Br. J. clin. Pharmac. (1978), **5**, 255–260

THE EFFECTS OF INTRAVENOUS ISOXSUPRINE ON BLOOD VISCOSITY IN PATIENTS WITH OCCLUSIVE PERIPHERAL ARTERIAL DISEASE

T. DI PERRI, S. FORCONI, D. AGNUSDEI, M. GUERRINI & F. LAGHI PASINI

Istituto di Semeiotica Medica, Facoltá di Medicina e Chirurgia, Universitá degli Studi di Siena, 53100 Siena, Italia

1 Blood viscosity is thought to be a major factor in disorders of the peripheral circulation.

2 Ten patients with obliterative arterial disease received an infusion of isoxsuprine of 20 μg kg^{-1} min^{-1} for 30 min. The infusion was followed by a highly significant and prolonged fall in blood, plasma and serum viscosity, in haematocrit and in plasma fibrinogen concentration. Blood lipid levels were also studied: total lipids and total cholesterol did not alter; triglyceride level was significantly lowered at the end of, and after, drug infusion.

3 There was no change in blood, plasma or serum viscosity in the four patients receiving a control infusion 2 days before the infusion of isoxsuprine.

4 The relationships between lowered viscosity and increasing peripheral tissue perfusion are discussed.

Introduction

Blood viscosity seems to be important in the dynamics of the circulation and especially of the microcirculation (Dintenfass, 1971). Changes in blood viscosity are dependent on haematocrit, which influences the viscosity of whole blood through the action of the packed red cells; on fibrinogen concentration, which influences plasma viscosity; and on lipid and protein concentration which influence serum viscosity.

Many experimental and clinical observations have stressed that human blood viscosity is very different in patients from that in normal subjects (Dintenfass, 1971).

These basic considerations and the observation that blood viscosity is significantly raised in most patients with either acute or chronic vascular disease such as myocardial infarction (Jan, Chien & Bigger, 1975), diabetic microangiopathy (Skovborg, Nielsen, Schlichtkrull & Ditzel, 1966; Muller, 1973; McMillan, 1974; Cogan, Merola & Laibson, 1961), and peripheral obstructive arterial disease (Dintenfass, 1971; Dintenfass, 1974; Dormandy, Hoare, Colley, Arrowsmith & Dormandy, 1973; Dormandy, Hoare, Khattab, Arrowsmith & Dormandy, 1973), lead to the hypothesis that this change may lead to the development of impaired circulation. Independently of the primary or secondary role of hyperviscosity in vascular diseases, it may lead to a progressive fall in tissue perfusion via a feed-back mechanism (Dintenfass, Julian & Miller, 1966).

It seems that drugs known to act on tissue perfusion may work by their effects on blood viscosity. Some substances which decrease blood viscosity *in vitro* have been investigated in this way. They may affect aggregation of red cells, like sodium linoleate (Ehrly, 1968), surface active agents (Miyauchi, Inouë & Paton, 1966), antimalarial drugs (Madow, 1960), dipyridamole (Dintenfass, 1970), and low molecular weight dextran (Gelin, 1962). They may affect the internal viscosity of red cells, like Tris or Tham (Moore, 1964), or bicarbonate therapy (Stewart, 1965). Some alter platelet aggregation, and some control plasma viscosity. In previous, not fully published research, we showed isoxsuprine hydrochloride to be a significant inhibitor of platelet aggregation induced by ADP, epinephrine or collagen (Di Perri, Forconi, Vittoria, Laghi-Pasini & De-Gori, 1974). The same drug also significantly lowers human whole blood, plasma and serum viscosity *in vitro*. The effect was the same whether immediately after adding the drug to the blood, serum or plasma sample, or after 30 or 60 min of incubation at 37°C. This activity was highly significant at the concentration of 7.4×10^{-4}M (Di Perri, Forconi, Guerrini & Agnusdei, 1977).

On the basis of these findings we studied the effects of a brief infusion of isoxsuprine on the whole blood, plasma and serum viscosity *in vivo*, in patients with occlusive peripheral arterial disease.

During the study, changes in the haematocrit, plasma fibrinogen concentration, serum total lipids, triglycerides and cholesterol concentration were measured.

Methods

Ten patients with obliterative arterial disease (seven males and three females, mean age 61 ± 6 years, mean weight 64 ± 3 kg) received an infusion of normal erythro-1-(*p*-hydroxyphenyl)-2-(1-methyl-2-phenoxyethylamine) propanol hydrochloride (isoxsuprine hydrochloride Duvadilan® Duphar) at the dose of 20 μg kg^{-1} min^{-1} for 30 min. The dilution was of 40 mg of isoxsuprine HCl in 100 ml of physiological saline solution (1 mg of active material/2.5 ml solution). Two days before the investigation, four of the ten patients were given a control infusion of 100 ml physiological saline only. Blood samples from an antecubital vein were withdrawn at the time 0 (before infusion), 30 min (end of infusion) and 60 and 90 min (30 and 60 min after the end of the infusion respectively). Samples for blood and plasma viscosity measurement were anticoagulated with EDTA 10% (0.1 ml in 8 ml of blood). The viscosity of blood plasma and serum was measured at 37°C of temperature by a Wells-Brookfield coneplate 1/4 RVT viscosimeter at shear rates of 750, 375, 150, 75, 37.5, 18.75 and 3.75 s^{-1}. All samples were subjected to measurements of packed red cell volume (PRCV), plasma fibrinogen, total lipids, cholesterol, triglycerides, and free fatty acids (FFA). The blood samples for plasma fibrinogen were anticoagulated with sodium citrate 3.8% (0.5 ml in 4.5 ml of blood). Plasma fibrinogen was estimated by nephelometry measuring the formation of antigen-antibody complexes by the fluornephelometer of an Autoanalyzer Technicon II, continuous flow AIP system. Total lipids and cholesterol were estimated by colorimetric methods using the methods of Zoellner & Kirsch (1962), and of Watson (1960), respectively, triglycerides by the enzymatic method of Eggstein (1966), and FFA by the colorimetric method as described by Lauwers (1969) and Duncombe (1964).

Results

Statistical analysis was performed applying Student's *t*-test for paired variates. Intravenous infusion of isoxsuprine hydrochloride at the dose of 20 μg kg^{-1} min^{-1} for 30 min was followed by a fall in whole blood, plasma and serum viscosity at all the shear rates tested, from 750 to 3.75 s^{-1}. At the shear rate of 3.75 s^{-1} blood viscosity decreased from 10.57 cP to 9.05 cP (14%) at the end of the infusion and to 8.28 cP (21%) 30 min afterwards. At the end of the observation period it was always 8.97 cP, a decrease of 15%. At this shear rate the hypoviscosimetric effect was very obvious, but the profile of viscosity changes was the same at all the shear rates tested (Figure 1). The drop in whole blood viscosity appears to be maximal and highly significant, 30 min after the end of the drug infusion. A significant

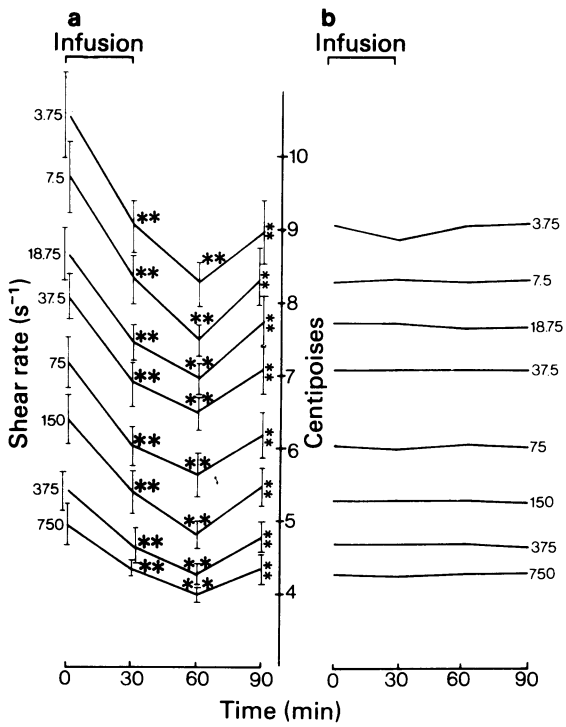

Figure 1 Mean ± s.e. mean changes in blood viscosity before and after treatment with (a) isoxsuprine hydrochloride (20 μg kg^{-1} min^{-1}, $n = 10$), or (b) physiological saline (100 ml 0.9% NaCl in 30 min, $n = 4$). ** $P < 0.001$.

fall was still present 60 min after the end of the infusion, despite the tendency of viscosity to return to the basal values.

The behaviour of plasma viscosity is very similar to that of blood. At the lower shear rate observed, 18.75 s^{-1}, the fall was 9.5% at the end of infusion, 15% and 8.3%, 30 min and 60 min later. The differences were significant (Figure 2).

Serum viscosity changed less. The maximal activity of the drug seemed to be at the end of the infusion. The decrease was 7.5% at the shear rate of 18.75 s^{-1} ($P < 0.05$) while later the differences were not always significant (Figure 3).

Haematocrit values decreased at the end of the infusion (from 44.3 to 43.1) and after 30 (42.7) and 60 min (42.4). Plasma fibrinogen concentration fell significantly at the end of the infusion (from 329 to 307 mg%–6%) and remained lower until 60 min later (Figure 4).

Blood total lipids and total cholesterol concentration had not changed significantly 30 min after the end of the infusion. Immediately after the infusion there

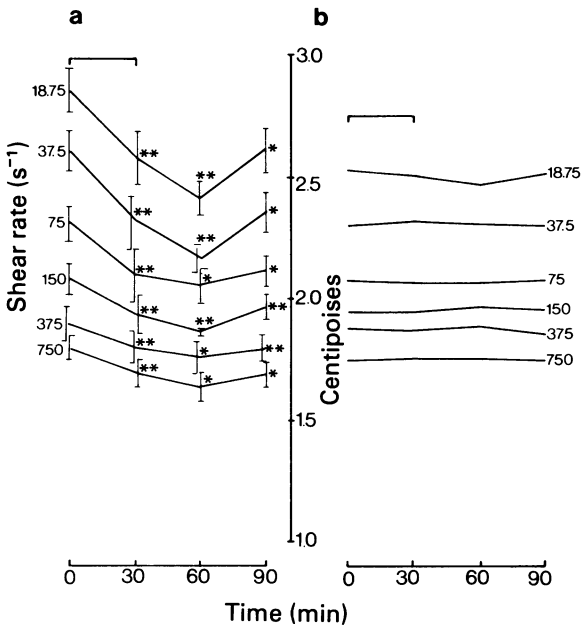

Figure 2 Mean ± s.e. mean changes in plasma viscosity before and after treatment with (a) isoxsuprine hydrochloride (20 μg kg^{-1} min^{-1}, $n=10$), or (b) physiological saline (100 ml 0.9% NaCl in 30 min, $n=4$). ** $P<0.001$; * $P<0.05$.

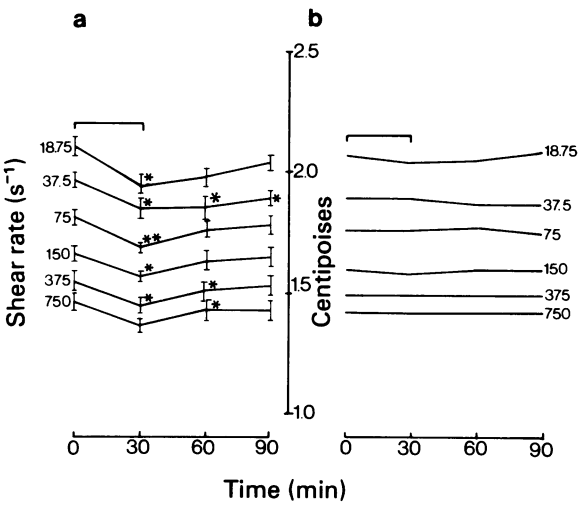

Figure 3 Mean ± s.e. mean changes in serum viscosity before and after treatment with (a) isoxsuprine hydrochloride (20 μg kg^{-1} min^{-1}, $n=10$), or (b) physiological saline (100 ml 0.9% NaCl in 30 min, $n=4$). ** $P<0.001$; * $P<0.05$.

was a significant rise in plasma FFA level (Table 1). There was no significant modification of the arterial blood pressure; diastolic pressure if anything tended to be lower. During the whole infusion period the heart rate quickened but never by more than 15 beats/min (basal rate was 70 to 80 beats/min). The tachycardia was noticed by the patients and stopped at the end of the infusion. Infusion was tolerated very well: slight headaches were reported very occasionally.

Control tests in the four patients with physiological saline only, administered in the same quantity and at the same perfusion rate, revealed no significant changes of viscosity in whole blood, plasma and serum, of PRCV or of plasma fibrinogen, total lipids, cholesterol, triglycerides and FFA level. The mean values for each parameter in each subject before the start of saline infusion was not significantly different from that before the start of isoxsuprine therapy (Table 1).

Discussion

The intravenous infusion of isoxsuprine hydrochloride in ten patients with peripheral occlusive disease at a

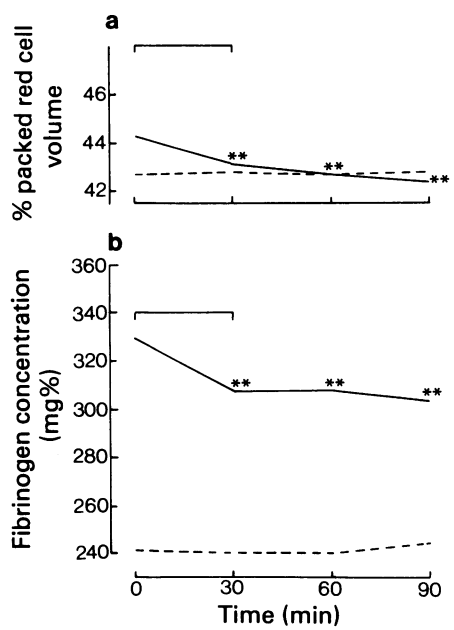

Figure 4 Changes in (a) packed red cell volume and (b) plasma fibrinogen concentration before and after treatment with isoxsuprine hydrochloride (20 μg kg^{-1} min^{-1}, $n=10$, solid line) or physiological saline (100 ml 0.9% NaCl in 30 min, $n=4$, dotted line). ** $P<0.001$.

Table 1 Mean ± s.e. mean changes in total lipids, cholesterol, triglycerides and free fatty acids concentrations in ten patients before and after treatment with isoxsuprine hydrochloride, and in four control patients after treatment with physiological saline. Statistical analysis was performed applying Student's *t*-test for paired variates. *t* concerns 30 min, *t'* 60 min and *t''* 90 min, all compared with 0 min.

Before and after infusion of isoxsuprine HCl 20 μg $kg^{-1}min^{-1}$ for 30 min

	0 min	*30 min*			*60 min*			*90 min*		
			t	*P*		*t'*	*P*		*t''*	*P*
Total lipids (mg %)	904.5 ± 57.6	892.8 ± 70.5	0.324	NS	896.5 ± 59.1	0.245	NS	854.8 ± 60.1	1,126	NS
Cholesterol (mg %)	226.7 ± 15.6	213.6 ± 13.6	1.802	NS	205.0 ± 19.7	1.440	NS	207.1 ± 16.5	1.887	NS
Triglycerides (mg %)	158.4 ± 16.4	136.6 ± 13.8	1.669	NS	122.6 ± 11.5	2.858	*	149.6 ± 21.2	0.533	NS
F.F.A. (mEq/l)	0.38 ± 0.07	0.58 ± 0.12	3.351	**	050 ± 0.13	1.442	NS	0.50 ± 0.10	1.531	NS

Before and after infusion of 100 ml physiological saline 0.9% in 30 min

	0 min	*30 min*			*60 min*			*90 min*		
			t	*P*		*t'*	*P*		*t''*	*P*
Total lipids (mg %)	699.0 ± 47.1	701.5 ± 55.0	0.212	NS	696.5 ± 44.0	0.385	NS	702.5 ± 47.6	0.456	NS
Cholesterol (mg %)	206.2 ± 19.5	207.5 ± 19.3	0.170	NS	210.7 ± 17.5	1.567	NS	200.5 ± 22.9	0.985	NS
Triglycerides (mg %)	105.7 ± 14.0	105.0 ± 13.1	0.056	NS	111.8 ± 14.7	1.308	NS	102.0 ± 13.0	1.379	NS
F.F.A. (mEq/l)	0.66 ± 0.33	0.67 ± 0.01	0.293	NS	0.69 ± 0.04	1.127	NS	0.66 ± 0.09	0.003	NS

* $P < 0.05$ and ** $P < 0.001$.

dose of 20 μg kg^{-1} min^{-1} for 30 min was followed by a significant and prolonged fall in whole blood, plasma and serum viscosity, in haematocrit value and in plasma fibrinogen. Lowering the viscosity was evident at very different shear rates, tested from 750 to 3.75 s^{-1}.

Lowering of whole blood viscosity may be related to changes in haematocrit or to a property of the red cell itself (aggregability or deformability); the reduction of plasma viscosity to falling fibrinogen concentration (Figure 4); the lowering of serum viscosity to changes in either protein or lipid components. Our findings appear to be in agreement with these biological relationships, as the statistical analysis of paired values reveals a very close correlation. The activity of isoxsuprine in inhibiting platelet aggregation (Di Perri *et al.*, 1974) may be an additional factor altering both whole blood and perhaps plasma viscosity. The significant fall in fibrinogen concentration secondary to isoxsuprine infusion may be an important cause of reduction in plasma viscosity.

The present findings indicate that the action of isoxsuprine is not likely to be a single one. Whole blood viscosity may be lower because of the fall in haematocrit, which is theoretically attributable to lower vessel wall permeability, and to a relative increase of plasma volume. The influence on red cell deformability or aggregability, possibly related to the fibrinogen decrease, should also be considered.

Isoxsuprine's effect on the plasma fibrinogen level must be emphasized. The fall is particularly significant and persists. This change cannot be attributed exclusively to an increase of circulating plasma volume, as the concentration of other organic plasma constituents does not change significantly. There is no evidence yet of a direct effect of the drug on fibrinogen metabolism. The significance of the fall in serum viscosity is suggestive of the involvement of some plasma factor other than fibrinogen. Although total lipid and total cholesterol content are not changed, triglyceride concentration is significantly lowered at the end of, and after the drug infusion.

There is some relationship between serum lipoprotein concentration and viscosity (Dintenfass, 1965) but it is difficult to isolate the influence of a single factor on the changes of a very complex phenomenon like blood hyperviscosity. In our findings the simultaneous fall in triglyceride concentration and rise in FFA suggests a lipolytic action of the drug. This is not easy to explain but could contribute to the fall in serum viscosity.

The action of isoxsuprine hydrochloride infusion does not finish immediately, but is still present 60 min after the end of the infusion. In a previous paper we have observed (Di Perri, Forconi, Agnusdei, Guerrini & Laghi Pasini, 1977) that in patients with peripheral arterial disease, isoxsuprine hydrochloride infusion at the same concentration and speed is followed by rise in peripheral muscular blood flow maximal after the end of the infusion and higher than the basal values for a long time thereafter.

The two actions, one on peripheral blood flow, the other on blood viscosity, appear to be closely interrelated, both in dynamic pattern and in duration. This agrees with the theory of Dormandy *et al.* (1973) that there is a pathophysiological link between higher blood viscosity and decreased peripheral tissue perfusion.

References

COGAN, D.C., MEROLA, L.B. & LAIBSON, P.R. (1961). Blood viscosity, serum hexosamine and diabetic retinopathy. *J. clin. Invest.*, **10**, 393–395.

DINTENFASS, L. (1965). Some observations on the viscosity of pathological human blood plasma. *Thromb. Diath. Haemorrh.*, **13**, 492–499.

DINTENFASS, L. (1970). Influence of ABO blood groups in the selective disaggregation of the red cells caused by drug RA433. *Med. J. Aust.*, **2**, 827–830.

DINTENFASS, L. (1971). *Blood microrheology, viscosity factors in blood flow, ischaemia and thrombosis.* London: Butterworths.

DINTENFASS, L. (1974). Blood rheology as a diagnostic and predictive tool in cardiovascular disease. *Angiology*, **25**, 365–372.

DINTENFASS, L., JULIAN, D.G. & MILLER, G. (1966). Viscosity of blood in normal subjects and in patients suffering from coronary occlusion and arterial thrombosis. *Am. Heart. J.*, **71**, 587–600.

DI PERRI, T., FORCONI, S., VITTORIA, A., LAGHI-PASINI, F. & DE-GORI, V. (1974). Action of isoxsuprine *in vitro* upon platelet aggregation by ADP, adrenaline and collagen. *Boll. Soc. Ital. Biol. Speriment.*, **50**, 1385–1390.

DI PERRI, T., FORCONI, S., AGNUSDEI, D., GUERRINI, M. & LAGHI PASINI, F. (1977). Interrelationship between blood flow increase and blood viscosity decrease after acute isoxsuprine hydrochloride (Duvadilan®) infusion in peripheral arterial disease. *Proceedings of the VII Congress of the European Society of Cadiology.*

DI PERRI, T., FORCONI, S., GUERRINI, M. & AGNUSDEI, D. (1977). *In vitro* activity of isoxsuprine on blood, plasma and serum viscosity. *Pharmatherapeutica*, **1**, 447–452.

DORMANDY, J.A., HOARE, E., COLLEY, J., ARROWSMITH, D.E. & DORMANDY, T.L. (1973). Clinical, haemodynamic, rheological and biochemical findings in 126 patients with intermittent claudication. *Br. med. J.*, **4**, 576–581.

DORMANDY, J.A., HOARE, E., KHATTAB, A.H., ARROWSMITH, D.E. & DORMANDY, T.L. (1973). Prognostic significance of rheological and biochemical findings in patients with intermittent claudication. *Br. med. J.*, **4**, 581–583.

DORMANDY, J.A. (1975). Hyperviscosity angina. *Lancet*, **i**, 679–680.

DUNCOMBE, W.G. (1964). The colorimetric microdetermination of non-esterified fatty acids in plasma. *Clinica Chim. Acta*, **9**, 122–125.

EGGSTEIN, M. (1966). An enzymatic method for triglyceride determination. *Klin. Wchr.*, **44**, 267–273.

EHRLY, A.M. (1968). Reduction in blood viscosity at low rates of shear by surface active substances; a new haemorrheologic phenomenon. *Biorheology*, **5**, 209–214.

GELIN, L.E. (1962). Rheological disturbances and the use of low viscosity dextran in surgery. *Rev. Surg.*, **19**, 285–290.

JAN, K.M., CHIEN, S., & BIGGER, J.T. (1975). Observations on blood viscosity changes after acute myocardial infarction. *Circulation*, **51**, 1079–1084.

LAUWERS, R. (1969). The determination of free fatty acids by colorimetry. *Analyt. Biochem.*, **32**, 331–333.

MADOW, B.P. (1960). Use of antimalarial drugs as 'desludging' agents in vascular disease process. *J. Am. med. Ass.*, **172**, 1630–1633.

McMILLAN, D.E. (1974). Disturbance of serum viscosity in diabetes mellitus. *J. clin. Invest.*, **53**, 1071–1079.

MIYAUCHI, Y., INOUE, T. & PATON, B.C. (1966). Adjunctive use of a surface-active agent in extracorporeal circulation. *Circulation*, **33**, suppl. 1, 71–77.

MOORE, F.D. (1964). Tris buffer, mannitol and low viscous dextrans: three new solutions for old problems. *Survey of Anaesthesiology*, **8**, 412–415.

MULLER, R. (1973). Diabetic angiopathy and blood viscosity. *Acta. Diabet. latin.*, **10**, 1311–1324.

SKOVBORG, F., NIELSEN, A.A.V., SCHLICHTKRULL, J. & DITZEL, J. (1966). Blood viscosity in diabetic patients. *Lancet*, **i**, 129–131.

STEWART, J.S.S. (1965). Bicarbonate therapy during embolectomy. *Lancet*, **ii**, 1320–1323.

WATSON, D. (1960). A simple method for the determination of serum cholesterol. *Clinica Chim. Acta*, **5**, 637–643.

ZOELLNER, N. & KIRSCH, K. (1962). On the quantitative determination of lipids (micro-method) by means of the general sulfophosphovanillin reaction of the many natural lipids (all known plasma lipids). *Z. Ges. Exp. Med.*, **135**, 545–561.

(Received December 14th 1976, revised July 18, 1977)