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Abnormalities of vascular prostaglandins in Henoch-Schönlein purpura

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SUMMARY The ability of plasma to support prostacyclin like activity from human umbilical arterial rings was studied in 17 patients with Henoch-Schönlein purpura and 17 controls matched for age and sex. Plasma from 13 of the 17 patients showed a diminished or absent ability to support prostacyclin like activity in vitro. Six patients whose plasma had a low or absent ability to support prostacyclin like activity showed evidence of inhibitory activity. Plasma from three of these patients also failed to preserve the effect of a stable prostacyclin like analogue (ZK36–374). The plasma concentration of prostacyclin metabolite and the serum concentration of thromboxane A_2 metabolite, thromboxane B_2, were measured simultaneously. The concentration of plasma prostacyclin metabolite in 10 of the 14 patients was decreased, and a positive correlation was found between the plasma prostacyclin metabolite values and the ability of the plasma to support prostacyclin like activity. There was no significant difference in the serum thromboxane A_2 metabolite concentrations between the patients and controls. These data suggest that abnormalities of vascular prostaglandin metabolism are involved in the pathophysiology of Henoch-Schönlein purpura.

Key for abbreviations used in text
PGI_2: prostacyclin.
PSA: estimation of ability of plasma to support vascular PGI_2 like activity.
PSAI: inhibition of vascular PGI_2 like activity.
PGI_2m: prostacyclin metabolite.
TxB_2: thromboxane A_2 metabolite.

Henoch-Schönlein purpura is the most commonly encountered type of vasculitis in childhood. The skin lesions are the most obvious sign, but visceral involvement carries a more serious prognosis. In two thirds of patients arthritis and gastrointestinal involvement occur, and in a minority of patients, in addition, the central nervous system is involved. Renal involvement, however, is potentially the most serious manifestation as chronic renal impairment may develop.[1 2] The aetiology and pathogenesis are obscure, but the primary manifestations are due to inflammation of small non-muscular vessels.[3] Henoch-Schönlein purpura may follow exposure to drugs or allergens[4] and is in many cases preceded by upper respiratory tract infection occasionally caused by group A β-haemolytic streptococci.[3] None of these factors, however, have been confirmed to be of pathogenetic importance.

Increased number of data are now available about the possible role of vascular prostaglandins prostacyclin (PGI_2), and thromboxane (TxA_2) in the pathogenesis of other vasculopathies. PGI_2 is an unstable substance with potent antiaggregatory and vasodilatory activity,[5] synthesised from arachidonic acid by the vessel well cycloxygenase system.[6] TxA_2 is also an unstable endoperoxide with vasoconstricting and platelet aggregatory activity,[7] derived from arachidonic acid predominantly by the platelet cycloxygenase system.[8] Remuzzi *et al* first showed deficient PGI_2 production in the haemolytic uraemic syndrome and the related disorder of thrombotic thrombocytopenic purpura. Plasma taken from these patients had a low capacity to support PGI_2 production in vitro from rat aortic rings.[9] Reduced umbilical and placental vascular PGI_2 has been observed in patients with severe pre-eclampsia.[10] Saldeen *et al* have reported an increased concentration of TxA_2 and PGI_2 metabolites in patients with deep venous thrombosis,[11] and increased TxA_2 synthesis by platelets has been shown in Kawasaki disease.[12] We report our preliminary findings on various aspects of vascular prostaglandin metabolism in the acute phase of Henoch-Schönlein purpura.

Patients

Seventeen patients with Henoch-Schönlein purpura aged between 2 and 13 years (mean (SD) 5·7 (2·5) years) and 17 controls matched for age and sex were studied. The control children were free of renal, cardiovascular, pulmonary, and inflammatory disease and were admitted for minor surgical operations. All patients had the classical purpuric rash, and in 13 cases the skin lesions were associated with joint manifestations. One child had recurrent purpura, and none of the patients had hypertension. In eight cases gastrointestinal symptoms were observed, and this included one patient who required operative intervention for ileocolic intussusception. Three patients had a mild illness without abnormal urinalysis or abdominal or joint manifestations. In eight patients haematuria (> 10 red blood cells/μl) and/or proteinuria (31 (SD 19) mg/hr/m^2), was observed. There was no significant difference in the creatinine clearance between those patients with abnormal urinalysis (94 (SD 33) ml/min/1·73 m^2) and those with normal urinalysis (100 (SD 21) ml/min/1·73 m^2). In all patients plasma albumin concentrations, plasma electrolyte concentrations, and blood film showed no abnormalities, and the mean platelet count was 357 (SD 54)$\times 10^9$/l.

Methods

After informed consent had been obtained 10 ml of venous blood was taken from the controls and from the patients during the acute phase of the disease, 1–5 days after the occurrence of the rash. Blood for estimation of the ability of the plasma to support PGI_2 like activity (PSA) was anticoagulated with 3·2% trisodium citrate in a ratio of 9:1. Samples for PGI_2 metabolite (PGI_2m) measurements were collected into ice cold plastic tubes anticoagulated with 1:9 volume 3·8% w/v trisodium citrate: 3×10^{-5}M indomethacin: 10^{-4}M adenosine. Tubes were kept on ice and spun within one hour at 4°C and 2500 *g*. Plasma was separated and stored at −70°C. Samples of this pool were included in each assay both at the beginning and end to determine reproducibility and to act as a standard. A known amount of 6-keto $PGF_1\alpha$ was added to an aliquot of this pooled plasma to estimate recovery.

Platelet poor plasma, obtained for PSA estimation, was prepared within 20 minutes of collection by centrifugation at 2000 *g* for 10 minutes at 4°C. Simultaneously, serum samples were separated for estimation of TxB_2 (stable metabolite of TxA_2). All plasma and serum samples were stored at −70°C until examination.

Platelet rich plasma for aggregation studies was prepared from normal adults by centrifugation of citrated plasma at 800 *g* for 10 minutes at room temperature. The final platelet count was adjusted to 250–300$\times 10^9$/l by dilution with autologous platelet poor plasma. No patient or control was taking any drug known to alter prostaglandin metabolism for at least two weeks before the study.

Estimation of ability of plasma to support vascular PGI_2 like activity (PSA). The ability of test plasma (patient and control) to support PGI_2 like activity was assessed by measurement of platelet antiaggregatory activity by the method of Moncada *et al.*[13] Human umbilical arterial rings were obtained from freshly delivered umbilical cords. The umbilical arteries were freed from all surrounding tissue, cut into rings 1 mm in length (30–50 mg wet weight) and kept in Ringer's buffer (pH 7·4) at 0°C for not more than 60 minutes. The rings were then incubated in 1 ml tromethamine buffer (pH 8·6) for five minutes at 37°C, and PGI_2 like activity (platelet antiaggregatory activity) of 100 μl of the supernatant was tested. This was added to 200 μl platelet rich plasma in a Servogor 120 dual channel aggregometer. The mixture was incubated for one minute at 37°C, then collagen (Hormonochemie), at a final concentration of 2 μg/ml, was added. The rate of aggregation was recorded using a Malin platelet aggregation recorder.[14] The rings were then washed several times with Ringer's buffer at 37°C until no antiaggregatory activity could be detected. These exhausted rings were then incubated with 1 ml of test platelet poor plasma at 37°C for 20 minutes. PGI_2 like activity was then assessed as before. The ability of each test platelet poor plasma to support PGI_2 like activity was expressed by calculation of the percentage difference in inhibition of platelet aggregation obtained by the same exhausted ring before and after the addition of test platelet poor plasma.

Detection of inhibition against PGI_2 like activity (PSAI). To detect inhibitory activity in the test plasma a modified method of Levin *et al* was used.[15] Fresh, unexhausted umbilical arterial rings were incubated in phosphate buffered saline (pH 7·4) at 37°C for five minutes. The PGI_2 like activity of the supernatant was compared with that produced by the same ring after five minutes incubation with test platelet poor plasma.

Preservation of PGI_2 like effect of stable PGI_2 analogue. The preservation of PGI_2 like effect of a stable PGI_2 analogue, ZK36-374 (Schering chemicals AG), was assessed using test platelet poor plasma. ZK36-374, at a final concentration of 100 ng/l, was

incubated with 300 µl of test platelet poor plasma at 37°C for five minutes. The concentration of ZK36-374 corresponded to that concentration known to have a definite antiplatelet effect when the drug is given by intravenous infusion.[16] Then 100 µl of the supernatant was removed and added to 200 µl platelet rich plasma. After one minute of incubation and stirring, platelet aggregation studies using collagen were carried out as before. After incubation of normal plasma with ZK36-374 total inhibition of platelet aggregation should be expected due to the protective effect of the PGI_2 analogue.[16]

Radioimmunoassay studies of plasma PGI_2m and serum TxB_2. Plasma concentrations of PGI_2m were assessed by radioimmunoassay using a modification of the method of Mitchell.[17] As only 40% of PGI_2 is metabolised to 6-keto $PGF_1\alpha$ we used an assay that measures in addition the more labile metabolites.[18] The measurement was carried out on unextracted plasma buffered with 0·1% bovine albumin, as many extraction techniques may add further impurities.[19] Our method gives good reproducibility and recovery of added PGI_2m and has a sensitivity of 5 pg/ml.[20] Serum TxB_2 was measured using a radioimmunoassay method as described by Granstrom and Kindahl.[21]

All platelet aggregation and radioimmunoassay studies were carried out in duplicate. Statistical analysis was carried out using the Student's *t* test and Spearman rank correlation test.

Results

Ability of plasma to support PGI_2 like activity was reduced in 13 of the 17 patients with Henoch-Schönlein purpura studied. In five cases PSA was undetectable (Fig. 1). The difference between the results of the patient group (21·3 (SD 20·5)%) and the control group (57·2 (SD 12·2)%) was significant ($p<0·001$). In addition, the results of those patients with abnormal urinalysis and gastrointestinal involvement (7·5 (SD 18·1)%) was significantly different from the results of those patients with neither of these manifestations, (32·3 (SD 23·5)%) ($p<0·01$).

Detection of inhibition against PGI_2 like activity (PSAI). Plasma from six patients in which the PSA was low or absent was further investigated to detect PSAI. Less PGI_2 like activity was produced by the unexhausted umbilical arterial rings when incubated with platelet poor plasma from the six patients than with either phosphate buffered saline or control platelet poor plasma (Table).

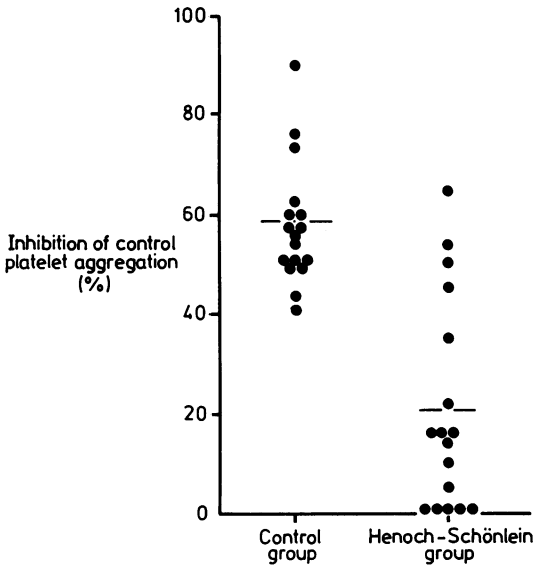

Fig. 1 *Ability of plasma to support vascular PGI_2 like activity (PSA) in patients with Henoch-Schönlein purpura.*

Table *Plasma factors influencing prostacyclin (PGI_2) like activity in Henoch-Schönlein purpura*

Case No	*PSA* (%)*	*PSAI†*	*Preservation of ZK 36-374‡ effect*
		Patients	
1	0	+	Normal
2	0	+	Decreased
3	0	+	Decreased
4	0	+	Decreased
5	0	+	Normal
6	23	+	Normal
		Controls	
1	62	−	Normal
2	57	−	Normal
3	65	−	Normal

*Estimation of ability of plasma to support vascular PGI_2 like activity.
†Inhibition of vascular PGI_2 like activity: +=present, −=absent.
‡A stable PGI_2 like analogue.

Preservation of PGI_2 like effect of stable PGI_2 analogue. The effect of ZK36–374 was noticeably depressed after incubation with platelet poor plasma from three of the six patients in whose plasma PSAI was present (Table).

Plasma PGI_2m and serum TxB_2. There was a significant difference between the results of plasma PGI_2m concentrations obtained from 14 patients with Henoch-Schönlein purpura (16·6 (SD 8·7) pg/ml) and the control group (35·3 (SD 7·5) pg/ml)

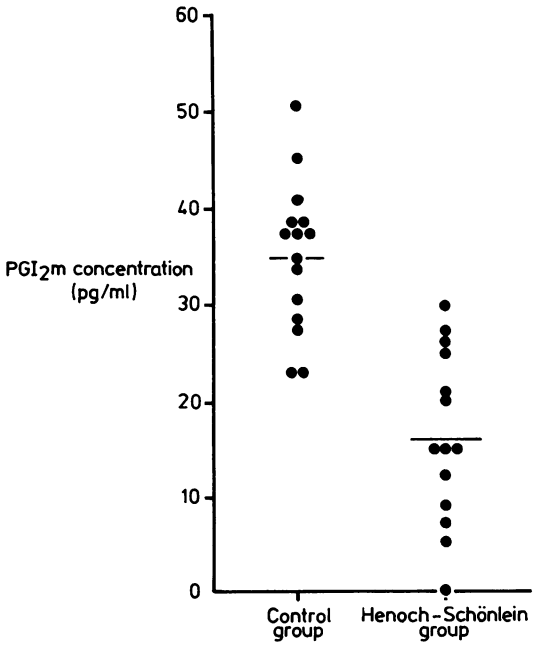

Fig. 2 *Plasma PGI_2 metabolite (PGI_2m) concentrations in patients with Henoch-Schönlein purpura.*

($p<0.05$) (Fig. 2). A positive correlation was found between PSA and PGI_2m values ($r=0.52$, $p<0.05$). There was no significant difference in serum TxB_2 concentration between the 16 patients (11·4 (SD 9·4) ng/ml) and the control group (13·2 (SD 8·6) ng/ml). No significant correlation was found between the results of the tests on serum TxB_2 and plasma PGI_2m concentrations.

Discussion

It is well known that the pathological defect in Henoch-Schönlein purpura is that of a predominantly small vessel vasculitis. Abnormalities of vascular prostaglandin metabolism have been documented in other vasculopathies, particularly in the haemolytic uraemic syndrome. There are, however, no published reports of studies in Henoch-Schönlein purpura. Conceivably, after an endothelial insult, such as deposition of immune complexes, a defect of PGI_2 generation may be present.

We have shown that plasma from patients with Henoch-Schönlein purpura in the acute phase of the illness has a diminished or absent ability to support PGI_2 generation in vitro from human umbilical arterial rings. Absent or depressed PSA concentrations may theoretically be due to a number of causes, occurring singly or in combination. Firstly, a decreased ability of the plasma to stimulate PGI_2 production;[9] secondly, the presence of inhibitory activity in the plasma either to the production[15] or to the effect of PGI_2;[22] and thirdly, rapid degradation of stimulated PGI_2.[23] All six patients in this study who had very low or absent concentrations of PSA, in whom further investigation was carried out, showed the presence of PSAI. The presence of PSAI may mean inhibitory activity against PGI_2 production, inhibitory activity against PGI_2 effect, or increased PGI_2 degradation. To attempt to differentiate these possibilities we looked at the ability of test plasma to preserve the effect of a stable PGI_2 analogue (ZK36-374) and found this to be absent in three of the six patients studied. The concentration of ZK36-374, although significantly higher than the physiological concentrations of PGI_2m in both patients and controls, was chosen because a definite antiplatelet effect was desired. It would be interesting to undertake further studies using different concentrations of the PGI_2 analogue; however, this was outwith the scope of this present study. On the basis of these data it would seem that in patients with low or absent concentrations of PSA inhibitory activity may exist to either PGI_2 production or PGI_2 effect.

We have also shown that most patients with Henoch-Schönlein purpura in this study had a decreased concentration of plasma PGI_2 metabolite (PGI_2m). It is of interest, however, that two patients showed a fairly high concentration of PGI_2m in combination with a low or absent PSA concentration and plasma inhibitory activity to the effect of the stable PGI_2 analogue. This would tend to confirm the presence of a circulating inhibitor to PGI_2 effect in these cases. These findings also show that platelet aggregation studies are more sensitive in detecting decreased PGI_2 like activity of plasma than determination of PGI_2m concentration.

We have found no significant difference in serum thromboxane (TxB_2) concentrations between the patient group and the control population. Furthermore, there was a poor correlation between the concentration of serum TxB_2 and plasma PGI_2m concentrations. These results do not suggest the presence of platelet activation in this syndrome. In view of the small numbers of patients in this study and the absence of any follow up data we felt unable to attempt a correlation between the demonstrated abnormalities and the clinical picture. It is of interest, however, that patients whose clinical picture included abnormal urinalysis and gastrointestinal involvement had a mean PSA concentration that was significantly depressed compared with

those patients in whom these clinical manifestations were absent.

The above findings suggest the abnormality of PGI_2 metabolism in Henoch-Schönlein purpura is rather complex and heterogenous. Further studies will be required to define the biochemical nature of the inhibitors and to establish whether low or absent PSA concentrations always reflect the presence of inhibitory activity and whether there is any evidence of rapid PGI_2 degradation. It seems likely that these abnormalities, having been shown in yet another vasculitic syndrome, are most probably a secondary manifestation of endothelial damage. The disturbances, however, may well be of importance in extending the primary microvascular insult.

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References

1. Habib R, Levy M. Anaphylactoid purpura nephritis. Observation with sixty childhood cases. *Clin Pediatr* 1973;**12**:445–6.
2. Meadow R. Schonlein-Henoch syndrome. *Arch Dis Child* 1979; **54**:822–4.
3. Vernier RL, Worthen HG, Peterson RD, Colle E, Good RA. Anaphylactoid purpura I. Pathology of the skin and kidney and frequency of streptococcal infection. *Pediatrics* 1961;**27**:181–93.
4. Ackroyd JF. Allergic purpura, including purpura due to foods, drugs and infections. *Am J Med* 1953;**14**:605–32.
5. O'Grady J, Warrington S, Moti MJ, *et al.* Effects of intravenous prostacyclin infusion in healthy volunteers—some preliminary observations. In: Vane JR, Bergstrom S, eds. *Prostacyclin*. New York: Raven Press, 1979:409.
6. Moncada S, Gryglewski R, Bunting S, Vane JR. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature* 1976;**263**:633–5.
7. Packham MA, Mustard JF. Pharmacology of platelet-affecting drugs. *Circulation* 1980;**62**(Suppl V):26–32.
8. Hamberg M, Svensson J, Samuelsson B. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc Natl Acad Sci* 1975;**72**:2994–8.
9. Remuzzi G, Marchesi D, Mecca G, Misiani R, Livio M, De Gaetano G. Haemolytic-uraemic syndrome: deficiency of plasma factor(s) regulating prostacyclin activity? *Lancet* 1978;**ii**: 871–2.
10. Remuzzi G, Marchesi D, Zoja C, *et al.* Reduced umbilical and placental vascular prostacyclin in severe pre-eclampsia. *Prostaglandins* 1980;**20**:105–10.
11. Saldeen P, Nilsson IM, Saldeen T. Increased synthesis of thromboxane B_2 and 6-keto-$PGF_{1\ alpha}$ in hand veins from patients with deep venous thrombosis. *Thromb Res* 1983;**32**:461–7.
12. Hidaka T, Nakano M, Ueta T, Komatsu Y, Yamanoto M. Increased synthesis of thromboxane A_2 by platelets from patients with Kawasaki disease. *J Pediatr* 1983;**102**:94–6.
13. Moncada S, Higgs EA, Vane JR. Human arterial and venous tissue generate prostacyclin (prostaglandin X) a potent inhibitor of platelet aggregation. *Lancet* 1977;**i**:18–20.
14. Born GVR. Aggregation of platelets by A D P and its reversal. *Nature* 1962;**194**:927–9.
15. Levin M, Elkon KB, Nokes TJC, *et al.* Inhibitor of prostacyclin production in sporadic haemolytic uraemic syndrome. *Arch Dis Child* 1983;**58**:703–8.
16. Belch JJF, Greer I, McLaren M, *et al.* The effects of intravenous ZK36-374 a stable prostacyclin analogue on normal volunteers. *Prostaglandins* 1984;**28**:67–75.
17. Mitchell MD. A sensitive radioimmunoassay for 6-keto-$PGF_{1\ alpha}$: preliminary observations on circulating concentrations. *Prostaglandins and Medicine* 1978;**1**:13–21.
18. McLaren M, Belch JJF, Forbes CD, Prentice CRM. Development of a radioimmunoassay for the measurement of prostacyclin metabolites in unextracted plasma. *Thromb Res* 1985;**37**:177–83.
19. Greaves M, Preston FE. Plasma 6-keto-$PGF_{1\ alpha}$: fact or fiction. *Thromb Res* 1982;**26**:145–57.
20. Belch JJF, Greer I, McLaren M, Walker JJ, Forbes CD. Measurement of prostacyclin metabolites. *Lancet* 1983;**ii**:1504.
21. Granstrom E, Kindahl H. Radio-immunoassay for prostaglandins and thromboxanes. In: Frolich JC, ed. *Advances in prostaglandin and thromboxane research*. New York: Raven Press, 1978;**5**:119.
22. Machin SJ, McVerry BA, Parry H, Marrow WJW. A plasma factor inhibiting prostacyclin-like activity in thrombotic thrombocytopenic purpura. *Acta Haematol* 1982;**67**:8–12.
23. Chen YC, McLeod B, Hall ER, Wu KK. Accelerated prostacyclin degradation in thrombotic thrombocytopenic purpura. *Lancet* 1981;**ii**:268–9.

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