Fibrinolysis and Urokinase

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PREFACE

Although 35 years old, anticoagulant therapy still presents problems to many physicians. It is therefore not surprising that the almost 20-year old fibrinolysis treatment gives rise to many problems today, even for those well versed in blood clotting. These include biochemistry, particularly that of activators and inhibitors, the question of dosage and supervision, above all by hard laboratory data, indications and contraindications. In this regard, streptokinase therapy is much better known than urokinase therapy. However, as an intrinsic activator of plasminogen, urokinase was discovered as a therapeutic agent at the same time as streptokinase.

Difficulties in manufacture and the initially enormous cost were responsible for the fact that fundamental work on urokinase has only been carried out in recent years. Meanwhile, our knowledge of urokinase has so increased that we can now pose many more and better directed questions concerning its activity, and thereby its biochemistry, dosage, indications etc. One thing is clear: therapy with urokinase is more easily controllable. Because of this and because it is an intrinsic body constituent, it causes fewer side-effects.

There are thus many therapeutic advantages to urokinase therapy in comparison with streptokinase, even if the cost is still distinctly higher. It is thus time not only to expound our knowledge of urokinase therapy, but to describe what we do not know, so that areas for further research can be indicated and guidelines can be given for the regulation of therapy in use at present.

October 1980 V. TILSNER

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INTRODUCTION
FIBRINOLYTIC THERAPY IN THROMBOSIS: PERSPECTIVES IN 1979

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An analysis of our knowledge of the place of fibrinolysis with urokinase is imaginative and timely. Many advances are at present being documented in the field of the prophylaxis and treatment of thrombosis, and it is most useful to have an up-dating of our knowledge and understanding of the biochemistry, pharmacology and clinical use of urokinase. The object of this introduction is to present a brief discussion of perspectives in the field of antithrombotic therapy.

Coronary heart disease and thrombo-embolic cerebro-vascular disease account for about 50% of deaths in the western world, and in community terms are the most important manifestations of thrombosis. For individual patients, however, there is a vast morbidity and a significant mortality from venous thrombo-embolic disease and the less common forms of thrombosis can also be devastating for the individuals involved.

Costs of Antithrombotic Treatment

There is much evidence to suggest that any agent which effectively controls pathological thrombosis will interfere with physiological haemostasis and the cost of effective pharmacological antithrombotic intervention is probably haemostatic failure and bleeding. Other obvious costs are the other side effects of drugs, the

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financial costs of the drugs themselves, of medical teams required to use them and the psychological and social costs involved in treatment.

Problems in the Clinical Trial of Antithrombotic Agents

There are few areas of therapeutics in which more effort has been invested than the medical treatment of thrombosis, and in some ways it must be admitted that the outcome is still disappointing. The period shortly after the war was high noon of the anticoagulant era and it is perhaps unfortunate that so much effort was invested in the clinical trial of anticoagulants before the principles underlying the design of good clinical trials had emerged. It was probably only in the 1960s that it became fully recognized that a good trial required adequate diagnostic criteria, a prospective design, concurrent controls, random allocation, standardization of other therapy, objective criteria for complications, and if possible a double-blind design. Probably no trial has ever met all these criteria, but over the years there has certainly been a striking move towards improvement of trial design.

Where the natural history of disease is very variable, as is usually the case with thromboembolic disease, very large numbers of patients are required for a good clinical trial. For example, using United States epidemiological statistics, Fletcher (1969) examined the possible outcome of a drug trial in patients with acute myocardial infarction in which a new drug was theoretically available which produced a genuine reduction in mortality of 25%. The drug was assumed to be tested with a prospective trial design with patients allocated at random either to the new drug or to conventional therapy, the end point being death. Fletcher calculated that after observing 16 deaths there would only be one chance in 100 of finding the true reduction in mortality of 25%; after 128 deaths there would be six chances in 100 and even after 500 deaths there would only be a roughly 50/50 chance of finding a true reduction in death rate of 25%. When it is remembered that the death rate in coronary care units is about 10%, the data indicate that even if 5000 patients are taken into a trial there is still a 50/50 chance of not finding a 25% reduction in death rate. These formidable figures indicate that there must be major reservations about drawing conclusions from trials based on small numbers of patients.

Prophylaxis of Venous Thrombo-Embolic Disease

The main prophylactic measures available at the present time are low-dose heparin given subcutaneously, oral anticoagulants, dextran and various physical methods, such as electrical calf stimulation or intermittent calf compression. At the present time probably the method of prophylaxis about which most data is available is subcutaneous low-dose heparin, in particular the large-scale international study organized by Kakkar from King's College Hospital Medical School in London (International Multicentre Trial, 1975). In this trial, in which more than 4000 patients underwent major surgery, there was random allocation to either a subcutaneous heparin or a placebo group. In parallel, with a striking reduction in the incidence of deep vein thrombosis, there was a very marked

reduction in death from pulmonary embolism, which fell from approximately 0.8% of the control group to 0.1% in the heparin-treated group. This clearly represents a major advance in the management of patients at risk to deep vein thrombosis and pulmonary embolism, but the important point to make in the context of the present discussion is that there is still a residue of patients with pulmonary embolism and deep vein thrombosis who require treatment, perhaps with urokinase.

However, the price to be paid for the reduction in deep vein thrombosis and pulmonary embolism was that the incidence of wound haematomas rose by about one third in the heparin-treated group as compared with the controls. It has been known for many years that oral anticoagulants given prophylactically reduce the incidence of deep vein thrombosis. Morris and Mitchell (1976a) confirmed the value of warfarin sodium in patients with fractured neck of femur, using isotope scanning as a diagnostic index. The incidence of positive scans was reduced by approximately 50% in patients given warfarin. While there were few deaths from pulmonary embolism in their trial, there was a very suggestive trend towards reduction in the incidence of fatal pulmonary embolism. The important point in this study is perhaps that even with well controlled warfarin therapy, there is still a large group of patients who develop, and will need treatment for, deep vein thrombosis.

Prophylaxis of Arterial Disease

In terms of the numbers of patients involved, the prophylaxis and treatment of arterial disease is much more important than venous disease. It must be admitted immediately that there are no satisfactory measures available for the primary prevention of arterial disease. Despite the enormous efforts put into the clofibrate trial, with well over 10 000 patients recruited for the study, the outcome was largely negative (Committee of Principal Investigators, 1978). It may be that if the whole population could be persuaded to stop smoking, to eat less, to take more exercise and to slow down the pace of life, atherosclerosis and hence thrombotic arterial disease might be reduced.

Secondary prevention, that is the use of drugs in patients who have already had an episode of arterial occlusion, is a more promising field for clinical study at the present time, largely because the patients have selected themselves as being at high risk. Of course it must be remembered that patients who survive to leave hospital after myocardial infarction die of a variety of causes, including pump failure. It would be naive to suppose that these patients would respond to treatment with agents which interfere with the haemostatic mechanism. Nonetheless very promising results have been obtained in secondary prevention studies.

The results of the Boston Drug Surveillance Program (Jick and Miettinen, 1976), although retrospective and not in any sense representing the outcome of a controlled trial, give a major stimulus to work in this field. In 25 000 patients included in the trial, regular aspirin users had about half the relative risk of leaving hospital with a diagnosis of myocardial infarction. While it is possible that the outcome might have resulted from, for example, a tendency of aspirin takers to die before they ever reached hospital or for diagnosis not to be made for one reason or another after admission in aspirin takers, the evidence was nonetheless

very promising and has stimulated much subsequent work. The outcome of many trials at present in progress, is awaited with much interest.

At present the only major trial on which data is published is the sulphinpyrazone (Anturan) (Anturane Reinfarction Trial Research Group, 1978). In this well designed multi-centre study nearly 1500 patients were included and given either sulphinpyrazone or a placebo; follow-up was for a minimum of eight months. There was a 50% reduction in cardiac deaths and approximately 50% in sudden deaths. There are a number of relatively minor ways in which this trial can be criticised, but the general conclusion must be accepted that for the particular patient group studied (volunteers in North America), sulphinpyrazone has a favourable effect on mortality rates after myocardial infarction for this first 8 month follow-up.

At the present time evidence suggests that aspirin is effective in the prophylaxis of deep vein thrombosis in male patients and is probably effective in stroke. Dipyridamole reduces thrombosis on plastic surfaces as does sulphinpyrazone. The effects of aspirin and dipyridamole in myocardial infarction are at present under scrutiny and the results should be available later this year.

Treatment of Established Venous Thrombo-embolic Disease

Agents available for the treatment of thrombo-embolic disease are anti-coagulants, both oral anticoagulants and heparin, fibrinolytic enzymes and surgery. There is a disappointing number of well designed, well conducted, well analysed, well reported, randomized, prospective clinical trials to compare the various methods of treatment, or indeed to establish if treatment is better than placebo. For example, there is only one prospective controlled trial of heparin in pulmonary embolism (Barritt and Jordan, 1960). In this study, patients in whom the diagnosis had been made by the then available techniques including history, physical examination, chest X-ray, ECG, but obviously not including lung scan or pulmonary angiography, were randomly allocated to either a rather small amount of heparin followed by coumarin, or to a placebo group. As is well known, in the control group there was a preponderance of deaths and apparently also a marked excess of non-fatal recurrences. Using today's criteria this trial is clearly very unsatisfactory, with inadequate numbers, probably inadequate randomization, inadequate criteria for non-fatal events and an unusual protocol for anticoagulant therapy. I quote the data to underline the inadequacies of our knowledge of the most widely used drug in the treatment of pulmonary embolism. Much further work remains to be done in this field in which urokinase has great promise. In particular, a comparison of urokinase and pulmonary embolectomy in a large series of patients with life-threatening pulmonary embolism is urgently needed.

Treatment of Established Arterial Thrombo-embolic Disease

In myocardial infarction, oral anticoagulants are generally accepted as being in the sunset years. There are a number of contexts in which surgery may be

valuable. However, it is probably in this field and in the treatment of established venous thrombo-embolic disease that fibrinolytic enzymes have most to offer.

Application of Existing Knowledge

Morris and Mitchell (1976b) obtained data from 411 orthopaedic surgeons in England about their use of prophylactic measures in patients with fractured femur, and despite the excellent evidence in favour of anticoagulants, only 4% of orthopaedic surgeons used prophylactic anticoagulants. It may well be that at the present time one of the most important problems is the application of existing knowledge. I am confident that this study will be stimulating and valuable, and look forward to a synopsis in which our new knowledge of urokinase will be presented, assessed, assimilated and seen in the perspective of the clinical problems and the inadequacy of the present measures available to deal with them.

REFERENCES

Anturane Reinfarction Trial Research Group (1978). Sulfinpyrazone in the prevention of cardiac death after myocardial infarction. *New England Journal of Medicine* **6**, 289–295.

Barritt, D. W. and Jordan, S. C. (1960). Anticoagulant drugs in the treatment of pulmonary embolism: a controlled trial. *Lancet* **1**, 1309–1312.

Committee of Principal Investigators (1978). A cooperative trial in the primary prevention of ischaemic heart disease using clofibrate. *British Heart Journal* **40**, 1069–1118.

Fletcher, A. P. (1969). *In* "Thrombosis" (eds Sherry, S., Brinkhous, K. M., Genton, E. and Stengle, J. M.), 710–723. National Academy of Sciences, Washington D.C.

International Multicentre Trial (1975). Prevention of fatal postoperative pulmonary embolism by low doses of heparin. *Lancet* **2**, 45–51.

Jick, H. and Miettinen, O. S. (1976). Regular aspirin use and myocardial infarction. *British Medical Journal* **1**, 1057.

Morris, G. K. and Mitchell, J. R. A. (1976a). Warfarin sodium in prevention of deep vein thrombosis in patients with fractured neck of femur. *Lancet* **2**, 869–872.

Morris, G. K. and Mitchell, J. R. A. (1976b). Prevention and diagnosis of venous thrombosis in patients with hip fractures. A survey of current practice. *Lancet* **2**, 867–869.

Section I
BIOCHEMICAL BACKGROUND