Heparin-like activity in uterine fluid

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Summary and conclusions

Uterine fluid was collected from a group of normal patients and a group of patients with menorrhagia. Heparin-like activity was detected in 34 out of 38 samples using an anti-Xa heparin assay. The heparin-like activity in uterine fluid was inhibited by adding the heparin antagonist hexadimethrine bromide to the assay. Concentrations of fibrinogen-fibrin degradation products (FDPs) were measured in five samples of uterine fluid. FDPs in the concentration detected had no effect on the anti-Xa assay. Heparin-like activity was higher in the group with menorrhagia, although the differences were not significant. Heparin-like activity increased throughout the menstrual cycle and decreased during menstruation, suggesting a possible cyclical variation in activity. There was no correlation between mast cell numbers in the endometrium and myometrium and heparin-like activity in uterine fluid and no correlation between the numbers and the stage in the menstrual cycle. In a few patients with intrauterine contraceptive devices (IUCDs) heparin-like activity was increased.

Introduction

The factors concerned in regulating menstrual blood loss are poorly understood. Mast cells are present in the human endometrium and more of them are found in most patients during menstruation.¹ Mast cells contain and release heparin.² Protamine sulphate and toluidine blue, which are both heparin antagonists, reduce menstrual blood loss.^{3,4} The purpose of this study was to determine whether heparin is present in uterine fluid.

Patients and methods

Thirty-eight samples of uterine fluid were collected from uteri removed at hysterectomy or from anaesthetised patients undergoing minor gynaecological procedures. Twenty-four of the patients com-

plained of menorrhagia and 14 patients had normal menstrual loss. Two patients in the normal group and four with menorrhagia had intrauterine contraceptive devices (IUCDs) in situ. In addition, a pool of uterine fluid was collected from 50 patients.

Uterine fluid was obtained through a fine polyethylene catheter (internal diameter 0.58 mm and external diameter 0.96 mm, Jencons, ref 800/100/200) inserted through the cervix until the fundus of the uterus was reached. The fluid was aspirated by means of a syringe attached to the tubing by a needle. The uterine fluid was then injected from the tubing into heparin-free microhaematocrit tubes (Hawksley and Sons), centrifuged to remove mucus and debris, and stored at -70°C in Eppendorf tubes. Plasma from peripheral blood collected in 3.8% sodium citrate was assayed for comparison in 10 cases.

Heparin-like activity in plasma and uterine fluid was assayed as described by Denson and Bonnar.⁵ In principle the method consists of incubating activated factor X with heated plasma and subsampling the mixture into factor-X-deficient plasma. In the presence of heparin the anti-Xa in the plasma is potentiated and more factor Xa is inhibited, giving rise to a prolongation of the clotting time in the substrate. The uterine fluid samples and a pooled plasma sample were heated for 15 minutes at 56°C to inactivate clotting factors and centrifuged at 2500 g for 10 minutes to remove precipitated fibrinogen. A 10- μl sample of uterine fluid supernatant was added to 90 μl of plasma, and the mixture was assayed for heparin-like activity; 10 μl of citrate-albumin-glycoxaline buffer in 90 μl of plasma served as a control. A standard heparin curve was prepared by adding heparin in buffer to plasma to give a final concentration of heparin in the range 0.02 U/ml to 0.2 U/ml.

Hexadimethrine bromide (Polybrene) (1 mg/ml), a heparin antagonist, was mixed in a one-to-one ratio with 12 individual samples of uterine fluid, and a sample taken from the pool of uterine fluid. Buffer in a one-to-one ratio with uterine fluid served as a control. 10 μl of the mixture was assayed as described above.

Hexadimethrine bromide and buffer each in a one-to-one ratio were added to pooled plasma samples containing known quantities of

FIG 1—Heparin-like activity in uterine fluid in normal patients and patients with menorrhagia; mean values are also shown. $\blacktriangle$ = Patients with IUCDs. Open symbols represent samples taken during menstruation.

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heparin in the range of 0 to 1 U/ml. 10 μ l of the mixtures was added to 90 μ l of pooled plasma and heparin was assayed as described above.

Concentrations of fibrinogen-fibrin degradation products (FDPs) were assayed as fibrinogen-fibrin related antigen (FR antigen) (Thrombo Wellco test) in five samples of uterine fluid, which included two samples collected during menstruation.

To determine the effect of FDPs on the heparin assay, FDPs were prepared by incubating 9.5 ml of pooled plasma and 10 U of lyso-fibrin (Porcine plasmin, Novo, Copenhagen) overnight at room temperature. Plasmin was inhibited with 0.2 molar aminocaproic acid and FDPs in plasma were measured before and after heating, giving ranges of 2500 to 3000 μ g/l and 120-160 mg/l respectively. 10- μ l samples of FDP-rich plasma were added to pooled normal plasma, and heparin-like activity was assayed as described above.

Mast cell counts were performed on sections taken from 21 uteri removed at hysterectomy. The indication for hysterectomy was menorrhagia in 16 patients and prolapse in five. Eleven patients had IUCDs in situ at the time of hysterectomy. Mast cells were counted in the myometrium of all the specimens and in the endometrium of 11, where the heparin-like activity of uterine fluid had been determined.

Two large blocks, including endometrium and myometrium, were taken from each uterus immediately after hysterectomy and fixed in formalin. For mast cell counting, 5- μ m paraffin sections were stained with 0.1% toluidine blue in 2% aluminium sulphate for 15 minutes or stained by the pinacyanol method.⁶

Mast cell counts were performed over microscope fields $\times 320$. In the myometrium the area chosen was two rows of contiguous fields running from the endometrium to the serosal surface. At least 50 fields were counted at the same magnification. The mean number ($\pm$ SD) of mast cells for the total number of fields counted was determined for the myometrium and endometrium.

Results

The volumes of uterine fluid were in the range of 30 μ l to 50 μ l; the larger volumes were obtained during menstruation. Heparin-like activity was detected in 34 of the 38 samples examined (mean ($\pm$ SD) 0.26 ± 0.18 , range 0 to 0.76 U/ml). Mean heparin-like activity in uterine fluid in the group with menorrhagia (0.285 ± 0.194 U/ml) was higher than in the normal group (0.215 ± 0.151), but the differences were not significant (fig 1). Mean heparin-like activity for the two groups counted was highest late in the menstrual cycle and lowest during menstruation. None of the differences were statistically significant (fig 2).

The highest individual concentrations of heparin-like activity were detected in patients who were menstruating and in patients with IUCDs, although no heparin-like activity was detected at all in three menstruating patients (fig 1). The number of patients with IUCDs was too small for statistical comparison with non-IUCD patients.

The addition of hexadimethrine bromide to plasma containing known quantities of heparin resulted in a complete reversal of the heparin effect on clotting times in the anti-Xa assay (fig 3). Hexa-

FIG 2—Mean heparin-like activity ($\pm$ SD) in uterine fluid throughout the menstrual cycle. Numbers of patients in each group are shown in parentheses.

FIG 3—Anti-Xa assay for heparin-like activity: clotting time in seconds plotted against heparin concentration (units/ml). $\bullet$ = Plasma samples containing equal volumes of heparin and buffer. $\times$ = Plasma containing heparin and hexadimethrine bromide. $\circ$ = Plasma containing uterine fluid (from pool of 50 patients) and buffer. $\blacktriangle$ = Uterine fluid and hexadimethrine bromide.

FIG 4—Anti-Xa assay for heparin-like activity in uterine fluid: clotting times in seconds and corresponding heparin concentration derived from plasma containing 0, 0.5, and 1.0 units of heparin/ml (broken lines). Plasma containing uterine fluid and buffer (Buffer). Plasma containing uterine fluid and hexadimethrine bromide (Hexadimethrine bromide).

dimethrine bromide, added to 12 individual samples of uterine fluid with a range of heparin-like activity of 0.02 to 0.28 U/ml completely inhibited this activity (fig 4). In plasma artificially depleted of all immunologically and functionally detectable antithrombin III (AT III), uterine fluid, like heparin, had no inhibitory effects in a factor Xa inhibition assay.

FDP concentrations in uterine fluid from five patients were in the range 20 to 1000 mg/l. FDPs at this concentration and higher (120 to 3000 mg/l) in prepared samples had no effect on the heparin assay.

Mast cells were detected in all the specimens examined; the range of mean values ($\pm$ SD) of about 50 fields counted was: 3.73 ± 2.34 to 24.02 ± 14.46 mast cells per field. There was no obvious correlation between the myometrial counts and the phase of the cycle, the presence or absence of an IUCD, or the heparin-like activity in uterine fluid. The mean count in the IUCD group (14.18 ± 10.33) was similar to that of the control group (12.57 ± 9.49).

Mast cells were detected in the endometrium of six out of 11

patients examined. The range 0.08 ± 0.24 to 1.34 ± 1.58 cells per field was considerably less than the range in the myometrium, and there was no correlation with the clinical data and heparin-like activity in uterine fluid.

Discussion

Heparin-like activity was detected in 34 of the samples of uterine fluid studied. The activity was within the range of heparin concentration in whole blood observed in patients receiving subcutaneous heparin.⁵ The inhibition of this activity by hexadimethrine bromide suggests that presence of free heparin in uterine fluid, although this needs final confirmation by biochemical methods. The finding of ATIII dependence for the anticoagulant activity of uterine fluid gives strong support for its identity with heparin, since only heparin and to a lesser extent heparan sulphate are known to depend on ATIII for augmentation of their anticoagulant effects.⁷

The mean heparin-like activity of uterine fluid increased throughout the menstrual cycle and decreased during menstruation, which suggested a possible cyclical activity. Mast cells are present in the connective tissue of most organs and contain heparin.² In the endometrium in humans and animals they undergo cyclical changes.¹⁻⁹ Increased numbers of mast cells and mast cell granules are found premenstrually, and degranulation occurs at the onset of menstruation. Vara⁸ postulated that mast cells may have a role in maintaining menstrual blood in a fluid state.

We found no correlation between the numbers of mast cells in the endometrium or myometrium and heparin-like activity in uterine fluid. A correlation between extracted concentrations of heparin and tissue mast cell numbers has been shown.¹⁰ If mast cells become degranulated, they may be unable to take up the routine stains that act primarily on their granules, so that a correlation between uterine fluid, heparin-like activity, and tissue mast cells may be more difficult to show than the correlation between mast cells and extracted tissue heparin.

The insertion of IUCDs in rats increases mast cell numbers,¹¹ and plastic spirals increase the content of acid mucopolysaccharides, including heparin in bovine endometrium.¹² In the few patients studied here there were no significant differences in mast cell numbers in the myometrium and endometrium of IUCD wearers and non-IUCD wearers. Nevertheless, heparin-like activity was higher in the IUCD wearers, which suggested that IUCDs might increase menstrual blood loss by increased heparin-like activity in uterine fluid.

The highest and lowest individual values of heparin-like activity in uterine fluid were found in patients who were menstruating, and heparin may be released during the initial processes of endometrial shedding and then diminish as

menstruation progresses. This large variability might also be explained by the presence of heparin-neutralising substances in menstrual blood. In menstruating patients whose platelets had been labelled with radiochromium we have failed to find preferential excretion of platelets in menstrual blood (unpublished work). If platelet factor 4 is released in parallel with β -thromboglobulin it might account for the variability in heparin-like activity during menstruation. Raised FDP concentrations had no effect on this activity, nor did they influence the inhibition of activated factor X by heparin.

The mean heparin-like activity in uterine fluid in patients with menorrhagia was higher than in normal patients, and increased heparin-like activity might be a factor in the pathogenesis of menorrhagia. A heparin-like anticoagulant effect in the circulation has been found in experimental anaphylactic shock in the dog,¹³ and in a single patient with plasma cell leukaemia,¹⁴ and Jacques *et al*¹⁰ suggest that mast cells may have a wider biological role than is generally accepted. We believe that our finding of heparin-like activity in uterine fluid gives support to this view.

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ONE HUNDRED YEARS AGO Dr Stone, in his letter upon prison dietaries, has referred to the loss of mineral salts in boiling fresh potatoes, and to the consequent superiority of preserved potato (which is not allowed to touch water) as an antiscorbutic. In experiments made by Dr Noad (described in Dr Lankester's *Lectures on Food*), it was proved that, after boiling one pound of potatoes the water contained seventeen grains of carbonate of potash; and the water in which cabbages were boiled contained twenty-one grains of sulphate of potash. The proportions of the other mineral salts and acids in the water are not stated; but, all being soluble, their retention in the cooked vegetable would depend upon the form of the vegetable substance. The tubers, from their form, lose less than the leafy vegetables like cabbages, etc; and we have here the main reason why potatoes are found so superior an antiscorbutic to other vegetables, and also why uncooked vegetables are so superior to cooked ones in preventing scurvy. Dr Ure, thirty years ago, when Mr Edwards first introduced preserved potato, pointed out (after analysing it) that it contained all the antiscorbutic properties of fresh raw potatoes, which

the late analysis of Professor Attfeld entirely confirms. Dr. Ure also recommended the use of Edwards's potato at home in the after-part of the season, when fresh potatoes become unsound from frost, growth, etc.

The reason why scurvy appeared in the late Arctic Expedition was that the ration of preserved potato was ridiculously small in proportion to the meat-ration. All the medical evidence agreed on this point. The comparison with prison diet is given in the appendix No. 25 to the Arctic Committee Report; viz: Convicts at home get ninety-six ounces of potatoes (or its equivalent of preserved during the season when potatoes are bad) to thirty-one ounces of meat. The seamen on board in the Arctic Expedition had only thirty ounces of potatoes, including all other vegetables, to one hundred and twelve ounces of meat; and on sledging parties, only fourteen ounces of potatoes to one hundred and forty ounces of meat. Any tyro in the chemistry of food might have predicted scurvy from such a diet. (*British Medical Journal*, 1878.)