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Oral nimodipine reduces prostaglandin and thromboxane production by arteries chronically exposed to a periarterial haematoma and the antifibrinolytic agent tranexamic acid

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SUMMARY The calcium antagonist nimodipine blocks the effects of many vasoconstrictors of cerebrovascular smooth muscle and may reduce the incidence of delayed cerebral ischaemia following subarachnoid haemorrhage though not necessarily by inhibiting the development of angiographic cerebral vasospasm. Post-haemorrhagic CSF contains abnormally large quantities of various eicosanoids that partly reflect enhanced production by cerebral arteries. Does nimodipine affect this process? The extra-arterial and intra-arterial production of PG6 keto-$F_1\alpha$, PGE_2, $PGF_2\alpha$ and TXB_2 were measured in perfused common carotid arteries taken from rabbits in which the arteries had been ensheathed by blood clot in vivo for 7 days. All rabbits were given the antifibrinolytic agent tranexamic acid to retard resolution of the clot, and half were given oral nimodipine (2 mg/kg/day) for 10 days. Nimodipine significantly reduced the extra-arterial production of TXB_2 during the third and fourth hours of perfusion and, less consistently, the production of $PGF_2\alpha$, PGE_2 and PG6 keto-$F_1\alpha$. Lutrol, the solvent for nimodipine, had no such effect.

The introduction of the calcium channel blocker nimodipine for the prophylactic treatment of cerebral ischaemia following subarachnoid haemorrhage (SAH), was based on the notion that it would inhibit the development of cerebral arterial narrowing ("vasospasm"). Cerebrovascular smooth muscle contractions in response to most agents, including haemorrhagic CSF, are dependent on extra-cellular calcium and such contractions are readily blocked by nimodipine.[1,2] However, whatever its merits in the prevention of post-haemorrhagic cerebral ischaemia prove to be in statistically valid trials,[3,4] nimodipine may not reduce the incidence or severity of cerebral vasospasm as demonstrated on angiography.[5–7]

The probability of delayed cerebral ischaemia after SAH increases both with the size of the periarterial haematoma seen on CT scan,[8,9] and with antifibrinolytic activity,[10] but the mechanisms by which such a blood clot causes abnormal vascular reactivity are still uncertain. Enhanced production of vasoconstrictor derivatives of arachidonic acid (eicosanoids) within or close to the arterial wall may be contributory. Increased concentrations of prostaglandins and thromboxane have been documented in post-haemorrhagic cerebrospinal fluid.[11] Some of these probably arise from the arteries themselves. We have shown previously that chronic exposure of the rabbit common carotid artery to a blood clot in vivo for one week, using either a sheath or the antifibrinolytic agent tranexamic acid to retard resolution of the clot, increases considerably the in vitro production of various eicosanoids by the arterial wall, particularly PGE_2.[12] In the present study we have examined the effects of oral administration of nimodipine on this process.

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Materials

Saffan: 0·9% alphaxalone/0·3% alphadolone acetate (Glaxovet, Uxbridge, England)
TEA: Cyklokapron 100 mg/ml (Kabivitrum, Middlesex)
Nimodipine: (Bayer, UK)
Lutrol: (Bayer, UK) (solvent for nimodipine)

Krebs-Ringer-Bicarbonate buffer containing glutathione (KRB) per litre: NaCl 7·24 g, KCl 0·372 g, KH_2PO_4 0·163 g, $NaHCO_3$ 2·184 g, glucose 1·8 g, $MgSO_4.7H_2O$ 0·32 g, $CaCl_2.2H_2O$ 0·184 g, glutathione 0·3072 g; pH adjusted to 7·4 after equilibrating with 95% O_2/5% CO_2.

Methods[12]

New Zealand white rabbits (both sexes) were anaesthetised with Saffan (1 ml/kg IV). Under sterile conditions a midline neck incision was made and the common carotid artery exposed and freed from surrounding tissues by careful blunt dissection. Three ml of fresh venous blood from the same animal was placed around the vessel and allowed to clot. The neck was then sutured in two layers using silk, and povidone iodine spray was applied to the wound.

Seven days later, the animals were anaesthetised with Saffan and heparinised. Approximately 3 cm from both common carotid arteries was removed and immersed in Krebs-Ringer-Bicarbonate buffer containing 1 mmol/l glutathione (KRB). Each artery was cannulated quickly at both ends and submerged in 4 ml of fresh buffer gassed with 95% O_2/5% CO_2 at 37°C. A 10 minute washing period followed during which KRB was also perfused through the lumen of the artery to wash out prostaglandins and free fatty acids. Following this, both perfusing and bathing buffers were changed. Bathing continued with KRB to which 40 g/l bovine serum albumin was added, and continuous perfusion at 1 ml min^{-1} was performed using a peristaltic pump and perfusing volume of 4–4·5 ml. All buffers were collected and replaced at 60 minute intervals. PGs and TXB_2 in each sample were extracted and assayed by radioimmunoassay (see Walker *et al* 1983[11] for details). Segments were taken from the vessel ends (before perfusion) and middle (after perfusion), fixed in formalin, stained with haematoxylin and eosin and examined histologically.

Quantification of inflammatory response Three areas were measured in duplicate transverse sections of each artery: Area 1 (lumen), Area 2 (lumen + intima + media) and Area 3 (lumen + intima + media + inflammation). A Leitz Dialux 22EB microscope with a VIDS II image analysis system (Analytical Measuring Systems Ltd, Saffron Walden, UK) was used. This is a semi-automatic image analyser in conjunction with an Apple IIe micro-processor and graphics tablet.

Drugs were all given orally for 10 days prior to the perfusion studies by addition to the drinking water bottle. Intakes were monitored to ensure that each animal received the correct dosage. Tranexamic acid (TEA; 5 ml cyklokapron containing 500 mg TEA) was given daily. This provides per kg body weight approximately twice the recommended human antifibrinolytic dose. Nimodipine was weighed out under sodium lamp illumination, and a stock solution of 10 mg/10 ml lutrol water was prepared fresh daily by heating the mixture with shaking. Both stock and drinking water bottles were darkened to protect the drug from light. A daily dose of 2 mg/kg was administered. Lutrol was given at a dose of 2 ml/kg daily.

Data are also presented from our earlier study[12] for arteries not exposed to clot from animals treated with TEA only for 10 days. The arteries were removed and perfused on the tenth day as above.

Statistical methods

The raw data consists of 96 sets of data. These were tested for normality using a Kolmogorov-Smirnov test. Seventy five sets of data did not differ significantly from the normal distribution. A log-transform was performed on all data and again the Kolmogorov-Smirnov test was used to test for normality. Only six out of the 96 data sets differed significantly from a normal distribution and hence the transformed data was analysed using Analysis of Variance. Where the calculated value of "F" was found to be significant ($P < 0.05$), the difference between means was examined using Duncan's Multiple Range Test. Where data were not normal in any one of the three groups at each time, an additional non-parametric test (Kruskal-Wallis) was also used.

Results

Administration of either TEA of nimodipine had no overt clinical effects on the animals which continued to eat, drink and behave normally.

Histology All seven arteries exposed to clot from animals treated with TEA only ("TEA arteries") were inflamed. There was a fibroblastic reaction around the arteries with granulation tissue around the more severely affected vessels. Macrophages, some containing haemosiderin (Prussian blue stain), and a few polymorphonuclear leukocytes were present and extended into the adventitia. In some vessels there was minimal oedema of the media. Treatment with nimodipine had no effect on the extent of the inflammatory changes. The area of inflammation (Area 3–Area 2) did not differ significantly between control and nimodipine groups (control: 0·69 mm^2 ± 0·11 (SEM); nimodipine: 0·64 mm^2 ± 0·05). There were no significant differences in the sizes of the arteries in the two groups (Area 1: 0·29 ± 0·10 and 0·29 ± 0·03 mm^2; Area 2: 0·74 ± 0·12 and 0·72 ± 0·04 mm^2 respectively). There was no evidence of inflammation or fibrosis in arteries not exposed to clot which were removed from animals treated with TEA for 10 days.

PROSTGLANDIN AND THROMBOXANE RELEASE

Animals Treated With TEA Only Arteries which had been dissected and exposed to blood clot for 7 days had enhanced prostanoid production when compared with control non-manipulated arteries (table). The difference was statistically significant for *intra-arterially* released PGE_2 from the second hour, and for $PGF_2\alpha$, and PG6 keto-$F_1\alpha$ by the fourth hour of perfusion, but TXB_2 was not increased. *Extra-arterially*, release of PGE_2 and TXB_2 was significantly increased from the second hour, and of $PGF_2\alpha$ and PG6 keto-$F_1\alpha$ from the third hour of perfusion. PGE_2 production by the inflamed arteries increased progressively.

Effect of Nimodipine Nimodipine treatment had no effect on the intra-arterial release of prostaglandins

Table *Prostaglandin production (mean and observed range: pg/mg wet weight) during four hours of perfusion in vitro by carotid arteries exposed to blood clot for seven days in vivo. Rabbits were treated with TEA only (n = 7) or TEA + NIMODIPINE (n = 8). Data is also presented for eight control arteries not exposed to clot, from rabbits treated with TEA for 10 days*

						Intra-arterial						
	$PGF_2\alpha$			PGE_2			PG6 keto-$F_1\alpha$			TXB_2		
Hour of perfusion	*TEA*	*TEA + NIM*	*TEA Controls*	*TEA*	*TEA + NIM*	*TEA Controls*	*TEA*	*TEA + NIM*	*TEA Controls*	*TEA*	*TEA + NIM*	*TEA Controls*
1	5	6	9	6	9	4	67	66	79	3	6	3
	2–14	3–13	4–24	2–16	4–16	2–11	18–215	23–213	24–175	1–7	2–26	2–5
2	5	7	4	15	18	4‡	65	58	40	3	7	2
	3–8	3–17	3–8	6–33	2–33	1–11	24–133	19–193	19–83	1–6	1–37	1–3
3	5	4	4	16	17	4‡	59	36	31	3	3	2
	3–8	3–6	2–7	7–27	5–33	1–10	21–102	18–60	16–68	2–6	1–7	1–3
4	6	4†	3‡	26	13	4‡	86	36†	26‡	3	3	2
	3–9	2–7	2–5	7–45	6–23	1–9	28–212	14–72	17–50	1–7	2–5	1–3
						Extra-arterial						
1	30	29	39	85	158	38	553	398	605	47	34	24
	16–75	10–40	20–60	21–169	50–257	15–64	253–1657	31–581	191–930	13–149	4–46	8–68
2	29	24	22	124	261	37‡	460	273	241	41	23	7‡
	14–69	11–40	7–39	35–215	76–332	4–73	159–1140	90–668	123–355	15–130	6–51	2–16
3	31	16†	16*	171	114	34*	432	166†	150*	48	18†	6‡
	14–77	7–23	5–27	51–320	17–211	3–53	180–1073	51–327	71–279	15–151	3–61	2–12
4	31	18	11‡	203	124	28‡	486	175§	94‡	43	21†	4‡
	16–55	6–28	4–15	64–329	13–222	4–50	191–1431	65–299	51–146	14–88	4–68	1–10

*p < 0·05, ‡p < 1 0·01: TEA control arteries compared with TEA arteries with clot.
†p < 0·05, §p < 1 0·01: "TEA" and "Nimodipine" arteries with clot compared.

and TX during the first three hours of perfusion. During the fourth hour, the increase in PG6 keto-$F_1\alpha$ observed for the TEA arteries did not occur, and PG6 keto-$F_1\alpha$ production was significantly less. Production of PGE_2 was also decreased, but not significantly. Similarly nimodipine had no significant effect on the early release of prostaglandins and thromboxane *extra-arterially*. However, during the third and fourth hours of perfusion production of all the prostanoids tended to decrease, and the difference from the TEA arteries was significant for $PGF_2\alpha$, PG6 keto-$F_1\alpha$ and TXB_2.

In two arteries from rabbits treated with lutrol, the solvent for nimodipine, the intra-arterial and the extra-arterial prostanoid release was similar to that of the TEA arteries and was not depressed (fourth hour mean values: intra-arterial: $PGF_2\alpha$ 6, E_2 34, 6 keto-$F_1\alpha$ 51, TXB_2 2 pg/mg wet weight; extra-arterial: $PGF_2\alpha$ 28, E_2 194, 6 keto-$F_1\alpha$ 256, TXB_2 107 pg/mg wet weight).

Discussion

In this study, carotid arteries from TEA-treated rabbits which were dissected and exposed to blood clot for 7 days had enhanced production of prostaglandins and thromboxane when perfused in vitro, and a progressive increase in PGE_2 was observed. Nimodipine treatment did not affect eicosanoid production during the first two hours of perfusion and, as for the the TEA arteries, production of PGE_2 and TX was markedly increased in comparison with arteries not dissected and exposed to clot. However, as perfusion was continued, production of all four eicosanoids by the nimodipine arteries decreased, whereas production by the TEA arteries was maintained at a high level or increased further (PGE_2). It seems, therefore, that an early prostanoid response could be mounted to arterial manipulation in vitro, but that this response was not sustained after chronic treatment with nimodipine. The dose of nimodipine used (2 mg/kg/day) was lower than that being used clinically (for example 5 mg/kg/day or more) but was chosen to avoid possible side effects, including hypotension. Published studies of the effects of a variety of calcium channel blocking agents on platelets and other tissues have examined only the acute effects of the drugs and have given conflicting results, some reporting decreased production of prostaglandins and/or thromboxane,[13–16] and others no change.[17–19] There is a clear need for study of more chronic effects of clinically relevant doses of nimodipine.

The initial step in prostanoid synthesis in response to cell membrane stimulation, is the release of arachidonic acid from membrane phospholipids by phospholipase A_2 or (in platelets) by the combined actions of phosphoinositide-specific phospholipase C and diglyceride lipase. Both phospholipases are activated by Ca^{++} possibly by a calcium-calmodulin mediated mechanism,[19–21] and an increase in concentration of free Ca^{++} in the cytoplasm is probably the primary intracellular mediator in the induction of prostaglandin and thromboxane production.[15 20] The

source of the Ca^{++} has remained controversial. Studies on porcine endothelial cells suggested that prostacyclin synthesis in response to bradykinin depended on an influx of extra-cellular Ca^{++}.[19] However, others have demonstrated that release of intracellular stored Ca^{++} may be a key mechanism for arachidonic acid liberation and metabolism in platelets,[21 22] in cultured vascular endothelial cells,[20] and in rat renal arteries stimulated by Angiotensin II.[18] An influx of extra-cellular Ca^{++} might in turn stimulate further calcium release from intra-cellular stores.[15]

Nimodipine could have reduced eicosanoid production in our study by suppressing the voltage-activated influx of calcium into arterial wall cells. However, this explanation seems unlikely since there was no attenuation of the early release of prostaglandin and thromboxane, the decline being delayed. Since dihydropyridine calcium antagonists bind reversibly at least to vascular smooth muscle,[23] any drug present on the external surfaces of the arterial wall cells in vivo would probably have been washed off fairly readily during in vitro perfusion, since the buffer did not contain nimodipine. An enhanced eicosanoid production might have been anticipated as the effect of the drug waned. The observed effects probably occurred through a different mechanism. It has become apparent that calcium channel blockers may have intra-cellular effects in addition to inhibiting influx of extra-cellular calcium.[24] They may interfere with calcium translocation across membrane of intra-cellular organelles, or with calcium release from the surface of intracellular membranes.[25] Of possible relevance is that using a histochemical technique, significant amounts of calcium were demonstrable on the inner surface of the plasma membranes from rabbit cerebral arteries.[26] Calcium antagonists may inhibit the refilling of calcium stores in cerebral and peripheral arteries.[26] This offers an attractive if speculative explanation for the findings in this study in which the arteries were chronically exposed to nimodipine.

Cells of the macrophage/monocyte series produce prostaglandins themselves and also stimulate production by adjacent connective tissue cells.[12 27] However, there was no histological evidence that nimodipine decreased the inflammatory response compared with the TEA arteries. Nimodipine treatment of monkeys (1–12 mg/kg/8 hours) for 7–14 days after subarachnoid haemorrhage, did not prevent the histopathological changes[28 29] (which in one study[28] included a collagenous reaction and macrophages in the adventitia), nor did nifedipine reverse delayed cerebral vasospasm in the two haemorrhage canine model.[30]

The findings in this study relate to a conducting artery. However, the profile of the eicosanoids released, including an increase in PGE_2 in response to clot, was similar to that of dog cerebral arteries incubated in vitro,[11] and the present findings are likely to be relevant to intracranial arteries. It is therefore of considerable interest that TEA reduced the incidence of rebleeding following SAH but increased the risk of delayed cerebral ischaemia.[10] The value of calcium channel blockers such as nimodipine for the prophylactic treatment of delayed cerebral ischaemia has not yet been proven in double-blind clinical trials of sufficient size to be statistically valid.[4] A diminished arterial capacity to produce vasoconstrictor eicosanoids might be one factor contributing to a beneficial effect.

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