The effect of intramedullary bone endoscopy
on the endosteal blood supply in long bones. An experimental study in sheep

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This study investigated whether the Intramedullary Bone Endoscopy (IBE) procedure within the cavity of an intact long bone will interfere with the local endosteal blood supply.

In a sheep model, 10 animals underwent the IBE procedure with complete perioperative anaesthesiology monitoring. After the femora were harvested, histological analysis was performed to examine destruction of the endosteum and consecutive reduction in perfusion.

Only one animal showed evidence of detachment of the endosteum with destruction of several microns of the endosteum, although this did not interfere with the cortical perfusion. None of the vessels were occluded by fat or other causes of occlusion, e.g. blood coagulation.

Our findings indicate that with the IBE procedure under visual control there is a potential risk to damage the endosteum. However, the interference was limited to a small part of the endosteum and did not lead to a reduction in the cortical perfusion. Clinical use could be in localized intramedullary lesions such as osteomyelitis or benign bone tumours.

Keywords : endoscopy ; bone ; intramedullary ; endosteal blood supply ; side effects.

INTRODUCTION

Endoscopic techniques are now standard procedures. Almost every "closed compartment" of the human body can be reached safely with special endoscopes. It is therefore not surprising to find reports about intramedullary inspection of long bone cavities using various endoscopes (*3,6,10,11,17*).

All of these procedures have one thing in common : the medullary canal had been prepared by drilling or was "opened" by another technique (e.g., removal of a stem in revision arthroplasty of the hip) before the endoscope was inserted. In contrast to these techniques, Intramedullary Bone Endos-

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copy (IBE) was introduced as a new approach to the closed compartment of an intact long bone. During IBE, the medullary canal is prepared by slowly pushing an endoscope distally under visual control and endoscopic preparation (13-16).

Given that any manipulation inside the medullary canal can potentially interfere with the endosteum and with the endosteal perfusion, we performed an animal experiment on sheep, in which the clinical set up of IBE was tested as to the potential risk of reduction of endosteal perfusion.

We hypothesised that IBE under visual control and gentle endoscopic preparation of the medullary canal will not lead to relevant interference with the endosteal perfusion.

MATERIALS AND METHODS

Animals : The IBE procedure was performed on ten female Merino sheep aged 9-13 months (mean 11), weighing 51-64 kg (mean 58). The whole experiment and the intraoperative set up was approved by the local ethics committee (Registered No. G-06/21). The entire experiment and all animal handling was performed according to German government guidelines for animal research projects.

Anaesthesia : The animals were starved for 1 day but given free access to water up to 12 hours before operation. The animals were placed in a right lateral inverse Trendelenburg position. Following preoxygenation anaesthesia was induced through a cephalic vein cannula with 2-4 mg/kg propofol (Propofol-Lipuro® 1%, B. Braun Melsungen AG, Melsungen, Germany) followed by endotracheal intubation. Anaesthesia was maintained by the inhalation of isoflurane (Forene®, Abbott, Wiesbaden, Germany) (end-tidal concentration of 1.8 vol%) delivered in a mixture of oxygen and air (FiO$_2$ 0.4). Analgesia was achieved by the preemptive injection of 0.002 mg/kg fentanyl (Fentanyl-Janssen®, Janssen-Cilag, Neuss, Germany) and 4 mg/kg carprofen (Rimadyl®, Pfizer, Karlsruhe, Germany) and additionally, by the infusion of 0.005 mg/kg/h fentanyl during the surgical procedure. All animals were mechanically ventilated with a Servo 900 C anaesthesia ventilator (Siemens-Elema, Solna, Sweden). At the end of the surgical procedure the sheep were weaned from ventilation, extubated and kept under postoperative care. Postoperatively, analgesia was maintained by subcutaneous injection of 4 mg/kg/d Carprofen for the next 2 days.

Endoscope : The endoscope for IBE (Wolf-Endoscopes, Knittlingen, Germany) was 369 mm in overall length with a 298 × 9 × 5.85 mm oval working canal. The shaft diameter was 13.7 mm. If necessary, the shaft could be covered by a trocar which was 1 mm larger in diameter. This created a circular space for intensive irrigation (Fig. 1 & 2). Several special endoscopic tools (suction tubes, forceps, chisel, bipolar coagulation hook) were available.

Surgical procedure : After anaesthesia, intubation and positioning of the animals, the entry region at the left femur was shaved. All operations were under sterile surgical conditions. Additionally, an intravenous short-time infusion of 2 g cefotiam (Spizef®, Grünenthal, Aachen, Germany) was given to all animals 30 minutes before skin incision.

Skin incision was done after identification by palpation of the tip of the greater trochanter just over the fossa piriformis. The muscle tissues were bluntly dissected down to the bone. The cortex was opened with a tapered awl and a guidewire was gently inserted for 2-3 cm inside the bone. The opening of the cortex was defined as the starting point for perioperative monitoring. Then the entry point was prepared by over-drilling the guidewire to a 12 mm diameter. In the next step, the endoscope was placed and the IBE procedure was performed for either 30 or 60 minutes.

During endoscopic intervention we removed the bone marrow and did a "clearing" of the medullary canal. We started this procedure proximally at the insertion point, preparing the canal distally as far as we could get during the intervention time (30 minutes and 60 minutes respectively). The procedure was performed in a combination of washing out the canal with irrigation water and removing fat and bone marrow with the endoscopic forceps. Following postoperative wound closure, extubation and complete recovery of the animals, the sheep were held in their boxes for 48 hours. The animals were then killed by an overdose of thiopental (50 mg/kg i.v.), followed by 2 mmol/kg KCl intravenously. Ten minutes before this overdose, five sheep (50%) had an i.v. injection of 100 mg/kg Procion-red-dye (Procionrot H-8BN, BASF, Ludwigshafen, Germany).

Histologic preparation and examination : Immediately after the sheep were killed, the left femur was explanted en bloc. Seven cm distal to the tip of the greater trochanter a representative slice of 3 mm bone was harvested on both the endoscopic and non-endoscopic side for further histological examination (Fig. 3).

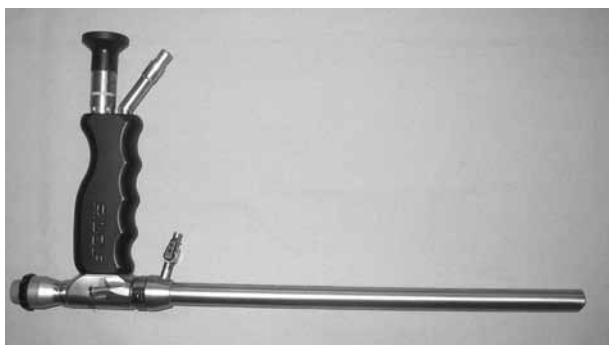The bone segments of the five sheep that had the Procion-red-dye prior to killing were processed as fol-

Fig. 1. — Endoscope for IBE (Wolf-Endoscopes, Knittlingen, Germany).

Fig. 2. — Distal end of the endoscope, covered with trocar
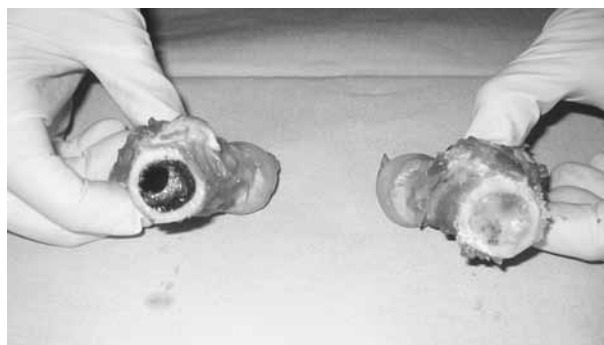

Fig. 3. — Macroscopic view of the bone section 7 cm distal to the tip of the greater trochanter. Left side : Cavity in bone marrow created by the endoscope ; right side : intact bone marrow.

lows : The bone segments were fixed in 4% buffered formalin (Merck, Darmstadt, Germany) for two weeks, then rinsed in tap water. Subsequently the samples were dehydrated according to the following schedule : 70% ethanol (2 days), 95% ethanol (2 days), 100% 2-propanol (twice for 1 day), and xylene (twice for 2 days). After dehydration, the samples were infiltrated with the plastic embedding mixture using a three-step protocol. Samples were infiltrated for one week in Solution I consisting of MMA (Merck, Darmstadt, Germany) and 15% dibuthylphtalat (Fluka, Selze, Germany). MMA Solution II was a mixture of MMA, 15% dibutylphtalat and 1,5% dry benzoylperoxid (Merck, Darmstadt, Germany) ; samples were infiltrated for one day. In the third step Solution III (mixture of MMA, 15% dibuthylphtalat and 3% dry benzoyl peroxide) was used for one week during which time samples were refrigerated (80° C). Polymerization was carried out at room temperature.

After trimming of the plastic blocks, 500 μm thick sections were prepared at room temperature with a microtome (Microslice®, IBS, Höhr-Grenzhausen, Germany) equipped with a knife with a diamond cutting edge. During sectioning, the knife and the blocks were kept moist with a sectioning fluid (WIV, Schwetzingen, Germany). The sections were transferred onto slides (Maertin, Darmstadt, Germany) and covered with a polyethylene foil, flattened with a rubber roller, pressed with a slide press, and dried for one day.

Thin sections were prepared using sandpaper (granularity 180 and 1200 ; Struers, Erkrath, Germany) to a section thickness of 80 μm. Polishing the specimens was accomplished using wet sandpaper (granularity 2400 and 4000 ; Struers, Erkrath, Germany).

The sections were evaluated under a fluorescence microscope (Axioplan 2 ; Carl Zeiss AG, Jena, Germany). For further evaluation the cortex was subdivided into an anterior, posterior, medial and lateral region, and within the thickness of the cortex ten subdivisions from endosteal to periosteal were made. Photographs were taken of the entire endosteum (AxioCam®, Carl Zeiss AG, Jena, Germany). The digital semiquantitative analysis was performed with the open source software program GIMP (Gimp ; GNU Image Manipulation Program, GNU General Public Licence, www.gip.org)

The bone segments of the five sheep without procionred-dye were processed as follows : They were fixed in 4% buffered formalin (Merck, Darmstadt, Germany) for two weeks, then rinsed in tap water and decalcified in Ossa Fixona® (mixture of trichloracetic acid, zinc chloride and formaldehyde ; Waldeck, Münster, Germany). Subsequently the samples were dehydrated according to the following schedule : 70% ethanol (2 days), 95% ethanol (2 days), 100% 2-propanol (twice for 1 day), and xylene (twice for 2 days) before embedded in paraffin wax using a standard protocol.

Fig. 4. — Intramedullary vision during IBE in sheep femur (suction tube at 21:00).

Sections of tissue blocks, 3 mm in thickness (using the microtome RM 2255 ; Leica, Nussloch, Germany) were deparaffinized, rehydrated, and stained with Mayer's Hematoxylin (2E 038 ; Shandon, Dreieich, Germany) and Eosin (6766007 ; Thermo Scientific, Waltham, MA, USA) and EvG (1.15974.001 ; Merck, Darmstadt, Germany) for histologic evaluation. Sections were mounted onto coated glass microscope slides (J1800AMNZ ; Menzel, Braunschweig, Germany).

Microscopic analyses (magnification 100×) were made in the same manner as mentioned above. Photographs were taken in complete cross section dimension from the whole endosteum at the levels of the osteotomy using the camera AxioCam® (Carl Zeiss AG, Jena, Germany) and semiquantitative analysis was performed.

RESULTS

All operations were performed without complications. No technical problems with the endoscope occurred. Under repetitive irrigation we achieved good visibility inside the medullary canal (Fig. 4).

Clinically, the animals appeared normal and showed no complications in the postoperative phase. During the postoperative recovery phase, all animals behaved normally while they recovered in their boxes. There were no infections.

To evaluate the endosteal disposition following the IBE procedure we had 5 compounds colored with procion-red and 5 compounds colored with HE and EvG respectively. In 9 out of 10 compounds we found no discontinuity in the endosteum (Fig. 5).

Under red (15Ex.546/12 nm wave length) and green light (09Ex.470/40 nm wave length) illumination, all the specimens presented an intense fluorescence of the periosteum and of the surrounding soft tissues, thus demonstrating a successful intravital staining of the perfused tissues.

Certain zones with minimal deficiencies in the staining of the intracortical vascular canals could be observed in all the cross-sections (< 3% of the whole area). Individual differences between the animals became apparent, but in all cases the cortical circulation of the femora investigated by the IBE procedure was not impaired significantly. Even when minimal endosteal damage was found no compromise of the cortical perfusion was detected.

None of the vessels were occluded by fat ore other causes of occlusion, e.g. blood coagulation (Fig. 6).

DISCUSSION

Our observations demonstrate that IBE under visual control and gentle endoscopic preparation will not lead to relevant interference with the endosteal perfusion.

After initial experimental tests, IBE was found to be a reliable and effective procedure to access the medullary cavity, especially when the technique is used in an "open system". In these cases the medullary canal is already opened by means of drilling or implant removal. Logically, in these cases there is no risk to interfere with the endosteal blood supply. IBE was found to be helpful for cement removal in revision total hip arthroplasty, removal of any lost objects inside the medullary canal and debridement, irrigation and resection of sequestra in osteomyelitis (11,16).

However, when using IBE to inspect the cavity of a bone, the endoscope would be used in an intact bone. In these cases as with other intramedullary manipulations such as reaming or intramedullary nailing in fracture treatment, there is the potential
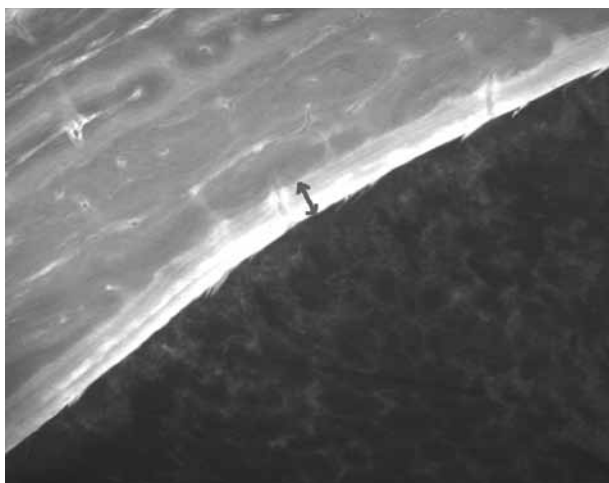
Fig. 5. — Bone section showing an intact endosteum with well perfused cortex (arrow ; animal 56, magnification 100× , Procion-red stained).

Fig. 6. — In one specimen, a small discontinuity about 0.2 mm in length was found in the endosteum.

risk of destroying the endosteum and reducing the cortical blood flow.

Therefore, the goal of this study was to determine if IBE within an intact bone interferes with the cortical blood flow of long bones. The animal experiment was performed on sheep, which is a widely used and accepted test animal for experiments of an orthopaedic or traumatologic nature (1,2,5).

As no special operative intervention was performed during IBE, no control group was established. The experiment was only designed to detect if there is an interference with the endosteum during IBE or not. For proper and valid statistical analysis, a study should have been performed with a very high number of test animals. However, this would have been impossible due to economical and ethical reasons. We are aware that in n = 10 animals the 95% confidence interval is 0-33.3% even if there are no positive incidents (i.e., no changes at the endosteum) during the investigation.

The operative procedure was standardized and all operations were performed by the senior author.

Laser Doppler Fluorometry is an accepted procedure to evaluate the local blood supply of long bones (7,12). However, this method is not suitable for detection of processes that take place inside the bone, because a Laser Doppler cannot reach this area.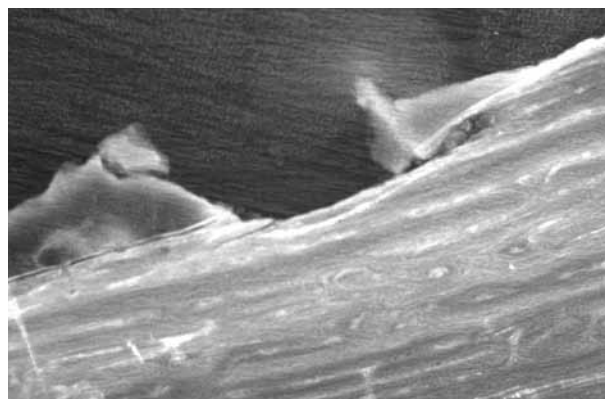 In contrast, detection of colored endothelial cells of blood vessels using fluoroscopy is an accepted method to quantify endosteal blood supply. In order to achieve sufficient dyeing, only a short circulation time of the stain in the blood circuit is necessary. The dye and its protein adhesion are very stable. It is not affected during the histological preparation or the decalcification of the specimens (7,12).

The negative effects of intramedullary manipulation to the endosteal blood supply are well known. As early as 1958, Kuentscher reported complete destruction of the endosteal surface in the diaphysis of long bones during intramedullary reaming (9). Klein *et al* noted destruction of the endosteal blood supply in 45-85% during reamed intramedullary nailing, as compared to unreamed nailing (7). Other authors reported negative effects of intramedullary reaming to the endosteal blood supply too (4,11,18,19,20). Grundnes and Reikeras found no reduction of the cortical blood flow in the diaphysis as long as the diameter of the reamer was smaller than the intramedullary cross section of the bone. However, when the diameter of the reamer was larger than the cross section of the bone, they detected an immediate reduction in cortical blood flow (4).

Our results demonstrate that controlled intramedullary bone endoscopy does not damage the endosteum in 9 out of 10 cases. Only in one single case did we find a discontinuity of the endosteum, and minimal intramedullary bleeding was easily

controlled with an endoscopic bipolar coagulation hook. Furthermore, this discontinuity was only 0.2 mm in length, and this minimal interference definitively did not affect the bone perfusion. This is in contrast with the damage to the endosteum that occurs during other common procedures such as reaming of the bone cavity or nailing in fracture treatment (*4,12*).

Considering that 90% of the femora had an intact endosteum, reduction of bone perfusion is not to be expected. In fact, the difference in luminance between the specimens studied (Fig. 5) would have made a comparison very difficult. Similarly, Müller also detected significant differences in fluorescence using Procion-red, that compromises reliability and validity of analysing the data (*12*). Fifty percent of the femora were stained witth EvG and HE, respectively (Procion-red was not available because of a manufacturer deficit). However, the intact endosteum was clearly visible in all compounds, regardless of the dyeing. Therefore differences in staining were not relevant, and the results of the study were not affected.

Given the small number of cases, results from the statistical analysis may have a modest value. In one of ten positive occurrences (one case of endosteal damaging), the predictive value for probability of such an incident is 10% : the confidence interval (Clopper-Pearson) is 0.003-0.445.

The same considerations are valid when looking at the intraoperative damage to the endosteum : In contrast to typical methods such as intramedullary nailing – where, as a rule, damage , including complete damage , is detected – using bone endoscopy causes none or only a minimal defect of the endosteum.

CONCLUSION

Our animal study demonstrated that during the IBE procedure, as with other techniques for intramedullary manipulation in long bones, there is a potential risk of interference between the endoscope and the endosteum.

However, the risk of a hazardous damage to the endosteum resulting in a reduced cortical blood supply is very low. In our investigation, only one out of 10 animals showed a disconnection of the endosteum and only of approximately 0.2 mm. This minimal disconnection did not lead to reduction of the endostal perfusion.

We conclude that the IBE procedure within the medullary cavity of an intact long bone is a safe procedure with regard to the cortical perfusion.

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