IN VITRO ANTIHEPARIN ACTION OF BERBERINE ON THE DOG AND HUMAN BLOOD

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Summary: 1—3 *mg* berberine has been shown to neutralize, *in vitro*, anticoagulant action of 50 i u heparin *ml* dog and human blood. Larger dose (10 *mg/ml*) has a paradoxical anticoagulant action. These effects resemble those produced by protamine sulphate and toluidine blue.

INTRODUCTION

While studying its "astringent" action on the plasma of heparinized blood of a dog, berberine, an alkaloid occurring in *Berberis aristata* was found to exert (like protamine and toluidine blue) heparin-neutralizing action. This has been studied in detail.

MATERIALS AND METHODS

Blood samples (5-15 *ml*) were drawn into dry syringes from the saphenous vein of 16 lightly anaesthetized (sodium pentobarbitone 20 *mg/kg* ip) or conscious dogs. Twelve *ml* blood was drawn into dry sterile syringes from the cubital vein of 2 human volunteers. Thus 18 blood samples were studied. The drawn blood was quickly distributed, in 1 *ml* volumes, into a series of dry clean test tubes (Corning, 1·3 x 10·0 *cm*) which contained 50 i u and, in a few experiments, 25 i u heparin. In each series there was one test tube without anticoagulant and it was used for observing the clotting time of the individual blood sample. For detection of clotting, the test tubes were kept at 37°C and gently tilted every min for the first 10 min. During the next 30 min they were observed every 5 min. In most of the experiments the test tubes were kept at 37°C, for occasional observation, for about 24 hr.

Heparin (Biological Evans, 5000 i u/*ml*) was used. It was diluted with distilled water, to 500 i u/*ml*, before use.

The effect of berberine sulphate (Unichem Laboratories, 5 *mg/ml* in distilled water) was studied in all the 18 samples. For testing toluidine blue (Merck, 10 *mg/ml* in distilled water) blood samples of 3 dogs and 2 humans were used; for protamine sulphate (Biological Evans, 10 *mg/ml* ampuled solution), blood samples of the 2 dogs and 2 humans were used. Usually 0·3, 1·0, 3·0 and 10·0 *mg* of each of these 3 substances were added within 10 min to the tubes of the individual blood samples.

Distilled water (0·3-5·0 *ml*), atropine sulphate (3-15 *mg*), morphine sulphate (3-15 *mg*), physostigmine sulphate (3 *mg*), strychnine hydrochloride (3 *mg*), quinidine sulphate (3 *mg*),

quinine sulphate (0·3-10.0 *mg*) and tannic acid (3-15 *mg*) were also added, in some experiments, to test tubes containing 1 *ml* heparinized blood.

The effect of different doses of berberine sulphate, toluidine blue and protamine sulphate on the normal blood clotting time was also studied.

The effect of berberine sulphate, toluidine blue and protamine sulphate was examined on the dog and human blood samples rendered incoagulable by EDTA (2-4 *mg/ml*) or potassium oxalate (3 *mg/ml*) or sodium citrate (4-10 *mg/ml*).

The antiheparin action of berberine hydrochloride (1 *mg/ml*) was studied in 1 experiment.

RESULTS

The control blood samples were clotted in less than 8 min. Fifty i u per *ml* of heparin completely prevented clotting of all the blood samples. After adding 3·0 *mg* of berberine sulphate the heparinized blood clotted within 10 min. In 6 of these experiments 0·3 or 1·0 *mg* dose of berberine sulphate slowly produced incomplete clotting over a period of 1/2 to 2 hr ; when 3 *mg* berberine sulphate was added to these samples there was prompt and complete clot formation. When 25 i u heparin was added to 1·0 *ml* blood, complete clot was usually formed with 0·5 *mg* berberine sulphate. At 1·0 and 3·0 *mg* doses, toluidine blue produced complete clotting in 2 out of 3 experiments; 0·3 and 1 *mg* doses of protamine sulphate produced complete clotting in 1 and 3 out of 4 experiments each. In all these experiments blood samples which did not at all clot within 2 hr failed to clot in the next 22 hr.

Distilled water, atropine, morphine, physostigmine, strychnine, quinidine, quinine and tannic acid did not neutralize the anticoagulant action of heparin ; however, when 3·0 *mg* berberine sulphate was added on the next day to those test tubes there was prompt and complete clotting.

Ten *mg* doses of each of berberine sulphate, toluidine blue and protamine sulphate did not induce clotting of heparinized blood samples. Also, at this dose berberine sulphate, toluidine blue and protamine sulphate were found by themselves to partially or completely inhibit normal clotting of 2 out of 3 blood samples.

Berberine sulphate (6 experiments), toluidine blue (3 experiments) and protamine sulphate (3 experiments) had no effect on the anticoagulant action of EDTA, potassium oxalate and sodium citrate.

In one experiment berberine hydrochloride was tried and found as potent antiheparin as the sulphate.

DISCUSSION

Protamine (M.W. 4000-12000 ; 1) and toluidine blue (M.W. 306) are known to neutralize, *in vitro* and *in vivo*, the anticoagulant action of heparin (11b) and, paradoxically, in large doses they themselves act as anticoagulants (3 b, 5 b, 11 b). The present work shows that berberine

(M.W. 353) also manifests antiheparin and direct anticoagulant action in lower and higher doses respectively which are close to those of protamine and toluidine blue.

Due to high content of esterified sulphuric acid heparin molecule is considered the strongest organic acid occurring within the body and its anticoagulant activity is partly due to this acidity (5 a). Protamines, the simple proteins obtained from the sperms of certain fish are strongly basic because they are rich in arginine (1). Therefore, they combine with strongly acidic heparin to form a complex which is ineffective as an anticoagulant (5 b). However, the structure of berberine (8) does not clearly indicate why it should act as a heparin antagonist (7).

Berberine induces hypotension in the anaesthetized dog and inhibits the action of histamine on the isolated guinea-pig ileum (8). These actions were not affected when berberine was used after being mixed, *in vitro*, with heparin (9).

Toluidine blue, unlike protamine, can be given orally in clinical practice and it has been claimed to benefit some cases of bleeding disorders including hypermenorrhoea (3 c, 4, 6). It is interesting to note that crude preparations of plants containing berberine have been traditionally used by the Hindus (2) and by the American Indians (*Hydrastis canadensis* ; 3 a) for conditions characterized by bleeding including hypermenorrhoea. On the other hand, there are conflicting clinical (2) and experimental (3 a; Hanzlik, 1918 quoted by 11a) reports which indicate that these prepartions may aggravate bleeding particularly at the site of application. It is possible that the antiheparin and direct anticoagulant actions of berberine at low and higher doses respectively might explain the conflicting reports.

One of the common and poorly understood side-effects of intrauterine contraceptive devices (IUCD, like Lippes's loop) is that they aggravate intensity and duration of the menstrual bleeding (10). In view of the present work on berberine and in view of the previous clinical reports on toluidine blue for hypermenorrhoea, it may be interesting to try orally berberine and toluidine blue for excessive bleeding which is secondary to IUCD. Work on this possibility is in progress.

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