Effect of Oral Anticoagulant (Marcumar) on Prothrombin and Related Components in Blood Coagulation

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Marcumar has been considered as an excellent anticoagulant for long term management. It was found that the effect of the drug on plasma levels of prothrombin and autoprothrombin I and autoprothrombin II differed from that of the drugs previously studied using the technics described. Autoprothrombin II was not reduced by Marcumar. This clotting factor, autoprothrombin II, has been found to have no influence on the Quick prothrombin time which is the test generally used to follow the effect of anticoagulants on the clotting mechanisms. The two coagulation factors which were found to decrease following the administration of Marcumar, prothrombin and autoprothrombin I, both influence the Quick prothrombin time.

It is now generally accepted that prothrombin itself is reduced several days after an initial dose of Dicumarol and anticoagulants of similar chemical structure, therefore the first prolongation of the prothrombin time must be due to the reduction of some other component. Most workers agree that the factor first affected by the anticoagulants is autoprothrombin I or factor VII. Yet it has been found that a third factor, autoprothrombin II or Christmas factor, as it is more commonly called, was decreased in plasma during anticoagulant therapy.

The conversion of purified prothrombin to autoprothrombin II has been carried out by the addition of purified thrombin to purified prothrombin. This reaction has not been reversed, and is not as well understood even as the formation of autoprothrombin I from prothrombin. It is interesting to note that these three factors have similar chemical properties so far as adsorbing agents and stability are concerned. Regardless of the interrelationships of these and other factors, the effect of the administration of anticoagulants on the clotting mechanisms is primarily on this complex of factors which may be derivatives of prothrombin.

The problem, however, is to measure these various activities with precision and to interpret the results in an accurate way in the light of present knowledge. The two-stage prothrombin assay of Ware and Seegers probably measures prothrombin activity as accurately as it is possible to do at this time. The lower prothrombin values are less accurate, of course, because of the effect of the antithrombin on the smaller amounts of formed thrombin. This assay was used throughout this investigation, and is considered by the authors to be the most precise determination used. The measurement of autoprothrombin I (factor VII) has always been much more difficult than that of prothrombin. Recently Seegers, Johnson and Penner have reported a two-stage method involving Russell viper venom. The results of the two-stage venom assay are presented here with the suggestion that the activity measured may be autoprothrombin I, and it may be influenced by plasma lipid concentration. This investigation also includes measurement of the autoprothrombin I in plasma and serum from patients undergoing anticoagulant treatment using the coaguloplastin test of Mann. The factor being measured by these methods is, certainly, different from that considered to be autoprothrombin II for the time sequence of the reduction of both factors differs.

The measurement of autoprothrombin II, with which this paper is mainly concerned, is much less controversial. Autoprothrombin II and the antihemophilic factor both, with platelets, activate purified prothrombin. The antihemophilic activity is not present in serum so the activity of these two factors can be
separated very easily. It is very simple to remove autoprothrombin II from plasma or serum by inorganic adsorbing agents.

It is possible that one prothrombin derivative can be converted to another especially when blood clots and plasma becomes serum. In addition to the conversion of prothrombin to thrombin, the increase found in serum autoprothrombin I over that of plasma autoprothrombin I indicates that some prothrombin is converted to autoprothrombin I when blood clots. It is possible, however, that the increase in serum autoprothrombin I is due to the liberation of platelet factors, particularly the platelet cothromboplastin of Lee, Johnson and Seegers.10 As yet there is no indication that under ordinary circumstances autoprothrombin II concentration increases when plasma is converted to serum.

The purpose of this present work was to determine which factors of the blood coagulation mechanisms were affected by the administration of Marcumar (3-(1-phenylpropyl)-4-hydroxycoumarin). Numerous reports attest to Marcumar as an effective anticoagulant.11 Long term anticoagulant therapy produced by Marcumar is easily controlled. It seems important to the authors to correlate this clinical observation with the effect of the drug on the coagulation factors using as precise determinations as are available.

Material and Methods

Purified Prothrombin. This material was prepared from bovine plasma by the method developed by Seegers and associates12 and made in Dr. Seegers' laboratory. Each product contained some small traces of Ac-globulin. Some purified prothrombin, prepared using essentially the same technique, was supplied by Dr. Miller.

Russell Viper Venom. This material was purchased from Burroughs Wellcome and Company and diluted to 0.001 per cent for use in the two-stage assay and as directed in the one-stage assay.

Reaction Mixture. This solution was prepared according to the method of Owen, Hurn and Mann.13

Platelet Preparation. Bovine platelets were collected according to the method of Schneider and associates.14

Thrombin. Topical thrombin of Parke, Davis and Company was used, after further purification, to defibrinate plasma for these tests. The thrombin as purchased had a specific activity of practically zero. Three vials were dissolved in 10 ml. of 25 per cent sodium citrate and left standing at room temperature for 2 hours, probably converting biothrombin to citrate thrombin. The citrate thrombin was then precipitated by 37 per cent sodium citrate in a 25 ml. volume for 45 min. and then centrifuged at 1500 G for 20 min. The resulting sediment was dissolved in distilled water and dialyzed against physiologic saline. This thrombin solution had a specific activity of 11,000 U./mg. tyrosine.

Fibrinogen. Dried fibrinogen, purchased from Armour, was made up in a solution of 10 per cent imidazole buffer15 in saline to a concentration of 1 Gm. per cent. This solution was tubed and quick frozen.

CaCl₂ in Imidazole Buffer. CaCl₂ was dissolved in imidazole buffer as follows: 2.396 Gm. CaCl₂ 2H₂O was dissolved in 100 ml. of solution containing 25 ml. of physiologic saline and 75 ml. of imidazole buffer (17.2 Gm. of imidazole dissolved in 900 ml. 0.1 N HCl and made up to a liter with distilled water, resulting in a pH 7.4). The buffer solution was tubed and quick frozen.

Plasma and Serum Samples. Plasma samples were collected in the following way. To begin with, 5.0 ml. of blood were drawn in the first syringe and left to clot to form serum. Then a second syringe was used to draw 4.5 ml. of blood and this blood was mixed with 0.5 ml. of 0.1 M sodium oxalate solution. The blood samples were then centrifuged for 10 min. at 500 G. The plasma was defibrinated by adding 0.4 ml. of physiologic saline, 0.1 ml. of thrombin solution to 0.5 ml. of oxalated plasma. The fibrin clot was wound out. The whole blood was permitted to clot for 2 hours at room temperature and then centrifuged for 10 min. at 300 G. The serum was diluted 1:1 with physiologic saline to give a solution equivalent to that of the defibrinated plasma.

One-Stage Prothrombin Time. This test was carried out according to the method of Quick.16

Two-Stage Prothrombin Determination. This test was carried out according to the method developed by Ware and Seegers.6 In the reaction mixture dextran replaced Aracia according to the method of Owen, Hurn and Mann.13

Autoprothrombin I Assay. This was carried out according to the method developed by Seegers, Johnson and Penner.7 A second method was used (that of the cothromboplastin assay of Mann) to compare the values. The latter method was adapted to the two-stage prothrombin method of Ware and Seegers as follows. Reaction mixture was made up according to Owen, Hurn and Mann13 using Difco Aplastin beef lung thromboplastin, 100 mg. incubated at 45 C. in 5 ml. of physiologic
saline for 20 min. The substrate plasma was defibrinated and diluted 1:25.

Autoprothrombin II Test. Both the antihemophilic factor and autoprothrombin II, with platelet factor 3 and calcium ions, activate purified prothrombin to thrombin. However, the antihemophilic activity disappears in serum so an assay on plasma includes both activities while an assay using serum measures essentially autoprothrombin II activity. By a difference, therefore, one can measure the antihemophilic activity in plasma also.

The following reaction mixture was set up:

- Purified prothrombin (3000 U./ml.) ................. 1.0 ml.
- Plasma or serum (2 X diluted in defibrination) ................. 0.5 ml.
- Calcium, imidazole ................. 0.5 ml.
- Platelet preparation suspension ................. 0.5 ml.
- Physiologic saline .......... 3.0 ml.

The thrombin formation was followed over a period of 1 hour and determined by the method of Seegers and Smith. The control assay on platelets alone, in the absence of both plasma and serum, never exceeded 200 U./ml. of thrombin. The patients used in this investigation were admitted to the hospital with acute manifestations of arteriosclerotic heart disease and given Marcumar. One plasma and serum sample was taken before therapy began, and one taken daily for periods of time varying from 2 weeks to 1 month. In some cases samples were studied from patients who had been under the administration of Marcumar for several years.

RESULTS

Presence of Autoprothrombin II in Marcumar Plasma and Serum. Although it has been shown that autoprothrombin II decreases in plasma when Dicumarol, Sintrom, Hedulin or Dipaxin were administered, following the administration of Marcumar there was no change in the autoprothrombin II levels in 10 patients studied (fig. 1). Johnson, Seegers, Koppel and Olwin have reported a considerable range for this factor in the plasma and serum of 35 normal medical students. The initial range before therapy began was considerable in the 10 patients reported here, but there was little or no change observed throughout the period of treatment. The plasma cofactor assay measures both the antihemophilic factor and the autoprothrombin II activity while the serum cofactor assay measures only the latter. By difference the antihemophilic activity can be determined. The antihemophilic activity has never been found to be affected by the anticoagulants and while it fluctuated considerably from day-to-day, it did not appear to be affected by Marcumar. In the group of 14...
patients who have been under anticoagulant control for more than 1 year all had autoprothrombin II values within the normal range.

Prothrombin Levels in Marcumar Plasma and Serum. These levels began to fall immediately after the administration of the drug, in most cases before the two-stage prothrombin levels had fallen (table 1). There was more autoprothrombin I in serum than in plasma, but the levels in plasma and serum fell parallel. The initial decrease in the Quick prothrombin time is perhaps due to the decrease in this factor. The autoprothrombin I was found to remain low as long as administration of the anticoagulant persisted for it was absent in all 14 patients studied who had been treated with anticoagulants for a long time.

**Discussion**

It has been reported that most anticoagulants decreased not only prothrombin and autoprothrombin I (factor VII), but also autoprothrombin II (Christmas factor). The change in the level of autoprothrombin II in plasma could, in part at least, account for the erratic action of some of these drugs. The Quick prothrombin time which is so widely used to follow anticoagulant therapy does not reflect changes in autoprothrombin II. This would involve, of course, assuming that concentrations of autoprothrombin II moved independently of both prothrombin and autoprothrombin I. This is a consideration to keep in mind for it is well established that plasma levels of autoprothrombin I fell before and therefore independently of plasma levels of prothrombin. The Quick prothrombin time, because tissue thromboplastin is used, reflects changes in factors which act with tissue thromboplastin, but not with factors which, in effect, replace tissue thromboplastin.

The ideal anticoagulant, from the point of view of easy control, would be, therefore, one which alters only factors measured by the Quick prothrombin time test. It appears to us now that Marcumar affects prothrombin itself, and autoprothrombin I, both of which are measured by the Quick prothrombin time, but not autoprothrombin II. This fact undoubtedly accounts, to some degree, for the long term stability of Marcumar management.

The role which autoprothrombin II plays in thrombosis is certainly not understood. The reason why some patients bleed with Christmas disease1,19 which is an apparent deficiency of this factor, and yet why 1 patient with myocardial infarction was found in this study with a deficiency of this factor is very obscure. When the discovery that autoprothrombin II was decreased by anticoagulants was made the role of this factor in anticoagulant therapy and possibly in thrombosis was considered to be very important. But when viewed in the light of this new data and in relation to patients with an initial deficiency the role of autoprothrombin II may be less important in intravascular thrombosis than previous investigations may have indicated.

These results appear to be more startling because in the cases previously studied by Johnson, Seegers, Koppel and Olwin18 when autoprothrombin II was affected by the anticoagulants it was usually reduced drastically, in fact to zero as measured by these assay procedures.

It is not to be suggested that all of the
variability observed throughout a period of anticoagulant therapy can be attributed to the changes in the levels of autoprotrombin II. As early as 1948, Link\textsuperscript{20} reported seven known factors that can influence the response to Dicumarol in a group of standardized laboratory rabbits. It is too frequently forgotten that changes in the environment such as nutrition can affect the response to anticoagulants.

**SUMMARY**

A detailed analysis of the effect of the oral anticoagulant Marcumar on the plasma and serum levels of the coagulation factors has shown that while prothrombin and autoprothrombin I (factor VII) are decreased very much, autoprothrombin II (Christmas factor, PTC) remained almost unchanged.

**SUMMARIO IN INTERLINGUA**

Un analyse detaliato del efecto del anticoagulante oral Marcumar super le nivellos plasmatic e serai del factores coagulatori ha monstrate que prothrombina, autoprothrombina I (factor VII) es reducite per multo, durante que autoprothrombina II (factor Christmas) remane aproximativamente stabile.

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Circ Res. 1957;5:252-256
doi: 10.1161/01.RES.5.3.252

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