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THE EFFECT OF CALCIUM PECTINATE GEL IMPLANTS ON THE HEALING OF EXPERIMENTAL DEFECTS IN THE FEMORA OF ALBINO RATS

by

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The history of the medicinal use of pectin goes back for several centuries. A printed recommendation for its use in the form of scraped apple appeared in an English book in 1775 as a remedy for diarrhoea and dysentry [Kertesz, 1951]. This is still its most important medicinal application and, combined with kaolin, it forms the basis of most antidiarrhoeal preparations. In recent times its range of medicinal uses has increased considerably. Parenterally administered solutions of pectin were shown to have haemostatic properties and to increase the rate of coagulation of blood [Violle and de Saint Rat, 1925]. Pectic substances also have an affinity for heavy metal ions, and are good antidotes for heavy metal poisoning [Stuewer and Olsen, 1940]. The action of many drugs is prolonged by injecting them in combination with a pectin solution which has the effect of retarding their rate of absorption [Kertesz, 1951].

Solutions of pectin have been effectively used as surgical dressings for the treatment of chronically infected soft tissue wounds, decubitus ulcers and osteomyelitic lesions [Tompkins *et al.*, 1941]. The beneficial effect of pectin in wound healing is attributed to its bactericidal properties [Haynes *et al.*, 1937, 1938]. During World War II solutions of pectin were successfully used as plasma replacement solutions in the treatment of shock in humans [Hartman *et al.*, 1941; Middleton and Wiggers, 1943 and Popper *et al.*, 1945]. The intravenous administration of pectin solutions to experimental dogs and rabbits was however shown to result in the accumulation of globular masses of pectin in the reticulo-endothelial cells of the liver, spleen, lung and bone marrow and the development of atheromatous lesions in various arteries [Heuper, 1942]. Autoclaving of pectin solutions was shown to result in partial depolymerisation of pectin macromolecules to a molecular weight approximately equal to that of plasma protein. This appeared to eliminate pectin deposition in reticulo-endothelial cells [Bryant *et al.*, 1942]. It was later shown that large volumes of intravenously administered autoclaved pectin solutions again resulted in pectin storage phenomena occurring in the phagocytic cells of the liver, spleen, kidney and lung in humans [Popper *et al.*, 1945]. In studies of the excretion of intravenously administered pectin Kozoll *et al.* [1946] showed that about half the pectin administered was rapidly excreted in the urine and the remainder could not be accounted for. They stated that until the fate of parenterally administered pectin was known, it could not be considered to be an innocuous substance.

In 1968 Retief *et al.*, described the use of a pectin gel implant (calcium pectinate) in which a soluble antibiotic had been incorporated for the treatment of chronic osteomyelitis. Barbakow [1972] successfully used a similar preparation for the endodontic treatment of chronic periapical lesions which did not respond to conventional therapy. The rationale of their therapy was based on the property of the gelled pectin to slowly revert to the sol phase in tissue fluid when implanted in tissues. They felt that this resulted in a slow release of the incorporated antibiotic and achieved a

prolonged local high concentration around the gel implant. The antibiotic, together with the natural bactericidal effects of pectin in solution, appeared to be an effective means of treating chronic infections. It was further postulated that the high molecular weight pectin molecules were hydrolysed in tissue fluids and that the smaller molecular weight products resulting from this produced a local increase in the osmotic pressure of fluid surrounding the implant. This local osmotic effect was believed to enhance transudation from the osseous vasculature, with reduction in oedema and increase fluid exchange and drainage from infected bone lesions.

The purpose of this investigation was (a) to study the effects of a calcium pectinate gel implant, with and without an antibiotic, on the healing of gel filled experimental bone defects, (b) to determine the effects on the surrounding soft tissues and (c) to assess the rate of resorption of the implant material during healing.

MATERIALS AND METHODS

Preparation of calcium pectinate gels. A commercially available low ester citrus pectin (W. J. Bush S.A. (Pty.) Ltd.) was used for the preparation of the calcium pectinate gels [Dreyer, 1972]. The powdered pectin was sterilized by tyndallization. Under aspetic conditions a 6% w/v pectin solution was prepared by slowly adding the powder to sterile, deionized water. As pectin is not readily soluble a mechanical stirrer was used to facilitate the preparation of the solution. The pectin solution was divided into two equal portions and to one of these 1.5 g of lincomycin hydrochloride monohydrate was added per 100 ml of pectin solution. To gel the pectin solution 0.5 ml aliquots of each of the two pectin solutions were then pipetted into separate saturated solutions of calcium chloride.

A film of calcium pectinate immediately formed on the surface of the pectin solution placed in the calcium chloride solution. Within 5 minutes the pectin solution was completely gelled and had formed into flattened discs of approximately 1.5 cm in diameter on the floor of the container holding the calcium chloride solution. The gel discs were then washed several times in deionized water to remove the superficial unreacted calcium chloride held in the pectin gel. The discs were drained and stored at 4°C in sterile petri dishes.

Pectin is a polysaccharide consisting of D-galacturonic acid molecules joined by α-1,4-glycosidic linkages. The physical change from sol to gel is produced by ionic cross-linkages of the carboxyl groups with calcium ions.

Experimental procedure. Thirty-six, two month old female Wistar strain albino rats weighing between 130 and 140 g were divided into two equal groups. The two groups were used to assess the effects of the calcium pectinate gel implants with and without antibiotic on the healing of experimental femoral defects. The rats were anaesthetized with a neurolept-analgesic preparation containing 0.05 mg/ml fentanyl and 2.5 mg/ml droperidol at a dosage of 2.5 ml/Kg intramuscularly. Using aseptic procedure the lateral femoral diaphyses of each rat were exposed by incising the skin overlying the femur and separating the vastus lateralis and biceps femoris muscles. Standard cortical defects 1.75 mm in diameter were made through the lateral mid-diaphyseal femoral cortices, using a slow-running saline-cooled No. 5 round dental bur. The left femoral defect was first prepared to serve as a control. After preparation the residual saline and bone debris was swabbed from the wound, the muscles re-apposed over the femur and the skin sutured. The right femoral defect was similarly prepared. Care was taken to arrest medullary hemorrhage from the defect by packing it with a pledget of cotton wool. This was necessary to prevent the gel implant being displaced by bleeding. The defect was then carefully filled by extruding the gel

implant material through a 22 gauge hypodermic needle attached to a 2 ml syringe. The right femoral defects of one group of eighteen rats were filled with calcium pectinate gel and the defects in the other groups filled with the gel containing lincomycin.

Retrieval of specimens. Three rats from each of the groups were killed at intervals of one, two, three and four weeks and three and six months after operation.

Tissue preparation. The femora were carefully dissected from each rat without disturbing the soft tissues overlying the control and implanted defects, and the femoral cortices sectioned on either side of the area of the defect with a dental separating disc to expose the medullary tissue. The specimens were then fixed in phosphate buffered formol saline, decalcified in 5% formic acid, processed routinely and embedded in paraplast. Serial sections, 7 μm thick, were cut transversely through the defects in the femora and stained with haematoxylin and eosin. Selected sections from the gel implanted defects were stained with toluidine blue to confirm the presence of pectic material. Pectic substances, being acidic polysaccharides, exhibit metachromasia when stained with toluidine blue.

RESULTS

The operative wounds in all the rats healed by primary intention and there were no deaths amongst the experimental groups during the experiment.

a) Healing pattern of the control femoral defects.

The healing pattern of the control defects in the two groups of rats confirmed the pattern previously described [Melcher and Irving, 1962; Pallusch, 1968; Wantenaar, 1970]. In this study the pattern was consistent in all the animals at the comparable time intervals.

One week. Endosteal callus arising from the cut walls of the defect had completely filled and bridged the defect. A mild periosteal response was evident around the margins of the defect with the deposition of a small amount of sub-periosteal bone (Fig. 1).

Two weeks. Marked proliferation of periosteal callus over the primary endosteal callus had resulted in complete periosteal bridging of the defect. Remodelling characterised by resorption of the medullary aspect of the endosteal callus was in progress (Fig. 2).

Three weeks. Further remodelling of the callus resulted in compact bone filling the outer half of the defect. Endosteal bone in the inner aspect of the defect had been further resorbed leaving only a few bone spicules. The intertrabecular spaces resulting from resorption were occupied by haemopoietic tissue.

Four weeks. The compact cortical bone bridging the defect was further reduced in thickness by resorption of the remainder of the endosteal bone in the central area of the defect which was bridged by the remaining periosteal bone. With the exception of small amounts of endosteal bone remaining on the walls of the defect most of the primary endosteal bone had been resorbed by this stage (Fig. 4).

Three months. The defects were almost completely filled by the inward deposition of lamellar bone on the remaining periosteal bone bridging the defect. Islands of haemopoietic tissue were still present in the new bone deposited in the inner aspect of the defect.

Six months. The cortical defects had been filled with lamellar bone and the cortical thickness was completely restored. The defect was still easily indentifiable by the irregular endosteal profile evident between the margins of the defect.

b) Healing pattern in the gel implanted femoral defects.

In this investigation it appeared that a similar healing pattern followed the implantation of the two types of gel irrespective of the inclusion of the antibiotic lincomycin. The study thus in fact became related only to the effect of the gel implant on bone healing. The results of the two groups of rats were combined and represented the general response obtained in six implanted femora at each time interval. The healing patterns in the implanted defects were not as consistent as in the controls. Variations in healing pattern were related to the amounts of pectic material retained in the defect and the position which it occupied during healing.

One week. The defects were almost completely filled with endosteal callus arising from the cut walls of the defects. A central area between the two masses of callus was occupied by pectic material surrounded by granulation tissue. This prevented complete endosteal bridging of the defect (Fig. 7).

Two weeks. Remodelling of the callus was in progress with resorption of the medullary aspect of the endosteal callus. Periosteal bone was proliferating over the endosteal callus present but could not bridge the central area which was occupied by small masses of pectic material (Fig. 8).

Three weeks. Resorption of the inner aspect of the medullary callus was advanced. The resorbed bone was replaced with active haemopoietic tissue. Periosteal bridging was blocked by the presence of pectic material in the middle of the defect (Fig. 9).

Four weeks. Most of the primary endosteal callus lying in the central area was resorbed by this stage. The defect was incompletely bridged by the remaining compact periosteal bone on either side of a central mass of pectic material (Fig. 10).

Three months. Bridging of the defect had occurred with lamellar bone either internally or externally around pectic material still present. The pectic material was enclosed in new bone or lay in small masses surrounded by haemopoietic tissue in the medulla, or surrounded by loose connective tissue in the outer aspect of the defect (Fig. 11).

Six months. By this stage all defects were bridged by compact lamellar bone which contained inclusions of pectic material resulting in an irregular pattern in bone structure in the defect area (Fig. 12).

Remarkably little tissue reaction to the gel implant material was noted during this study. The main reaction observed was fibroblastic proliferation around the pectic material lying in the outer aspect of the defect. This was evident from two weeks onwards.

DISCUSSION

The persistence of pectic material in healing bone defects for up to six months suggests that it is not a readily biodegradable and resorbable implant material. Although the amount of pectic material remaining in the defects was very much less than the original amount implanted it is suspected that this decrease may have been largely due to physical displacement of the gel. The defect was not covered after implantation of the gel and was in contact with the movement of the overlying muscles. Extrusion of the gel through the hypodermic needle used for filling the defect also caused a physical disruption of the gel structure. The defect was thus filled with a mass of small fragments of gel rather than a homogenous gel implant.

Enzymes which hydrolyse the α-1,4-glycosidic linkages in pectates and other polygalacturonates have only been described as occurring in plant cells, bacteria, yeasts and in certain invertebrates [Dixon and Webb, 1966]. Pectic substances can be degraded by non-enzymatic means such as by the action of strong acids, dilute alkalis, ion exchangers, heavy metal ions, oxidizing and reducing agents, irradiation

and mechanical treatment such as grinding [Deuel and Stutz, 1958]. The non-enzymatic degradation of pectin or pectic substances *in vivo* is also a possibility but the known conditions under which such degradations occur are too severe to take place *in vivo*.

Since the fate of parentally administered pectic substances is unknown and orally administered pectin passes practically unaltered through the human gastro-intestinal tract, the assumption that a local hydrolytic degradation of the pectin gel can occur in tissues cannot at this stage be supported.

The induced local transudation which has been observed to follow the implantation of pectin gels prepared by the method used in this study is suggested as being mainly due to the osmotic effect of unreacted calcium chloride in solution which is taken up by the gel during its preparation and not to the hydrolysis of pectin. A saturated solution of calcium chloride contains 130 parts of calcium chloride in 100 parts of water at 20°C [Lange, 1946]. In view of this consideration it was not surprising that transitory pain was experienced by patients in whom a pectin-lincomycin gel similarly prepared was sealed in the root canals of teeth [Barbakow, 1972]. The osmotic effect of the hypertonic calcium chloride solution held in the gel would produce a local oedema in the periapical area. The increase in pressure in this confined space would be expected to produce pain.

The persistence of the calcium pectinate gel implants in healing experimental bone defects for up to six months and the physical barrier they present to bone healing, suggests that this material should not be implanted into sites in which bone regeneration is expected to occur.

The similar results in the healing pattern obtained with pectin gel with and without antibiotic would suggest that the lincomycin does not either retard or promote bone healing under these experimental conditions. In clinical conditions involving chronic infective bone lesions, the local administration of lincomycin may be achieved by this means.

SUMMARY

The effects of calcium pectinate gel implants with and without antibiotic on the healing of experimental defects in the femora of thirty six albino rats have been studied. There were no differences between the healing patterns of the experimental defects filled with the two types of calcium pectinate implant, suggesting that local application of lincomycin to bone by this means did not promote or retard bone healing. The implants were well tolerated by the host animals and produced little local tissue reaction. Calcium pectinate gels did not appear to be easily degraded and resorbed when implanted into experimental bone defects, and healing of the implanted defects was impeded by remnants of the implant material which persisted in the defects for up to six months.

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PLATE 1

Fig. 1. Control defect in rat femur 1 week after operation. The defect is filled with endosteal callus over which periosteal callus is beginning to proliferate. Arrows indicate the margin of the defect. (× 120).
Fig. 2. Control defect 2 weeks after operation. The outer aspect of callus is maturing into compact bone while resorption of the inner endosteal callus has begun. (× 120).
Fig. 3. Control defect 3 weeks after operation. The outer aspect of defect is bridged by compact bone and advanced resorption of the inner medullary callus is evident. (× 120).

PLATE 2

Fig. 4. Control defect 4 weeks after operation. Defect bridged by periosteal bone with resorption of all the endosteal callus in the central area of the defect. (× 120).
Fig. 5. Control defect 3 months after operation. The inner aspect of the defect has been restored by deposition of lamellar bone. Islands of haemopoietic tissue still persist. (× 120).
Fig. 6. Control defect 6 months after operation. Defect completely filled with compact bone. (× 120).

PLATE 3

Fig. 7. Implanted defect 1 week after operation. Pectic material (P) lies between endosteal callus arising from the cut walls of the defect. (× 120).
Fig. 8. Implanted defect 2 weeks after operation. Bridging of the maturing callus is prevented by the presence of pectic material (P). (x 120).
Fig. 9. Implanted defect 3 weeks after operation. Resorption of the medullary aspect of the callus is evident. Pectic material (P) has prevented complete periosteal bridging of the endosteal callus. (× 120).

PLATE 4

Fig. 10. Implanted defects 4 weeks after operation. Defect is incompletely bridged by periosteal bone. Gap in the defect is occupied by pectic material (P). (× 120).
Fig. 11. Implanted defect 3 months after operation. Presence of pectic material has prevented regeneration of the bone in the central area of the defect although the gap has been bridged internally. Islands of pectic material (P) are at present surrounded by bone. (× 120).
Fig. 12. Implanted defect 6 months after operation. Regeneration of the bone is incomplete. Islands of pectic (P) are still present in the new bone. Separation of the new bone from the margins of the defect are artefacts. (× 120).