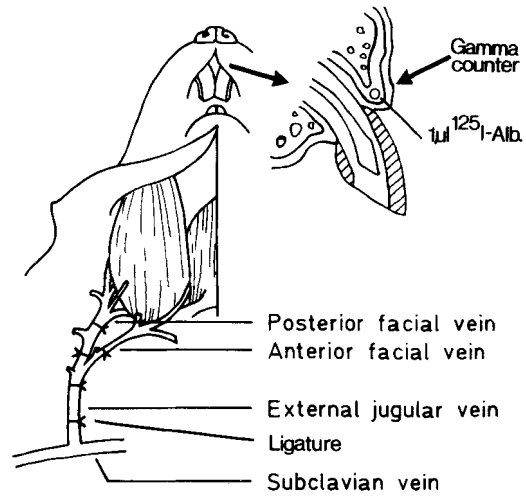

Fig. 1. Schematic illustration showing the position of ligatures on the external jugular vein, the position of the injected albumin depot, and the detection of radioactivity over the point of injection.

Venous ligation

In the 20 experimental animals an incision was made from the clavicle in cranial direction up to the mandible on each side of the neck. The external jugular vein was carefully exposed, and ligations were placed on five different levels bilaterally (Fig. 1). In the SOC the surgical procedure was identical, except that no ligations were made. The incisions were closed with sutures, and the rats were returned to their cages, where they recovered from anesthesia during the course of 1–2 h. Food and water were available ad libitum.

Series I: measurements of P_i and P_v

P_i was measured by the micropuncture technique, using a sharpened glass pipette

(diameter, 2–4 μm) connected to a servo-controlled counterpressure system as described by Widerhielm et al. (10) with a setup slightly modified from that of Intaglietta et al. (11). In all animals P_i was measured by puncture through the intact epithelium of free and attached gingiva around incisors. For measurements of P_v a small incision was made cranially to the ligatures, and the facial vein was carefully exposed. The pressure in the anterior facial vein (P_v) was thereafter measured by the micropuncture technique. P_v and P_i were measured daily for up to 4 days, starting on the day of operation.

Series II: removal rate of albumin

On day 1 a depot of 1 μl ^{125}I-labeled human serum albumin (Institute for Energy Technology, Kjeller, Norway) was injected into the gingiva during 2 min with a special syringe for small samples. Radioactivity was counted by external gamma-counting equipment (Canberra, Conn., USA) for periods of 5 min over the point of injection (Fig. 1) for at least four half times of elimination ($T_{1/2}$). Background activity was measured every day for 1 h, and values obtained in control and experimental rats were corrected for background activity. Fractional removal rate (k_{Alb}) was obtained from the radioactivity measurements as $k_{Alb} = \ln 2/T_{1/2} = 0.693/T_{1/2}$, where $T_{1/2}$ is the half time of removal of albumin.

Series III: measurements of COP

On day 1 the rats were reanesthetized, and a blood sample was taken from the saphenous vein for measurement of COP_p. Thereafter the animals were killed by an intracardiac injection of KCl and immediately transferred to an infant incubator with 100% humidity for implantation of dry nylon wicks (12). Two wicks were placed in the molar region of the buccal gingiva, and two wicks were placed on the back. After 15 min the nylon wicks were removed and transferred to plastic vials, and wick fluid was isolated by centrifugation. The COP of plasma and wick fluid was measured with a colloid osmometer for submicroliter samples (13).

Statistics

Student's t test was used for statistical analysis. All results are presented as means ± SD, unless otherwise indicated.

Results

Preoperative measurements

Body weights averaged 301 ± 15 g both in experimental and sham-operated control rats. Hematocrit was 0.456 (mean value of all rats). Plasma COP was 19.8 ± 0.5 mm Hg.

Clinical observations

During the first 24 h after surgery the 20 experimental animals consistently developed a marked edema. The edema was clinically visible in skin and subcutaneous tissue in the head and neck regions, whereas there was no visible edema in free or attached gingiva. The edema lasted throughout the experiments but did not seem to affect breathing, feeding, or normal behavior. No clinical edema was present in the 19 sham-operated control rats.

Series I (effect of venous stasis on P_v and P_i)

In the six sham-operated control rats there was no significant change in the measured variables during the 4 days of the experiment (Table 1). The mean value of P_v in the SOC was 2.7 mm Hg, whereas P_i was 3.7 mm Hg in free gingiva and 6.2 mm Hg in attached gingiva (average values for days 0 to 4) (Fig. 2). In the five experimental rats measurements performed within 10 min after ligation showed that P_v was considerably increased and stayed at this level on day 1, before a gradual decrease towards control level (Table 1). Venous stasis also markedly increased P_i. Both in free and attached gingiva P_i was nearly doubled on days 0 and 1, before a gradual decrease towards control level (Table 1, Fig. 2).

Table 1. Effect of venous stasis on interstitial fluid pressure, venous pressure (series I), and albumin clearance (series II)

	P_v (mm Hg)		P_i free (mm Hg)		P_i att., (mm Hg)		k_{Alb} (h^{-1})	
Days	SOC	Exp.	SOC	Exp.	SOC	Exp.	SOC	Exp.
Series I								
0	2.5	15.8*	3.5	6.3*	6.0	11.1*		
	(0.3)	(2.8)	(0.4)	(0.7)	(0.7	(2.1)		
1	2.9	15.9*	3.4	5.3*	6.3	14.2*		
	(0.4)	(2.1)	(0.8)	(1.1)	(0.8)	(2.0)		
2	2.8	9.0*	4.5	4.8	6.5	8.4		
	(1.0)	(0.9)	(0.7)	(0.6)	(0.7)	(1.8)		
3	2.8	7.9*	3.8	5.1	6.5	7.4		
	(0.8)	(1.1)	(1.8)	(0.9)	(0.9)	(1.1)		
4	2.5	7.4*	3.5	4.4	5.5	7.9		
	(1.0)	(0.9)	(1.0)	(0.9)	(1.0)	(2.6)		
Series II								
1	2.8	14.5*	3.1	4.4*	6.4	12.4*	0.073	0.211*
	(0.5)	(2.8)	(0.4)	(0.8)	(1.0)	(2.2)	(0.01)	(0.06)

Abbreviations: P_i free = interstitial fluid pressure in free gingiva; P_i att. = interstitial fluid pressure in attached gingiva; P_v = venous pressure; k_{Alb} = albumin clearance; SOC = sham-operated control. Numbers in parentheses are ± SD.

* $P < 0.05$ when compared with its corresponding control.

Series II (effect of venous stasis on k_{Alb})

Compared with the SOC, venous ligation resulted in an almost threefold increase in the removal rate of isotope-labeled albumin 1 day after surgery. Fig. 3 exemplifies the semilogarithmic plot from one control rat and one experimental rat, with k_{Alb} values of 0.072 h^{-1} and 0.242 h^{-1}, respectively. Average k_{Alb} value in the six experimental rats was 0.211 h^{-1}, compared with 0.073 h^{-1} in the control group (Table 1). As is evident from Table 1, P_v and P_i in series II were not significantly different from the corresponding measurements in series I, indicating approximately equal transcapillary fluid flow in the two series.

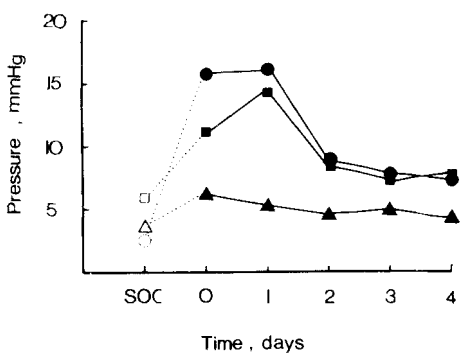

Fig. 2. Venous pressure (P_v) and interstitial fluid pressure (P_i) in free and attached gingiva of sham-operated controls (SOC) (open symbols) and experimental rats (solid symbols) during days 0–4 (series I). Circles represent P_v. Squares = P_i in attached gingiva; triangles = P_i in free gingiva.

Series III (effect of venous stasis on COP)

There was no significant difference between COP measured in fluid samples from gingiva and in subcutaneous tissue from the back in the SOC. Colloid osmotic pressure in wick fluid from gingiva was 10.6 mm Hg, and the COP_i of subcutaneous tissue from the back was 10.7 mm Hg, with COP of plasma being 20.7 ± 1.6 mm Hg. However, venous stasis caused a more

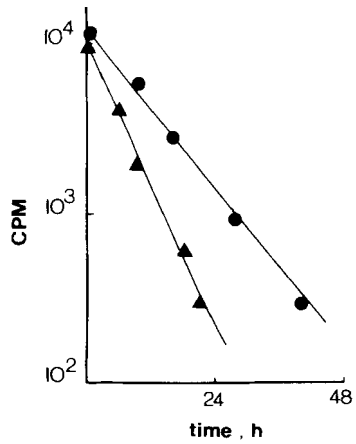

Fig. 3. Semilogarithmic plot of counts per minute (^{125}I-albumin) versus time from one control rat (circles) and one experimental rat (triangles) with k_{Alb} values of 0.072 h^{-1} and 0.242 h^{-1}, respectively.

than 60% fall in COP_i in wick fluid from gingiva—that is, from 10.6 ± 1.4 to 4.5 ± 0.4 mm Hg, whereas COP_i of subcutaneous tissue from the back and COP_p did not change significantly when compared with the SOC.

Discussion

By means of extensive bilateral ligation of the external jugular veins we produced an elevation of venous pressure in the head which lasted for at least 4 days. This prolonged pressure increase led to a rise in capillary pressure with increased fluid filtration, resulting in a marked visible edema formation in most high-compliant tissues of the head and neck region, such as skin and subcutaneous tissue. On the other hand, no clinical edema was observed in gingiva, probably due to a relatively low compliance in this tissue (14). The observation corresponds well with findings in other low-compliant tissues like dental pulp (4), rat brain (3), and rat tail (2, 5).

P_i and hydrostatic buffering

During the sustained venous stasis P_v rose by about 12 to 13 mm Hg, giving an increase in P_i in attached gingiva of 5 mm Hg, whereas P_i in free gingiva rose by 2–3 mm Hg (average values on days 0 and 1). Assuming that the capillary filtration coefficient and reflection coefficient for proteins were unchanged and also that P_c will change in proportion to P_v, the measured increase in P_i in attached gingiva can be calculated to have counteracted about 60% of the filtration caused by the rise in P_c.

The present finding of a somewhat greater increase in P_i in attached than in free gingiva suggests that attached gingiva is less distensible—that is, has lower compliance. In contrast, in more high-compliant tissues, such as skin and muscle, a considerable increase in interstitial fluid volume will not increase P_i by more than 1–2 mm Hg (7, 8). Probably due to development of collateral veins, P_v began to fall after the 2nd day. The accompanying fall in P_i most likely resulted from a reduction in capillary pressure, but removal of fluid and proteins by leakage into the crevicular fluid and/or enhanced lymph flow could also have contributed. The latter explanation is supported by our finding of increased turnover of injected albumin on the 2nd day of the venous stasis.

COP_i and osmotic buffering

In rats with venous stasis we measured a decrease in gingival COP_i to about 40% of the control values. This fall in COP_i will add further edema prevention to the already mentioned hydrostatic buffering.

Several mechanisms might be responsible for the present finding of a drop in COP_i during venous stasis. In theory, the decrease in gingival COP_i might be due to dilution of interstitial proteins caused by increased capillary net filtration. In the present study, however, this seems less likely, as no edema was visible in the gingiva, and thus probably very small volume changes have taken place. A more acceptable explanation of the fall in COP_i would be increased lymph flow, an assumption favored by our measurements of a threefold increase in k_{Alb} during venous stasis. Our hypothesis is therefore that increased net filtration, leading to increased tissue pressure, will initiate increased vol-

ume lymph flow and washout of interstitial proteins. Thus the increased net filtration is balanced by enhanced lymph flow, giving a new steady state with constant volume and COP in the gingiva. However, as pointed out by Szabo (16), another possibility for albumin removal from the interstitium could be back-diffusion of proteins through the vessel wall. Although there is, in fact, a considerable gradient for tracer albumin to diffuse back to plasma, it seems incomprehensible that a net diffusion of unlabeled albumin might occur against a concentration gradient. In support of this, in skeletal muscle it has been shown (17) that radiolabeled albumin can be transported to blood at a rate that is less than 30% of that in the opposite direction.

As reviewed by Pashley (18), enhanced formation of crevicular fluid seems to be due to increased gingival capillary pressure. A possibly increased removal of proteins by augmented formation of crevicular fluid might thus further contribute to maintain a low COP_i in the gingiva. In fact, none of the above-mentioned explanations seem to be completely mutually exclusive.

In brief, the present study has shown that increased filtration pressure causes a rise in P_i and a decrease in COP_i in the gingiva in rats. The measured threefold increase in the removal rate of locally injected ^{125}I-albumin during venous stasis indicates a corresponding increase in lymph flow.

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