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Scanning Electron Microscopic Comparison Between the Thrombogenic Properties of Heparinized and Non-Heparinized Vena Cava Catheters

A Long-Term Study

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Summary. In an experimental comparative study on rabbits, the efficacy of the reduction of surface thrombus formation was investigated using heparinized central venous catheters compared with *non-heparinized.* Up to a central indwelling period of ten days, there was a reduction of the thrombus layer thickness on the heparinized catheters of 80% (with a trial duration of three days this was as much as 90%).

The action of the stabilized surface heparin, which leads to a substantial thromboresistance, is complex in nature. 1) By intensified adsorption of plasma proteins in the early phase (proteins consist of antithrombin III, factor IX, X and XI), there is very much less thrombocyte adhesion and aggregation on the heparinized catheter surfaces. 2) The externally orientated negative net charge of the heparin shows a similar effect. 3) If there is thrombocyte adhesion despite this, further heparin effects become more prominent. Thus the release of ThF 3 and ADP, which leads to aggregation, is markedly inhibited. On the one hand, this leads to easier dissolution of already aggregated thrombocytes and on the other to a reduction of the catalytic thrombocyte surface for the plasma clotting factors. 4) This effect is supported by the increased adsorption of antithrombin III onto the control surface. 5) Finally there is also an inhibitory effect of heparin on the activated Stuart-Prower factor.

Local thrombosis on the venous catheter surface can thus be effectively reduced by local therapy, without greater risks for the intensive care patient.

Key words: Intensive medicine – Central venous catheters – Heparin – Surface heparinization – Reduction of thrombogenesis

Introduction

The problem of the use of artifical non-physiological surfaces in today at the forefront of clinical and experimental medicine. After Opderbecke had intro-

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duced the vena cava catheter in the field of intensive medicine in Germany in 1961, there were occasional warnings concerning the not inappreciable risks of central catheterization and pleas for stricter establishment of the indications [1, 9, 16, 19]. In contrast to the early complications (vessel and cardiac wall perforations, arrythmias, catheter embolism, haemothorax and pneumothorax etc.) resulting from the technique of application, secondary complications (thrombosis and thromboembolism) caused by the material used have been investigated experimentally in the present study.

Pathological anatomy studies have shown that the *actual* incidence of thrombogenesis with central indwelling venous catheters is appreciably higher than the rate of clinically diagnosed thrombi suggests [6]. Haag et al. [7] reported a rate of thrombosis of 28.3% in the superior vena cava system. This incidence is within the range of autopsy results of other authors [14, 18, 19]. Burri and Gasser [1] verified thrombosis venographically in about 30% of the cases in subclavian catheters. Eldh and Jacobsson [5] investigated catheter surfaces after radiodiagnostic intervention. They were able to detect thrombus material on the catheter in about 75% with the "washout method" and in about 80% with "pullout" angiography. These data clearly underline the need for biochemical and biotechnological improvement of vena cava catheters.

We were therefore concerned to study the physiological and pathological clotting behavior of heparinized surfaces in the form of vascular catheters (manufacturer: Braun Melsungen) in the central venous bloodstream over a long period of time.

Experimental Method

I. Surgery

All studies were in vivo experiments carried out on 30 New Zealand rabbits with a weight of between 2–3 kg at an age of six months. After Nembutal anaesthesia (1 ml/kg) with the addition of 0.25 mg atropine, we proceeded as follows:

1) the jugular vein and internal carotid artery were dissected out,

2) the vein was ligated distally with a haemostatic clamp,

3) the catheter was introduced into the vessel by means of an incision.

This was performed for the following reasons: a) there was a danger that the experimental results could be masked or falsified by the possible occurrence of puncture damage, b) it was important not to damage the superficial heparin film by too small a puncture opening.

4) the catheter was fixed in position with a tobacco pouch suture and external knotting (catheter is about 2–4 cm deep),

5) subcutaneous suturing was afterwards carried out in the fat and perivascular connective tissue. This was again performed for two reasons: a) subcutaneous suturing ensured good protection from infection against organisms from outside, and b) relative positional stability of the catheter in the intravascular space was attained in this way.

Infection prophylaxis with Tardomyocel® Bayer was performed postoperatively in all experimental animals at a dosage of 8,000 IU/kg. Heparinized and *non-heparinized* polyethylene catheters from the company Braun Melsungen with the dimensions 1.2×1.7 mm, length 10 cm (sterilized and vacuumclosed) were used to perform the experiments.

In the individual experimental groups, the catheters were left in position for periods of 4 h, 24 h, 48 h, 72 h, six days, ten days, 14 days, 20 days and 30 days.

At the respective deadlines, the catheters were removed in toto with the corresponding vessel sections. The removal of the catheter and vessel was performed in vivo in the (again non-lethally)

anesthetized animal. This tedious procedure appeared to us to be justified in order to be able to exclude postmortem thombogenesis and further postmortem alterations in and on the vessel itself.

II. Histology

The following procedure was adopted: 1) Slow fixation in 2.5% glutaraldehyde (buffered); duration 2 h; 2) washing out in buffer (6–8 times); 3) immersion in osmium tetroxide (for 2 h), elevation of conductivity; 4) dehydration by ascending isopropanol series 30–100% (15 min per step); 5) alcohol-Freon exchange (30 min); 6) desiccation at the critical point (2 h); 7) sputtering with gold (2 min 400 A at 15 mA).

The preparations were evaluated on a scanning electron microscope (SEM) from the Cambridge company (Stereoscan) and on the SEM of the company Hitachi (S 450). The experimental preparations were measured on the screen of the SEM, taking into account the respective magnification steps and using a special balancing device which permitted precise adjustment of angular degrees.

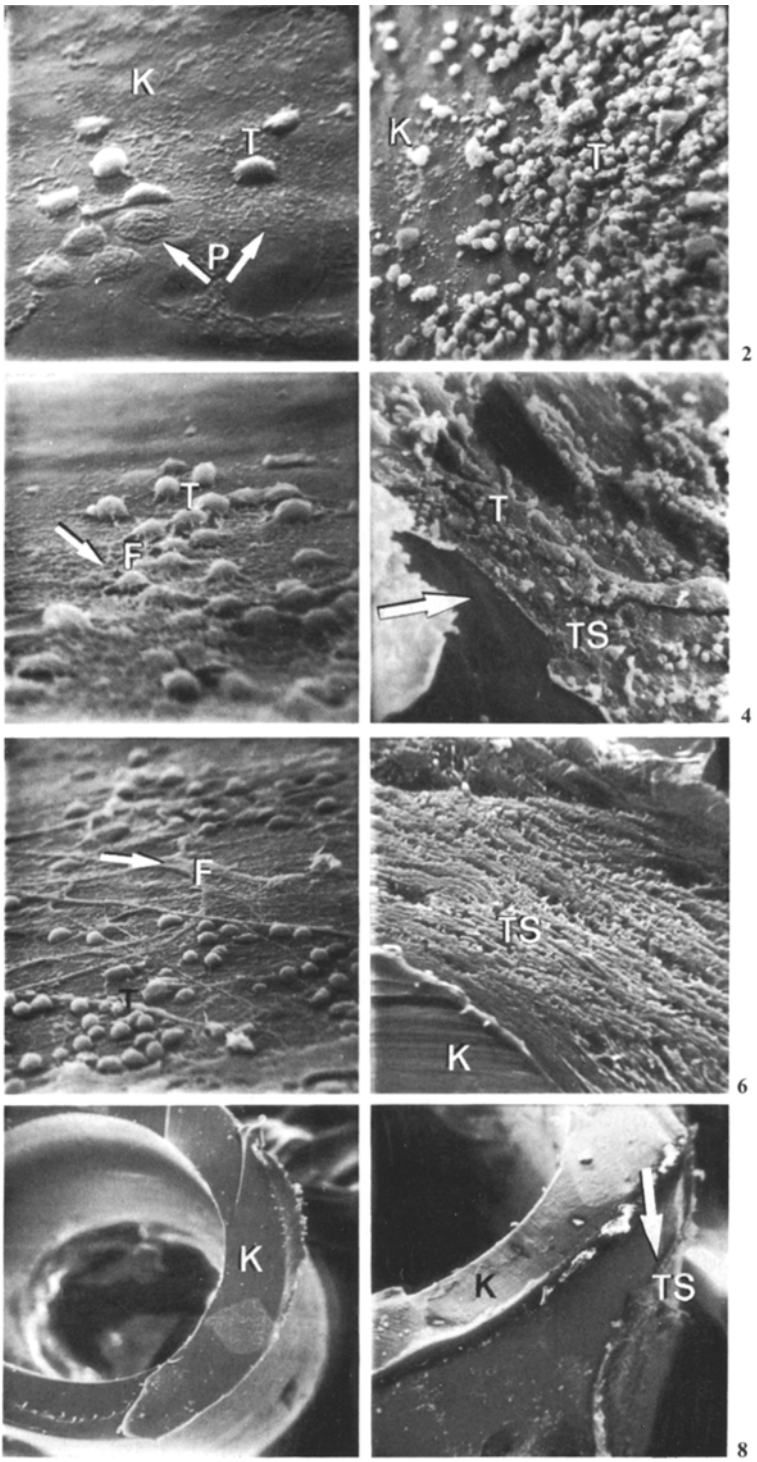
Experimental Results

After an experimental duration of 4 h, a morphologically detectable film could be demonstrated on the heparinized catheter surfaces in the SEM: this probably consisted of adsorbed plasma proteins (Fig. 1). Only very occasional thrombocytes adhere to this very thin layer; with regard to their clotting activity, no morphological substrate can be demonstrated. After the passage of 24 h, the number of adhering thrombocytes has considerably increased (Fig. 3). At this time, the platelet clotting activities can also be documented very well in the oblique scanning electron microscope. This activity consists of production of fibrin and its linkage to the thrombocyte surface. Reticulate fibrin structures on the heparinized catheters in which thrombocytic and leukocytic cells have been caught can only be recognized after 36 h experiment duration (Fig. 5). This exceedingly thin thrombus carpet now grows by layers to a thickness which leads to obliteration of the affected vessel (Fig. 6). In contrast to the heparinized surfaces, we were able to demonstrate a thick thrombocyte coat on the *non-heparinized* control surfaces even after passage of 4 h; this transformed to a firm thrombus formation after 24 h (Figs. 2 and 4). A fissured and rough surface is displayed here.

However, the more thromboresistant early behavior of the heparinized catheters in the experiment compared with the non-pretreated vascular catheters, was further shown in the results of quantitative measurements of thrombus layer thicknesses up to period of 30 days, when the catheters were left in the vein.

Quantitative SEM comparison between experimental and control catheters shows a paper thin protein layer with occasional thrombocytes on the heparinized surfaces after only 24 h at high light microscopic magnifications (Fig. 7). Thrombus formation from fibrin, erythrocytes, thrombocytes and leukocytes with an average thickness of 20 ± 10 μm has already developed on the control catheters after a period of 24 h (Fig. 8).

After 48 h, this phenomenon is even more pronounced. Whereas a very thin, fragile, carpetlike thrombocyte film develops on the heparinized surfaces (Fig. 9) thrombus formation on the control surfaces attains a thickness of 130 ± 30 μm. In this stage, roughly 500% greater growth of the thrombus materi-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al on the non-pretreated catheters occurs compared with the 24 h period of placement (Fig. 10).

The following reaction was shown after 72 h: whereas on the heparinized catheters a loose fibrin-thrombocyte film, which could only be extracted with a very careful dissection technique, could be detected (Fig. 11), a massive thrombus ring with a thickness of 340 ± 40 µm was deposited on the corresponding control catheters (Fig. 12).

A firm fibrin-thrombocyte ring formed on the heparinized venous catheter only between the third and sixth day. On the sixth day (i.e. after a period of 144 h within the central vascular bed), it attains an average layer thickness of 135 ± 25 µm (Fig. 13). Thrombus formation on the non-heparinized catheter has already attained a comparable extent after 48 h. The centrally situated control catheter shows a layer thickness of 440 ± 40 µm after six days (Fig. 14).

After a period of ten days in the vascular bed of the common jugular vein, a thrombus layer thicknesses averaging 155 ± 15 µm can be measured on the stabilized heparinized plastic surfaces. It appears as if surface thrombogenesis stagnated in the transition from the sixth to the tenth day and that in this stage an equilibrium was established between thrombogenesis and thrombolysis. The control catheter displayed a corresponding experimental behaviour: it also failed to show any appreciable increase in thrombus layer thickness in this period.

The phenomenon of stationary thrombogenesis could not be followed further in the course of the experiment in the major portion of the material. In control catheters for example, the thrombus substance reached the opposite wall of the jugular vein after an experimental period of 20 days (Fig. 16), leading to occlusion of the vessel. The layer thickness of the thrombus on the heparinized plastic surfaces was 460 ± 14 µm at this time and the vascular bed proved to

Fig. 1. Heparinized catheter surface after an experimental period of 4 h. Occasional thrombocytes on adsorbed protein layer. AR no. 558. Magn. ×1,250

Fig. 2. Dense thrombocyte coat on control catheter after an experimental period of 4 h. AR no. 574. Magn. ×500. *T*, thrombocyte; *K*, catheter surface; *P*, Plasma proteins; *F*, Fibrin

Fig. 3. Heparinized catheter surface after 24 h. Thrombocytes show morphologically detectable clotting activity. AR no. 557. Agn. ×1,250

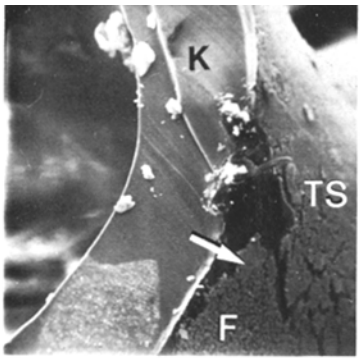
Fig. 4. Carpetlike thrombus formation on control catheter after passage of 24 h. AR no. 465. Magn. ×400. *TS*, thrombus layer; *T*, thrombocyte; *F*, fibrin

Fig. 5. Heparinized catheter surface after 36 h. Netlike fibrin structure. AR no. 559. Magn. ×700

Fig. 6. Lamellar layering of thrombus after 30 days on control catheter. AR no. 903. Magn. ×50. *TS*, thrombus layer; *T*, thrombocyte; *F*, fibrin

Fig. 7. Appearance of morphological reaction after 24 h (heparinized catheter). AR no. 552. Magn. ×25

Fig. 8. Appearance of morphological reaction after 24 h (control catheter). AR no. 564. Magn. ×50. *TS*, thrombus layer; *K*, catheter



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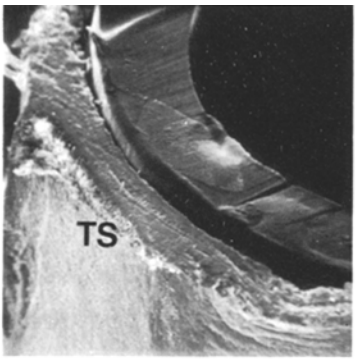

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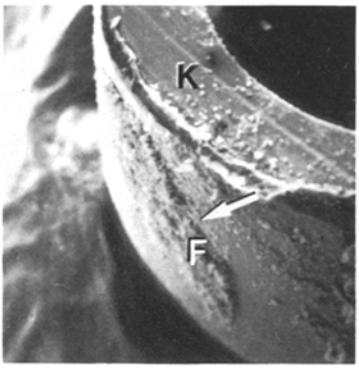

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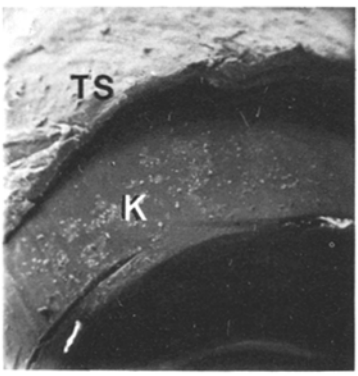

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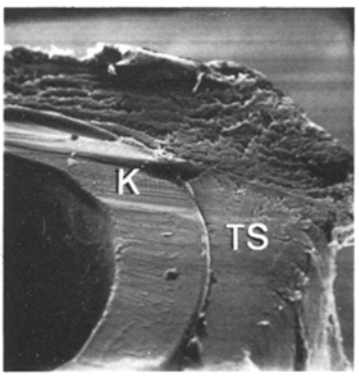

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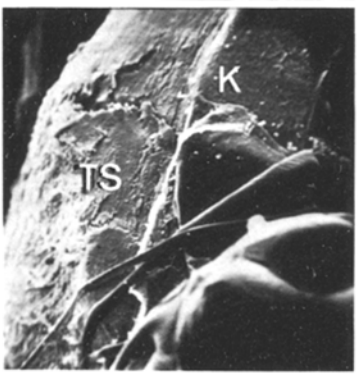

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be still patent in this experimental group (Fig. 15). This patency of the jugular vein was interrupted by the developing thrombus after an experimental period of 30 days, even in the heparinized catheters (Precise measurement of the thrombus layer thickness was no longer possible, since the vessel wall had been reached.)

Discussion

The experimental results show very convincingly that heparinized central venous catheters are more thromboresistant than non-heparinized control catheters. With a period of indwelling of up to 10 days, there is a reduction of thrombotic material on the respective heparinized catheters by 80% compared with the control catheters. This experimental behavior is even more pronounced when a comparison is made between catheters left in for only three days when decrease of the thrombus layer thickness on the heparinized catheters by 90% compared to the non-pretreated control catheters is shown. We believe that there is now hardly any doubt as to the basic efficacy of this surface heparinization.

The documented results are also consistent with the experimental results of Jacobsson and Schlosman [8], Olsson et al. [15] and Larsson et al. [12]. Whereas these authors preferred very brief trials (5 min to 40 min) and also mainly preferred in vitro observations, there is nevertheless good agreement with our experimental results. In addition, we wished to investigate the long-term behavior and the practical applicability of these central venous catheters. On the basis of the above results, we would give the use of heparinized catheters for up to 10 to 12 days absolute preference compared to the untreated conventional polyethylene type. After these ten days had passed, there was a more intense thrombus development on this type of catheter (Fig. 15) which in our opinion, is due to either a) complete consumption of bound heparin or b)

Fig. 9. Appearance of morphological reaction after second experimental day (heparinized catheter). AR no. 566. Magn. ×50

Fig. 10. Corresponding reaction appearance after second experimental day (control catheter). AR no. 569. Magn. ×50. *K*, catheter; *TS*, thrombus layer; *F*, fibrin

Fig. 11. Appearance of morphological reaction after third experimental day (heparinized catheter). AR no. 571. Magn. ×50

Fig. 12. Corresponding reaction appearance after third experimental day (control catheter). AR no. 596. Magn. ×50. *K*, catheter; *TS*, thrombus layer; F, fibrin

Fig. 13. Appearance of morphological reaction after sixth experimental day (heparinized catheter). AR no. 605. Magn. ×50

Fig. 14. Corresponding reaction appearance after sixth experimental day (control catheter). AR no. 900. Magn. ×50. *K*, catheter; *TS*, thrombus layer

Fig. 15. Appearance of morphological reaction after 20th experimental day (heparinized catheter). AR no. AR no. 897. Magn. ×50

Fig. 16. Corresponding appearance after 20th experimental day (control catheter). AR no. 884. Magn. ×25. *K*, catheter; *TS*, thrombus layer; *LS*, lamellar layering

the fact that the heparin bound to the surface is prevented from acting by the thicker thrombus layer. Larsson et al. describe in their studies with glutaraldehyde-stabilized and with ^{35}S-labeled heparin an initial heparin desorption of 3% in experiments performed in vitro. Corresponding in vivo trials showed an initial decrease of the superficial heparin concentration by 12%, which then remained constant. We were unable to confirm this mode of reaction of the heparin layer, especially with regard to the period beyond the tenth day. The thrombus formations develop both on the heparinized catheters and on the control catheters in layers on the artifical surface, until complete occlusion of the vessel occurs after about 30 days due to the continuous lamellar growth (Fig. 6). Especially in the early phase of the experiments, the effect of heparin was very pronounced in the SEM photographs. Whereas massive thrombocyte adhesion and thrombocyte aggregation has occurred after four to 24 h on the non-pretreated catheters, only occasional thrombocyte adhesions can be discerned on the heparinized surfaces. The mechanism by which glutardialdehyde-stabilized heparinized surfaces are able to prevent thrombus formation are not yet known. In our view, the action of heparin in this connection is very complex:

1) As already stated above, obligatory thrombocyte adhesion on the foreign surface is *always* preceded by adsorption of plasma proteins. Larsson et al. (1977) demonstrated a larger protein layer on the heparinized surfaces than on the control surfaces. These proteins are probably a) antithrombin III, b) factor IX, c) factor X and d) factor XI (Danishefsky and Tzeng 1974). It is probably precisely this adsorbed protein layer which leads in the early stage to a reduction of thrombocyte adhesion and thrombocyte aggregation.

2) The externally orientated negative net charge of the heparin is likely to show a corresponding effect (Sawyer and Pate 1963). (Negatively charged like that of heparin and thrombocytes repel each other.)

3) If an adhesion of the thrombocytes on the surface has nevertheless occurred, further mechanisms of the action of heparin may become more prominent. Thus is doubtless an inhibition of the release of ThF 3 and ADP (which lead physiologically to an aggregation; Deutsch 1977). In our opinion, this inhibition of aggregation has two advantages: a) the already adhering thrombocytes become more readily soluble and b) with the reduction of thrombocyte surface area the catalytic reaction mediator of the plasma coagulation steps is lacking.

Walsh and Biggs [21] demonstrated that the clotting factors adsorbed on the thrombocyte surface and this produce thrombin more rapidly here than it can be neutralized by antithrombins.

4) The high adsorption of antithrombin III on the catheter surface is likely to limit thrombin production substantially.

5) However, it is not only the effect of heparin in relation to antithrombin III alone which is important, but also the complex inhibitory action on factor Xa (Walker and Esmon 1979).

Furthermore, it is to be observed that the method of surface heparinization achieves very much better results with regard to reduction of thrombus formation than can be attained with corresponding systemic heparinization. Lawin [13] and Burri [2] were unable to reduce local thrombus formation on vena cava

catheters by a systemic antocoagulation. Lagergren et al. [10] made a similar observation from their experimental studies on artificial AV shunts made of polyethylene. It therefore appears appropriate also to attempt to deal with local thrombus formation with local therapy. On the one hand, this procedure has greater efficacy, and on the other no additional risks or dangerous burdens must be accepted for patients undergoing intensive medical care.

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