JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 9 (1998) 333–336

Experimental evaluation of a percutaneous injectable biomaterial used in radio-interventional bone-filling procedures

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Interventional radiology is beginning to be used in bone pathology. An injectable biomaterial Ethibloc*, composed of alcohol and a vegetal protein (zein), has been successfully used for percutaneous treatment of benign bone lesions. The reasons for this success remained unknown and needed to be understood. In this study, using a rabbit model, an evaluation was made of bone formation and tissue reaction during the first weeks after the injection of this biomaterial. Ethibloc* was injected percutaneously into bone defects in rabbit distal femurs. Three time intervals were studied: 2, 4 and 8 wks. The three control groups constituted unfilled, polymethylmethacrylate (PMMA), and alcohol. Undecalcified bone technique was used for a qualitative analysis and histomorphometric evaluation. A low bone formation was found which was less than in the control groups (PMMA and unfilled). The ''Ethibloc group'' was characterized by an early inflammatory reaction. Good clinical results obtained with Ethibloc* probably arose, after an initial vascular thrombosis, from a secondary bone reaction and spontaneous osteogenesis obtained after the disappearance of vascular or hyperpression reaction.

1. Introduction

Biomaterials have an ever-growing place in bone pathology. New percutaneous techniques, less aggressive than surgical methods, have been developed over recent years in spine pathology and in paediatric bone pathology.

Ethibloc* has been used successfully for percutaneous treatment of benign bone lesions, such as unicameral or anevrysmal bone cysts, by several workers [1, 2]. This material was primarily employed for the embolization of vascularized tumours [3–5] such as pancreatic carcinoma, renal tumours or brain tumours [6]. Contact of this material with blood cells leads to a thrombosis by a fibrotic reaction.

Ethibloc* has also been employed as an antibiotic carrier in the treatment of chronic osteomyelitis [7]. For these authors, this material was replaced in bone and muscular tissues by connective and fibrous proliferation tissue. Some workers have used it for percutaneous treatment of bone tumours, especially in spinal or pelvic locations, or in children. Unicameral bone cysts, as well as giant bone cysts and aneurysmal bone cysts, have also been treated [1]. Tumour growth was stopped after direct injection of this material into cystic defects. A slow healing with bony reconstruction was seen with a cortical and septal thickening, a shrinking of cavities and a peripherical osteosclerosis. In all cases, with a follow-up ranging from 12–18 mon, no local complication or tumoral recurrence was found. In spite of these interesting clinical results, no experimental results are available and the bone properties of this material remain unknown.

The aim of this study was to evaluate bone formation and bone reaction after injection of this material into an experimental bone defect. These results were compared with three control groups: unfilled control defect, filling with PMMA-filled defect (orthopaedic low-viscosity cement), and an alcohol group.

2. Materials and methods

2.1. Biomaterials

*2.1.1. Ethibloc**

Ethibloc* (ethnor Laboratories, Ethicon) is a radio-opaque solution which is a mixture of zein protein in alcohol. Ethibloc* contains in 1 ml: 210 mg zein, 162 mg sodium aminotrizoate tetrahydrate (contrast medium), 145 mg papaverin oleum, 6 mg propylene glycol in a 38.3% by volume ethanol solution.

Zein is an amino acid extracted from corn: its chemical structure is characterized by a solubility in alcohol

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(ethanol) which represents 30% of Ethibloc* volume. This hydrophilic liquid material takes on a harder consistency when it is placed in water. This partial hardening of Ethibloc* is obtained within several minutes: after a transition stage of gummy consistency, it becomes a firm material but never as hard as a polymethacrylate orthopaedic cement (PMMA).

The high viscosity of Ethibloc* (200 cP at 37 °C) allows the filling of cavities without any serious spilling over into surrounding tissues, as usually occurs with liquids. Ethibloc* is biodegradable; its cleavage by different amino-acid enzymes is obtained within 11–25 d [1, 3].

This solution was packaged in a sterile single disposable syringe of 7.5 ml.

2.1.2. PMMA

The tested PMMA was an orthopaedic low-viscosity cement (Cerafix* from Ceraver-Osteal Corporation, France) used in human pathology for arthroplasties or percutaneous vertebroplasties [8]. This cement is obtained by mixing two premeasured sterilized components: one solid, the other liquid. (1) Liquid component (20 ml): methylmethacrylate 16.89 ml; *N*-butyl methacrylate 2.71 ml; *N*-*N* dimethyl-*P*-toluidine 0.40 ml. (2) Solid component (46.5 g): polymethylmethacrylate 41.75 g; zirconium dioide 4.30 g; active benzoyl peroxide 0.45 g.

The liquid component was sterilized by ethylene oxide and packaged in a vial. The solid one was sterilized by gamma irradiation and packaged in a pouch.

2.1.3. Alcohol

A solution of 35% ethanol was obtained by adding 178 ml sterile water to 100 ml 95% ethanol.

2.2. Animals

Adult female New Zealand White rabbits (36 in all, of controlled sanitary status) with a mean weight of 3.820 kg (range 3.450–4.260 kg) were used in this study. Rabbits were fed in standard conditions. National Institutes of Health and CRWU guidelines for the care and use of laboratory animals were observed.

2.3. Methods

2.3.1. Group constitution

Four different groups were composed and compared in a randomized trail: (i) an “Ethibloc group” to test the material, and three “control” groups, (ii) an “unfilled group” studied the spontaneous evolution of the bone defect, (iii) an “alcohol group” tested the alone alcoholic component, (iv) a “PMMA group” constituted to compare the global effect of Ethibloc* with another material used in human percutaneous filling and which is considered as a reference bioinert material.

For each group, three time intervals were tested: 2 wk (W2), 4 wk (W4), and 8 wk (W8). Six femora were studied at the 2 wk delay, five femora at the 4 wk delay, and seven femora at the 8 wk delay.

2.3.2. Surgical procedure

2.3.2.1. Bone defect creation. A bone defect was created in the distal femoral extremity as recommended by Katthagen [9] and modified by us previously [10]. Both femurs were done at the same surgical time. After a surgical approach made via the lateral side, a bone square was made and removed. A 6 mm diameter bone defect was then hollowed out in the transverse intercondylar axis of the femur. This defect was created in cancellous bone without any communication with the intra-articular space or the intramedullary canal. After washing and cleaning, the bone defect was closed with the cortical bone square which was replaced on the lateral side without osteosynthesis.

2.3.2.2. Percutaneous injection (groups “Ethibloc”, “PMMA” and “Alcohol”).* Into this closed defect, the percutaneous injection was carried out from the medial side through an internal needle, as described previously [10]. Filling of the defect was done progressively with slow rotation of the needle.

After surgery, the animals were allowed to move freely in their cages without joint immobilization. The animals were sacrificed by an intravenous overdose of sodium pentobarbital (Nesdonal*).

2.3.3. Microscopic study

2.3.3.1. Technical preparation. After sacrifice, the distal femurs were harvested, cleaned of soft tissue, resected and fixed. The bone segment was dehydrated in ethanol baths and embedded in polymethacrylate. The undecalcified bone technique was used to obtain stained sections for a qualitative analysis and microradiographs for a quantitative histomorphometric evaluation.

(a) *Stained sections*: 7 μm thick sagittal sections were prepared with a Jung microtone (Jouan, France) and stained with May-Grünwald’s reagent.

(b) *Microradiographs*: 1000 μm thick sagittal sections were cut (with cooling) with a Leitz 1600 (Microm, France). Two sections were made in three sectors: external, middle, and internal.

Microradiographs of the sections were made on high-resolution Kodak SO343 film (Kodak corporation).

2.3.3.2. Analytical procedure.

(a) Qualitative analysis: local inflammatory reaction, vascular proliferation and new bone in the defect were assessed.

(b) Quantitative histomorphometric evaluation: histomorphometric parameters were measured by an image analysing computer (Vidas 3D, Kontron, France). The magnification used was × 6.3, and the scale was millimetric. Some sections were excluded: sections with articular or intra-medullary communications, first sections of external and internal sectors.

Two parameters were measured: the defect area (mm^2) and the bone area in the defect (mm^2). Bone formation in the defect (BF, %) was obtained from the ratio "bone area in defect/defect area".

2.3.4. Statistical analysis

Comparison of bone formation rate was made with a non-parametric Kruskall–Wallis test, where the tested factors were "application" and "time interval". A minimum of $p < 0.05$ was required for significance.

3. Results (Fig. 1)

3.1. Control groups

3.1.1. "Unfilled group"

A post-traumatic bony reaction was seen with an early bone proliferation and a secondary bone remodelling. No inflammatory reaction was seen; a vascular proliferation was observed on the periphery. The natural evolution of empty defects was first, an early bone invasion of the defect, followed by an organization of this peripherical bone invasion with a local reinforcement of peripheral bone texture. The bone invasion was larger at the periphery of the defect than in its central part. The quantitative analysis evaluated the spontaneous new bone formation which was decreasing with time even if the comparison of bone formation with the time intervals was never significant. At 2 wk, BF was evaluated at 15.1% (± 8.2%), going to 12.4% (± 1.9%) at 4 wk and 9.9% (± 3%) at 8 wk.

3.1.2. "PMMA group"

The bone defect was never completely filled by injected PMMA. Even if the injection was done slowly with a rotating needle at a low injection pressure, the PMMA mass appeared irregular with folds. In these spaces without PMMA, new bone was often found. A low fibrous reaction with connective tissue was seen on the interface "bone–PMMA". This small peripheral fibrotic reaction did not stop the invasion of PMMA gaps by new bone.

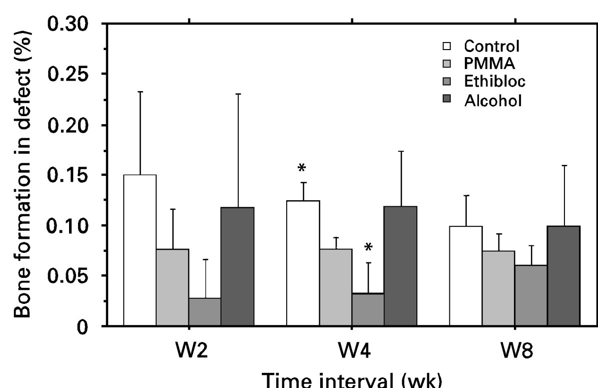

Figure 1 Variations in the delay of bone formation in the different groups. Bone formation is expressed in a per cent obtained by the ratio "bone area in defect/defect area". Bars represent the mean of each group with its standard deviation. At "4 wk," delay, "Ethibloc group" and "unfilled group" differed significantly: $p < 0.05$.

In the quantitative evaluation, BF in this group was constant with time. 7.6% (±4%) at 2 wk, 7.5% (±1.2%) at 4 wk, 7.3% (±1.8%) at 8 wk.

3.1.3. "Alcohol"

An inflammatory reaction was seen in the cancellous bone surrounding the defect at 2 and 4 wk. At 8 wk, the bone growth in the defect was very similar to that in the "unfilled group". This new bone formation was greater at the periphery of the bone defect.

BF was similar at 2 wk and 4 wk, but was smaller at 8 wk even though no significant difference was found between interval groups. BF was close to the "unfilled group": 11.8% (±11.3%) at 2 wk, 11.9% (±5.5%) at 4 wk, and 9.8% (±6.1%) at 8 wk.

3.2. "Ethibloc group"

3.2.1. Qualitative analysis (Figs 2 and 3)

Zein appeared, at every time interval, as a compact mass in the defect with waves at low magnification (Fig. 2). No bone cell was seen in this mass, no vascular proliferation was seen in the defect nor in the bordering bone.

An important fibrous and connective reaction was seen with a poor vascular proliferation and a light thrombosis of vessels. An important fibrotic reaction was seen at the early time intervals (2 and 4 wk). The defect was never completely filled by Ethibloc*, but the space between bone and Ethibloc* was invaded early by fibrosis (Fig. 3). This fibrosis was neither seen in the "unfilled group" nor in the "PMMA group". This phenomenon was the same in the "Ethibloc group" and the "alcohol group".

3.2.2. Quantitative analysis

BF was very low. After a poor early bone reaction with 2.7% (±1.8%) at 2 wk and 3.1% (±3.1%) at 4 wk, the bone formation increased a little with time: 5.9% (±2.5%) at 8 wk. No significant statistical difference between time intervals was noted.

In this group, BF never reached the values obtained in control groups. Furthermore, BF was significantly lower at 4 wk than with the "unfilled group".

4. Discussion

This study found no bone formation which was induced by Ethibloc*. A very low bone formation was seen, less than in the control groups: the "Ethibloc group" was characterized by an early inflammatory reaction.

The model used in this study was created to analyse the bone reaction after the injection of liquid materials in a spongy bone defect. It has been previously shown that this model was reproducible, with no spontaneous bone repair of the defect. In the empty control cavities, the bone formation was limited to 10% at 8 wk [11]. In the present study, bone formation was evaluated as 15.1% at 2 wk, but there is no significant difference between 2 and 8 wk results.

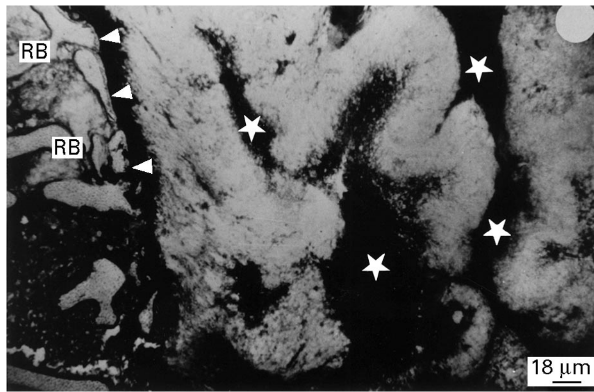

Figure 2 Microradiographed part of a section of an Ethibloc* group (with an 8 wk delay). The bone cavity (limits marked with white arrow heads) is invaded with a fibrous tissue (white stars) between Ethibloc* waves without new bone. r.b. = rabbit bone.

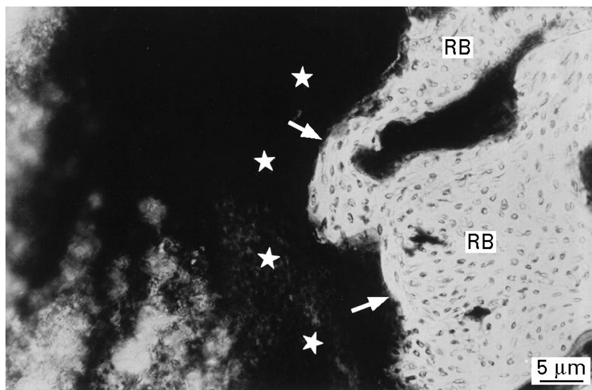

Figure 3 Microradiographed part of a section of an Ethibloc* group at a higher magnification. The space between bone and Ethibloc* is large. No contact is seen between bone and this biomaterial, and there is no aspect of local bone ingrowth. r.b. = rabbit bone.

These experimental results with Ethibloc* are not in agreement with the good clinical results described in the literature [1, 4, 6]. Good results were obtained especially in the treatment of aneurysmal bone cysts in children. In these cases, CT scans made after Ethibloc* injection showed a decreasing vascular reaction, then an osteocondensation. In fact, this evolution is not opposed to our experimental results: we observed an early sclerosis and vessel thrombosis. This state could correspond to the last stages of evolution of these vascular lesions after which a normal bone remodelling can be restored. Our results showed this kind of evolution with a poor early BF coupled with the inflammatory reaction during the early time intervals, and then followed by a decreased inflammatory reaction with the beginning of BF. So, the therapeutic bone effect of Ethibloc* appears to be non-specific and a consequence of the fibrous effect. In our study, secondary BF remains small, this can be explained by several factors. Our experimentation was done on adult rabbits, whereas most clinical results emanate from child pathology. Our time intervals were short to quantify bone remodelling or bone cicatrization phenomenon compared to the observations made after filling of an aneurysmal bone cyst. No hypervascular phenomenon was studied in our experimental model, as was the case in bone cysts.

Fibrotic reaction was equivalent in the "Ethibloc group" and the "Alcohol group". The alcoholic component of Ethibloc* could thus explain the main effect of Ethibloc*. In fact, alcohol is used successfully in the treatment of bone cysts and vertebral haemangioma [11].

However, in this study, BF was not the same with alcohol alone as with Ethibloc. In Ethibloc, the other major component, zein (which filled the defect) with its proteinic mass, modifies the inflammatory reaction. In fact, these two properties, sclerosing effect and mass-filling effect, characterized the different groups studied in this work: a sclerosing effect for Ethibloc and alcohol; a mass filling effect for Ethibloc and PMMA. These properties could explain the BF and the inflammatory reaction observed in each group.

5. Conclusion

The results of our study strongly argue against specific osteogenic properties of Ethibloc*. As in soft tissues, vessels and arterio–venous malformations, Ethibloc* is a powerful sclerosing and fibrosing material in bone. The clinical results could be explained by the following process. There is, at first, an early sclerosing vascular effect with an inflammatory reaction, then the vascular phenomena decrease and allow a normal bone turn-over. These results are in agreement with the slow improvement reported by Adamsbaum *et al.* [1].

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Received 26 February
and accepted 25 September 1997