Effects of a carbonic anhydrase inhibitor on cerebral blood flow in geriatric patients

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SYNOPSIS A carbonic anhydrase inhibitor (UK-12,130) was shown to increase cerebral blood flow in mildly demented geriatric patients. Oral administration caused a significant increase in blood flow at two different dose levels; this persisted for at least six weeks, which was the duration of the longest study. There was no consistent improvement in mentation during treatment. Blood flow was measured by the washout of ^{133}Xe after inhalation of this inert gas.

Carbonic anhydrase inhibitors (CAI) have been investigated for their vasodilatory effect because it seemed likely that vessels might react in the same way as they do to increases in PaCO_2 . Carbonic anhydrase catalyses the reversible hydration of CO_2 in erythrocytes and in the glial cells in choroid plexus, but not in the neurones of the brain (Maren, 1967); this would be expected to result in changes in the extracellular pH in the brain with resultant cerebral vasodilatation. One such CAI drug, acetazolamide (Diamox) can increase cerebral blood flow (CBF) by as much as inhalation of 5% CO_2 (Ehrenreich *et al.*, 1961) both in experimental animals (Severinghaus and Lassen, 1967; Lassen, 1968; Cotev *et al.*, 1968; Kong *et al.*, 1969) and in patients suffering from cerebrovascular disease (Posner and Plum, 1960; Ehrenreich *et al.*, 1961; Gotoh *et al.*, 1966). At effective doses, however, other tissues containing carbonic anhydrase are also affected (Maren, 1963), and a marked diuresis occurs because of the renal effect (Maren, 1967). A recently produced CAI (UK-12,130) has a more selective action on the brain, because it crosses the blood-brain barrier more readily it is more lipophilic and has lower ionization compared with acetazolamide. At doses producing a similar increase in CBF in dogs the diuresis was four times less

than with acetazolamide. The only study in man showed a significant increase in CBF after intravenous dosage, using the intracarotid injection of radioactive xenon to measure the CBF (Skinhøj, 1975). This invasive method has limited application and it is difficult to justify repeated measurements. We have therefore used the recently developed inhalation method to make repeated measurements of CBF before and during oral administration of UK-12,130 in demented geriatric patients—a group for whom the drug has been recommended as of possible therapeutic benefit.

METHOD

Seven mildly demented but cooperative female patients aged 67 to 91 years were chosen at the Department of Geriatric Medicine, Royal Alexandra Infirmary, and consent for their inclusion in the trial obtained in writing from relatives. They were brought by car to the Institute of Neurological Sciences, Southern General Hospital, Glasgow at weekly intervals for measurements.

Three separate dosage regimens were employed:

Regimen 1 Initial loading dose of 100 mg UK-12, 130. This was followed (four hours later) by 50 mg three times daily. This dosage was continued for two weeks.

Regimen 2 Initial loading dose of 100 mg UK-12, 130. This was followed four hours later by 25 mg three times daily. This dosage was continued for two weeks.

Regimen 3 Initial loading dose of 100 mg, followed four hours later by 50 mg three times daily for six weeks.

All doses were taken orally.

A minimum of four weeks elapsed between the end of one course and the start of another. The purpose of regimen 3 was to investigate possible escape from the effects of the drug.

There were six patients in regimen 1, five of these plus one new patient in regimen 2, and four in regimen 3.

MEASUREMENT OF CEREBRAL BLOOD FLOW Cerebral blood flow was measured using the two-minute slope ^{133}Xe inhalation technique (Wyper *et al.*, 1976). The patient, who was sitting or lying on a couch, breathed through a rubber mouthpiece. Once a steady respiratory pattern had been obtained, a 32-second count of background radioactivity was recorded; then the two-minute saturation phase began, during which a mixture of ^{133}Xe in air at a concentration of 1 mCi/l. was inhaled. Clearance of ^{133}Xe from the head was then recorded for 2½ minutes, during air breathing using four 2.5 cm (1 in) diameter collimated scintillation detectors. Two detectors were positioned to view the hemispheres from front to back and the other two were positioned on the left side viewing the temporal and parietal regions. The end-expired air concentration of ^{133}Xe was also recorded using a well-type scintillation counter, in order to allow a correction to be made for recirculating xenon. The end-tidal PCO_2 was measured using an infra-red CO_2 analyser.

CBF measurements were made as follows:

Regimen 1 Two measurements before the loading dose then two hours and four hours after the loading dose. One measurement after one week on the regular dosage immediately before that day's first dose. One measurement two hours after this. One measurement after a further week on the regular dose immediately before that day's first dose repeated two hours after this.

Regimen 2 Measurements as in regimen 1.

Regimen 3 One measurement before the loading dose of 100 mg. One measurement after two weeks on a regular dosage of 50 mg three times daily. One measurement after a further four weeks on the same dosage.

RESULTS

No significant regional variations were encountered in CBF between different detectors; the CBF values presented are the averages for the four detectors.

1. Before starting the treatment in these patients the average CBF was 31.3 ± 2.3 ml/100 g/min compared with 50.1 ± 7.0 ml/100 g/min for a group of 29 normal subjects aged 20 to 50 years; both were at a similar, normal level of PCO_2 .

2. As two control measurements of CBF were made on each patient (Tables 1 and 2) the precision of the technique when applied to this particular group of patients can be assessed. If the distribution of the values of the difference between the first and second measurements is assumed to be Gaussian (not a critical assumption as the range of CBF values is small), the standard deviation of this distribution gives a measure of the precision of the technique. Any two measurements of CBF must differ by twice this standard deviation to be significantly different at the $P < 0.05$ confidence level.

TABLE 1
CBF/ PCO_2 FOR 50 MG THRICE DAILY STUDY*

	Patient					
	JF	SR	EMcM	FM	FMcD	ER
Control	30/40	33/29	32/36	37/40	31/32	36/36
	27/40	28/29	30/34	31/40	27/32	32/36
2 h	31/39	33/29	27/35	33/40	29/32	29/35
4 h	23/40	33/29	29/35	32/36	26/30	32/35
1 week	40/31	34/22	37/36	44/31	33/29	54/26
	40/29	40/22	34/30	46/32	33/29	54/25
2 weeks	42/29	36/21	38/29	41/34	35/31	48/26
	34/27	39/20	45/30	40/32	36/28	46/25

*CBF in ml/100g/min and end-tidal PCO_2 in mmHg.

TABLE 2
CBF/ PCO_2 FOR 25 MG THRICE DAILY STUDY*

	Patient					
	EMcM	ER	FMcD	FM	AD	SR
Control	34/33	31/37	26/38	37/35	44/37	35/31
	30/38	26/38	28/39	37/35	44/37	35/31
2 h	42/37	39/38	28/36	36/34	43/34	28/30
4 h	35/35	34/35	33/35	41/35	43/35	37/29
1 week	40/32	41/31	36/30	43/32	46/36	33/24
	36/32	41/31	37/29	40/32	50/32	31/24
2 weeks	32/23	43/21	30/20	45/29	43/36	42/25
	32/32	43/31	34/29	45/29	45/33	37/23

*CBF in ml/100g/min and end-tidal PCO_2 in mmHg.

Analysed in this way, the precision of the technique is ± 5 ml/100 g/min or 16% (twice the standard deviation of the differences between control measurements).

3. REGIMEN 1 50 mg three times daily (Table 1). Cerebral blood flow was not significantly increased two or four hours after the initial oral dose, but after a week CBF was found to have increased by $31.5 \pm 6.4\%$ (mean and standard error); after a second week CBF was still significantly above the pre-treatment level ($28.6 \pm 2.9\%$) (Fig. 1a). For each patient, the average CBF increase after one and two weeks is statistically significant on an individual basis ($P < 0.05$ as described above).

End-tidal PCO_2 was not significantly changed two or four hours after the loading dose but was reduced after one and two weeks by $18.7 \pm 3.7\%$ and $19.0 \pm 4.3\%$ respectively (Fig. 1b).

4. REGIMEN 2 25 mg three times daily (Table 2). After the initial loading dose the increases in CBF were $12.6 \pm 9.6\%$ at two hours (not significant because of the scatter of results) and $11.2 \pm 3.7\%$ at four hours ($P < 0.05$) (Fig. 1a). After one and two weeks the increases in CBF were $19.5 \pm 7.9\%$ and $19.3 \pm 8.2\%$ respectively, compared with the control values ($P < 0.05$).

Although these increases were lower than the corresponding values for the 50 mg thrice daily

study, this was not significant on a statistical basis because of large individual differences of the five subjects common to regimens 1 and 2; two responded equally to the 25 mg thrice daily dose as to the 50 mg thrice daily dose.

End-tidal PCO_2 did not change significantly after two hours but fell by $6.7 \pm 1.4\%$ after four hours ($P < 0.02$), by $16.2 \pm 2.3\%$ after one week ($P < 0.002$), and $17.3 \pm 2.9\%$ after two weeks ($P < 0.002$).

5. REGIMEN 3 Six week study. The increase in CBF two weeks after starting on a regular 50 mg three times daily dosage was $25.0 \pm 6.8\%$. After an additional four weeks CBF was $21.5 \pm 4.3\%$ above control (Fig. 2a). There was no significant difference between the values of two weeks and six weeks (Table 3).

TABLE 3
CBF/ PCO_2 FOR SIX WEEK STUDY*

	Patient			
	FM	FMcD	ER	SR
Control	33/40	29/37	30/37	32/26
2 weeks	44/29	33/25	42/32	36/24
6 weeks	44/26	34/27	37/26	36/22

*CBF in ml/100g/min and end-tidal PCO_2 in mmHg.

FIG. 1 (a) Cerebral blood flow (CBF) and (b) end-tidal PCO_2 (PetCO_2) as a percentage of control for regimen 1 (50 mg thrice daily dosage) ■, and regimen 2 (25 mg thrice daily dosage) ○. Error bars indicate standard error of mean of six measurements.

End-tidal PCO₂ fell by $20.5 \pm 5.7\%$ and $26.7 \pm 4.3\%$ after two and six weeks respectively (Fig. 2b).

DISCUSSION

This study shows that a significant and persisting increase in the cerebral blood flow occurs after the oral administration of UK-12,130. Repeatability studies indicate that these changes would not be expected in these patients if they did not receive a drug. There was no consistent improvement in mentation associated with the increase in cerebral blood flow.

Increases found in CBF tended to be accompanied by decreases in end-tidal PCO₂, but as these were attributed to the effect of the drug it was not considered appropriate to apply corrections to the CBF values to compensate for changes in PCO₂. When erythrocyte carbonic anhydrase is inhibited the normal criteria for measuring blood and end-tidal PCO₂ do not apply. In the presence of CAI the normally rapid hydration of CO₂ (at the tissue capillary) and its dehydration (at the alveolar capillary) is incomplete, resulting in an increased gradient of CO₂ from tissue to alveolus, since CO₂ cannot reach equilibrium with H₂CO₃ (Maren, 1967). Thus, tissue and capillary PCO₂ and arterial H₂CO₃ will rise and alveolar PCO₂ falls.

An increase in CBF was observed four hours after the loading dose of UK-12,130 in the

second study, but not in the first. This can be explained by the high affinity of the erythrocyte for UK-12,130. It can be shown that after the first loading dose of 100 mg approximately 70% of the UK-12,130 is bound by the erythrocyte carbonic anhydrase, and about 20% is bound to plasma proteins and is not available for enzyme inhibition. Thus the plasma concentration, which reflects the availability to other tissues including brain, is very low. Data from young normal volunteers showed that after 100 mg of UK-12,130, plasma levels were undetectable, whereas erythrocyte levels were 20–30 µg/ml.

The clearance of UK-12,130 from the erythrocyte is slow ($T_{1/2} \approx$ two months) whereas from plasma $T_{1/2}$ is approximately 12 hours. The second study began between four and seven weeks after the first two week treatment of 50 mg three times daily and so there would be little drug in plasma but between 50% and 75% of the maximum concentration in erythrocyte. Administration of 100 mg UK-12,130 under these conditions would saturate the remaining erythrocyte binding sites and leave a significant amount of the drug in plasma. Consistent with this was the finding that administration of a second dose of UK-12,130 to the normal volunteers, one week after the first, produced significant plasma levels (0.5 µg/ml).

The persistent high level of CBF after six weeks in regimen 3 indicates that there is no significant tolerance effect.

CONCLUSIONS

UK-12,130 produces a significant increase in cerebral blood flow in demented geriatric patients which is persistent over some weeks.

It was found that it was practicable to use the inhalation method of measuring blood flow in this group of patients. It promises to be of value in other investigations calling for repeated measurements over a period of time, and in patients in whom invasive methods would not be justified.

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