The effects of saline-induced edema in the human nasal mucosa on laser Doppler flowmetry*

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SUMMARY *The nasal mucosa on the anteromedial surface of the inferior turbinate was studied with laser Doppler flowmetry in ten patients who were under general anaesthesia. A specially designed adapter was used, which held an injection needle with a diameter of 0.4 mm in a fixed position to the tip of the probe. The tip of the needle was inserted to a depth of 0.7 mm below the surface of the mucosa, while the tip of the probe was held at a distance of 0.3 mm from the mucosa. The laser Doppler parameters of perfusion, concentration of moving blood cells (CMBC) and velocity were recorded before and after the injection of 0.8 ml saline, thus inducing an experimental edema. After the injection no change in perfusion was detected but CMBC decreased and velocity increased. The findings agreed with the view that an increase in mucosal edema would reduce CMBC. When studying unanaesthesised subjects we normally use both rhinostereometry and laser Doppler flowmetry. It is then possible to measure the degree of mucosal congestion and micro circulation simultaneously, thus permitting study of the effects of a change in interstitial fluid content on mucosal congestion.*

Key words: nasal mucosa, rhinostereometry, laser Doppler flowmetry, edema

INTRODUCTION

In most types of rhinitis, infectious, allergic and non-allergic, the major presenting symptom is difficulty in breathing through the nose because of nasal congestion. Congestion of the nasal mucosa is caused by changes in the mucosal micro circulation and it is often treated with drugs that act on the vessels. To understand the physiology and pathophysiology of rhinitis and the pharmacological effects on the mucosa, one should have a method of studying the relation between nasal mucosal congestion and the microcirculatory events in the mucosa causing changes in congestion.

The combination of rhinostereometry (RSM) (Juto, 1985) and laser Doppler flowmetry (LDF) has the advantage of using two non-invasive methods permitting direct and simultaneous measurements of congestion and the micro circulation since with rhinostereometry the laser probe can be in the nose when measuring congestion (Grudemo and Juto, 1997a). With RSM the position of the probe can also be checked exactly (Grudemo and Juto, 1997b). With LDF continuous recordings can be made of the perfusion, the concentration of moving blood cells (CMBC), and the velocity of flow. The perfusion is the product of CMBC and velocity.

In the first study using this combination, one of the events in the LDF that took place following histamine challenge was a decrease in CMBC (Grudemo and Juto, 1997a).

CMBC reflects the number of blood cells moving within the part of the tissue reached by the laser light and reflected back to the probe. It therefore seems probable that a decrease in CMBC reflects an increase in the amount of interstitial fluid between the vessels. This means that the percentage of tissue consisting of vessels containing moving blood cells would be reduced. Such an increase is equivalent to an interstitial edema, a well known component in an inflammatory reaction. The cardinal signs of inflammation, rubor, calor, tumor and dolor are attributed to the Roman encyclopaedist Celsus (30BC-38 AD). "Tumor" is partly the effect of increased vascular permeability.

One of the many effects of histamine in the human nasal mucosa, is to increase the permeability of the micro vessels (Svensson et al, 1989, Raphael et al, 1989). The sites of plasma leakage have been shown to be in the small post capillary venules (Majno and Palade, 1961a, Majno and Palade, 1961b). These studies also demonstrated the formation of intercellular gaps between the epithelial cells at the sites of intercellular tight junctions. Later findings include indirect evidence of histamine receptors in the

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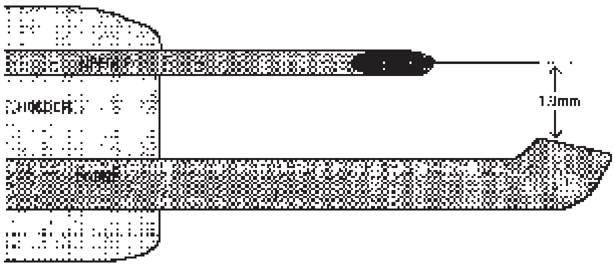

Figure 1. Adapter constructed to hold the needle and probe in position.

region of the intercellular tight junctions (Heltianu et al, 1982, Simionescu et al, 1982).
We therefore assumed that interstitial edema should be induced in the nasal mucosa by histamine challenge and that this might be detected with LDF as a decrease in CMBC.

AIM OF THE STUDY

In this study we combined RSM and LDF to study the effects on various LDF parameters of experimentally induced edema in the human nasal mucosa, by injecting isotonic saline into the superficial part of the mucosa studied with laser Doppler. Thus we produced an artificial edema without causing other histamine-induced changes in the mucosa.

MATERIALS AND METHODS

Ten patients participated. They were prepared for ENT surgery, such as tonsillectomy, septoplasty or sinus surgery. They were placed in a dorsal position and under general anaesthesia.
RSM (Rhinomed, Sweden) is a direct optical method with the subject secured to a frame by means of an individually adapted dental splint. The frame holds a micrometer table supporting a surgical microscope with a small depth of focus. The micrometer table has four microgauges, three of which define the position of the microscope in the perpendicular directions up-down, anterio-posterior and right-to-left and the fourth the rotation around a vertical axis. The eye-piece has a horizontal millimetre scale. The position of the surface of the mucosa is determined by defining the point at which it intersects a horizontal line in the frontal plane defined as the intersection of the plane of focus of the microscope and the horizontal plane defined by the millimetre scale.
LDF is performed using a PERIFLUX 4001 (Perimed, SWEDEN). The wavelength of the laser beam is 780 nm. The signal is fed into an IBM-compatible computer using the PERISOFT software program (Perimed, SWEDEN).
The specially designed probe has an outer diameter of 1.6 mm and a fibre separation of 0.5 mm. The end surface of the probe is at an angle of 15 degrees to the line of sight, to ensure that it is parallel to the surface of the mucosa.
Normally we perform RSM and LDF together using a micromanipulator (Rhinomed, Sweden) to keep the probe in a steady position and at a fixed distance from the surface of the mucosa. The position of the probe is changed if the congestive state of the mucosa alters. Artifacts from a change in the distance between the probe and mucosa can then be avoided (Grudemo and Juto, 1997b).
Since the patients in this study were under general anaesthesia, we could not use the usual RSM apparatus with the tooth splint. Instead, we used an operating microscope (OPMI 9, Zeiss) equipped with an eye-piece containing a mm-scale. The interior of the nose was examined with a linear magnification of 18 times. Each scale division then corresponded to a distance of 0.1 mm in the nose. Instead of using a micromanipulator, the examiner held the probe in his hand, using support against the face of the subject.
An injection needle with a diameter of 0.4 mm (27G) was attached to the probe by means of a specially constructed adapter. The end of the probe was positioned 2 mm past the tip of the needle and 1 mm from the longitudinal axis of the needle (Figure 1).
A 1 ml syringe with scale divisions of 0.1 ml was attached to the needle and filled with 0.8 ml of isotonic saline at body temperature. The needle was inserted about 3 mm into the mucosa until the tip of the probe was 0.3 mm from the surface. The site of injection was the anteromedial surface of the inferior turbinate, where RSM measurements are usually made. The needle tip was then approximately 0.7 mm below the surface. When the needle was in place, the LDF recording was started and continued until the readings stabilised. The injection was then given during 5 to 10 seconds while the LDF recording continued.

STATISTICAL ANALYSIS

The LDF parameters of perfusion, CMBC and velocity were recorded and analysed immediately before, and at the end of the injection. The Wilcoxon matched pairs test was used to determine the significance in changes in LDF parameters.

RESULTS

During the injection the mucosa underlying the probe became swollen and pale. The speed of injection however, was adjusted to prevent the mucosa from touching the probe. The distance between the tip of the probe and the mucosa was kept between 0.1 and 0.3 mm. Within these limits no significant changes occurred in the LDF recordings because of a change in the measuring distance (Grudemo and Juto 1997b).
The CMBC decreased from 112 (SEM 4.6) CU before to 73 (SEM 5.2) CU after the injection ($p=0.005$) as shown in Figure 2.

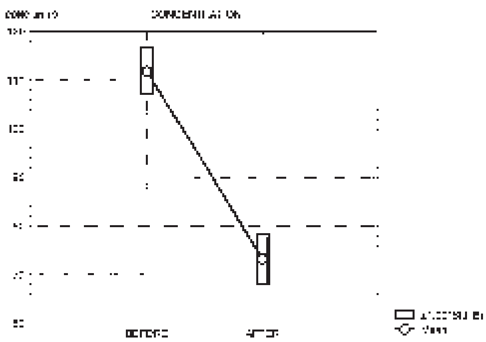

Figure 2. CMBC before and after injection of saline.

The perfusion was 274 (SEM 33) PU before, and 264 (SEM 23) PU after the injection (Figure 3). There was no significant change after the injection (p =0.72).

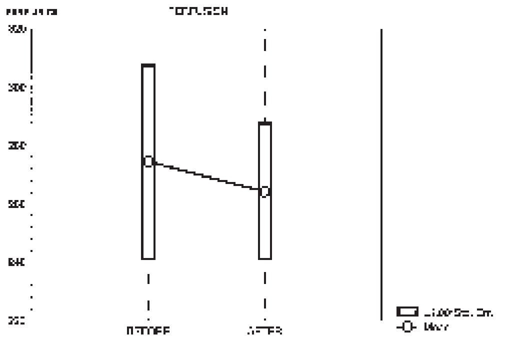

Figure 3. Perfusion before and after injection of saline.

The velocity increased from 248 (SEM 30) VU before to 380 (SEM 45) VU after the injection (p=0.017) (Figure 4).

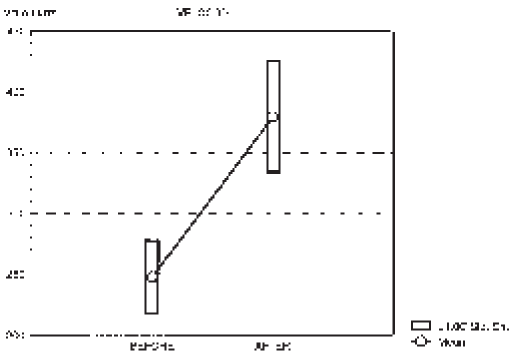

Figure 4. Velocity before and after injection of saline.

DISCUSSION

The diameter of the injection needle (0.4 mm) was considerably greater than the diameter of vessels in the superficial part of the nasal mucosa. The injection was carried out while advancing the needle slowly during a short period. It therefore seems likely that the saline was deposited interstitially and not into the lumen of a vessel. On a few occasions there was bleeding from the injection site. We then made the measurements in the other side of the nose.

The changes in the mucosa seen with the microscope consisted of a localised swelling and pallor.

A paler mucosa means less blood content in the tissue. This can be caused either by an edema or by vasoconstriction. Swelling on the other hand, can be caused by an increase in interstitial fluid or by vasodilatation. Therefore, the only plausible explanation of the changes seen in the mucosa is an increase in the amount of interstitial fluid, i.e. edema.

According to the LDF measurements, after the injection perfusion was unchanged, but CMBC decreased significantly and velocity increased significantly.

The decrease in CMBC is plausible since the vessels are spread apart by an increased amount of interstitial fluid. Fewer vessels containing moving blood cells are then reached by the laser beam.

The change in velocity is more difficult to explain. However, it could be due to an increase in the interstitial tissue pressure, causing a slight compression of the vessels. Since the level of the blood pressure through the injected tissue remained relatively constant, this could increase the velocity. Because the diameter of the needle is much larger than that of the vessels, haemodilution as an explanation of the increased velocity seems less likely.

A slight straightening of small tortuous vessels could maybe result in a more laminar flow, increasing the velocity. Another explanation could be that the injection has a more pronounced compressive effect on veins with lower velocity of flow as compared to arteries, resulting in an increase in average velocity as measured by laser Doppler.

The change in velocity must however be interpreted with caution, since velocity is the least well studied of the LDF parameters. Perfusion and CMBC are measured by the software program and the velocity is then calculated by dividing the perfusion with the CMBC.

The injection of saline could cause a local effect of stretching of the small vessels. Stretching or traction on vessels is known to cause vascular reactions from muscular elements or endothelium through release of endotheline and norepinephrine (Yao et al, 1991). This would however induce contraction of the vessels, which would lead to a decrease in velocity of flow and not an increase as was seen in this study. In a previous study of the effects of oxymethazoline, a well known constrictor of vessels, we saw a pronounced decrease in perfusion and velocity, but no change in CMBC.

The relationship between the probe tip and the surface of the mucosa was under visual control using the microscope during the time the micro circulation was recorded. The tip of the probe was kept parallel to the mucosa at a distance between 0.1 and 0.3 mm to avoid artifacts from changes in geometrical conditions. Within this distance there are no changes in the readings of microcirculatory parameters because of change in distance between probe tip and mucosal surface (Grudemo and Juto, 1997b). It was, however, not possible to keep conditions stable for a long enough period to determine the time it took for the edema to be resorbed.

CONCLUSION

We recorded the changes in laser Doppler flowmetry in the human nasal mucosa during intramucosal injection of saline, which caused an experimental edema. The results support the view that the development of interstitial edema reduces the laser Doppler parameter CMBC. Since interstitial edema is part of the inflammatory reaction involved in allergic and hyperreactive nasal disorders and also in changes induced by intranasal challenges with inflammatory mediators, we believe that changes in CMBC may be of value in interpreting the microcirculatory findings in the nasal mucosa after, for instance, a histamine challenge.

By combining RSM and LDF it is also possible to study the relation between changes in CMBC and mucosal congestion by simultaneously measuring micro circulation and congestion.

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