Eur J Pediatr (1996) 155:117–119

Andrew Whitelaw
Marie-Christine Mowinckel
Vineta Fellman
Ulrich Abildgaard

Endogenous tissue plasminogen activator in neonatal cerebrospinal fluid

Received: 16 February 1995
Accepted: 7 June 1995

A. Whitelaw (✉)
Paediatric Department,
Aker University Hospital,
N-0514 Oslo, Norway
Tel.: 47-22-894599
Fax: 47-22-468467

M.-C. Mowinckel · U. Abildgaard
Haematology Research Laboratory,
Aker Hospital, University of Oslo,
Oslo, Norway

V. Fellman
Children's Hospital,
University of Helsinki, Helsinki, Finland

Abstract Tissue type plasminogen activator (tPA) plays a role in differentiation of neurones and activity-dependent structural changes in neurones. We hypothesised that tPA would also be present in CSF during fibrinolysis after intraventricular haemorrhage. We measured tPA antigen in CSF from 13 normal newborn infants and 14 infants with post-haemorrhagic ventricular dilatation (PHVD). tPA was undetectable or at the limit of detection (1 µg/l) in normal CSF. The CSF tPA concentration ranged from 1.3 to 3.5 µg/l in the infants with PHVD. Serial tapping in one infant showed persistence of tPA in the CSF from 3 to 8 weeks of age. We conclude that endogenous tPA may be part of the physiological response to intraventricular haemorrhage or may be present as a result of passive diffusion into the CSF.

Key words Newborn infant · Plasminogen activator · Cerebrospinal fluid · Intraventricular haemorrhage · Hydrocephalus

Abbreviations *IVH* intraventricular haemorrhage · *LTP* long-term potentiation · *PHVD* post-haemorrhagic ventricular dilatation · *tPA* tissue plasminogen activator

Introduction

Intraventricular haemorrhage (IVH) is a common complication of extremely preterm birth [3]. Only a small minority (usually with the largest haemorrhages) go on to develop post-haemorrhagic hydrocephalus. It is thought that multiple blood clots in the CSF can lead to hydrocephalus by obstructing the circulation and reabsorption of CSF [6, 7]. We have previously demonstrated that CSF from neonates without IVH showed no fibrinolytic activity on fibrin plate [17] but the majority of apparently normal neonatal CSF samples did contain small amounts of fibrin degradation products [18]. Following IVH, fibrin degradation products increased [18] and fibrinolytic activity became demonstrable after 2 or more weeks in the majority of cases [17]. We concluded that the neonate had considerable capacity for endogenous fibrinolytic activity in the CSF but that the process was not always adequate to clear large amounts of blood. In blood, fibrinolysis is normally initiated by the activation of the inactive pro-enzyme, plasminogen, to the active enzyme plasmin. Plasmin lyses fibrin into smaller degradation products. Physiologically, tissue type plasminogen activator (tPA) and urokinase type plasminogen activator are the enzymes which bring about the conversion of plasminogen to plasmin. Human tPA has been synthesised by recombinant DNA technology and has been used for the treatment of arterial thromboses for some years.

Intrathecal administration of human recombinant tPA has been widely used in adults with subarachnoid haemorrhage or IVH, to lyse blood clots in the CSF and prevent cerebral vasospasm, [4, 5, 9, 11, 15, 19] with an acceptably low rate of secondary bleeding. There is experimental animal evidence that intrathecal or intraventricular administration of tPA or urokinase can prevent post-haemorrhagic hydrocephalus [1, 12]. Prior to the possible therapeutic use of tPA intraventricularly in infants it seemed to

us essential to study the physiology and pathophysiology of tPA in relation to the CSF.

tPA has other important functions in the brain. Neurones and glial cells can produce tPA and there is evidence that tPA has a functional role in the morphological differentiation of neurones [10, 13]. Furthermore tPA is induced by an immediate-early gene in the dentate gyrus during long-term potentiation (LTP) [14] . LTP is the mechanism by which repeated stimulation of a pathway leads to a sustained increase in the resulting action potential. LTP has implications for learning and memory. It is postulated that tPA could contribute to activity-related structural changes in the brain by: (1) altering adhesive contacts between neurones; (2) changing the spatial and temporal interactions bewteen proteases and protease inhibitors; (3) activating a potential receptor [14].

In spite of tPA's important functions, it is not known whether tPA is present in neonatal CSF. The object of this study was to determine whether tPA was detectable in CSF from a) normal neonates and b) infants with post-haemorrhagic ventricular dilatation (PHVD).

Methods

CSF was obtained by lumbar or ventricular puncture for clinical indications and the CSF which was surplus to the bacteriology and clinical chemistry laboratories' requirements was frozen until tPA analysis. Because no prolongation or additional procedure was involved, no specific consent for tPA analysis was obtained from the parents. "Normal" CSF (without visible colouring) was studied from 13 infants with gestational ages 28–40 weeks aged 1 day–1 month who underwent lumbar puncture to exclude meningitis and were retrospectively found to be free of meningitis or intracranial haemorrhage. Lumbar CSF was collected from 14 infants with PHVD. These infants ranged in gestational age from 24 to 38 weeks and their postnatal age ranged from 8 days to 8 weeks. Large IVH (grade 3 or 4) had been documented by ultrasound scanning and lateral ventricular width had increased to 4 mm over the 97th percentile for postmenstrual age (gestational age + postnatal age) [8]. These diagnostic criteria were the same as those used in the multicentre trial of early tapping as treatment for PHVD [16]. In one infant with PHVD a series of 14 ventricular taps was carried out over a period of 5 weeks to reduce symptoms from raised intracranial pressure as the infant was unsuitable for surgical shunt insertion.

tPA assay

CSF was frozen a few minutes after collection. After thawing for analysis, the CSF was centrifuged and the supernatant analysed. tPA was measured by a double antibody enzyme-linked technique (Biopool TintElize tPA, Biopool AB, Umeå, Sweden). From each sample, 20 µl aliquots were added to two adjacent wells: one containing 2 µg normal goat IgG and the other containing 2 µg anti-human tPA. The samples were incubated at 25° C for 3 h. Any tPA in the sample then became linked to the anti tPA IgG. 50 µl of horseradish peroxidase labelled anti tPA IgG was then added to each well and incubated at 25° C for 3 h. The contents of each well were then discarded and the wells washed four times with buffer and dried. Orthophenylenediamine dihydrochloride and hydrogen peroxide (substrate for peroxidase) (200 µl) were then added and incubated at 25°C. After 15 min, 50 µl of 3.0 mol/l sulphuric acid was added to stop the reaction and the absorbance measured at 492 nm on a spectrophotometer. False-positive reactions can occur from the presence of anti-goat antibodies in the sample but with this method, for each sample, the difference between the readings from each well is specific for tPA. A standard curve was obtained using samples with known tPA concentrations of 0, 1.5, 3, 6, 12, 18, 24, and 30 µg/l. The limit of detection is 1.0 µg/l. The coefficient of variation intra-assay is about 6% and inter assay about 10%.

Results

tPA in the CSF from the normal infants was below the limit of detection, 1.0 µg/l, in 11 cases and around the limit of detection (1.0 µg/l and 1.1 µg/l) in 2 cases. CSF from the infants with PHVD showed detectable tPA in all cases with a range of 1.3–3.5 µg/l (median 2.3 µg/l) (Fig. 1). Serial ventricular tapping in one infant (Fig. 2) showed CSF tPA persisting from 3 to 8 weeks of age. The first four CSF samples from this infant showed detectable tPA but no detectable fibrinolytic activity when tested on a fibrin agar plate [17].

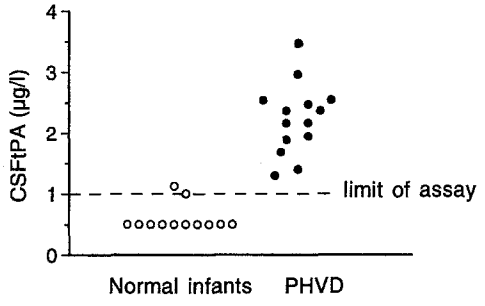

Fig. 1 CSF concentrations of tPA in 13 neonates without infection or haemorrhage (○) and 14 infants with PHVD (●)

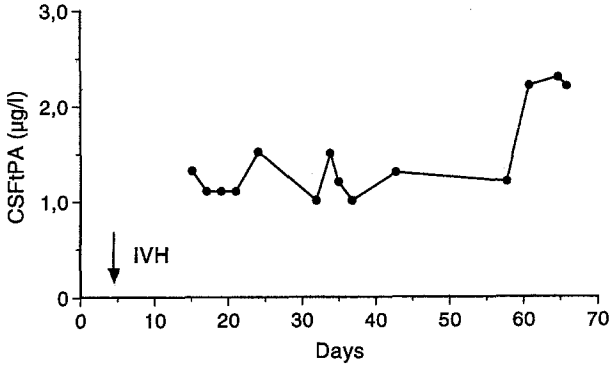

Fig. 2 Serial ventricular endogenous CSF tPA concentrations in one infant with PHVD. Fourteen ventricular punctures were carried out to control intracranial pressure between 2 and 9 weeks of age

Discussion

tPA was virtually undetectable in the CSF of normal neonates despite its involvement in differentiation and learning, both vitally important processes in newborn infants. tPA has a molecular weight of 70000 [10] and although it is secreted into the extracellular space in the brain [13], it would not be expected to easily diffuse from brain to CSF with an intact ependyma. The absence of tPA from normal CSF is in agreement with the absence of fibrinolytic activity we previously found in normal neonatal CSF [17].

tPA was found in all the CSF samples from infants with PHVD with a trend for the endogenous tPA CSF concentration to increase with time from 2 to 8 weeks after IVH. This is compatible with the observation that most infants with PHVD demonstrate fibrinolytic activity in the CSF several weeks after the haemorrhage and that it persists for some weeks [17]. The fact that four CSF samples had demonstrable tPA antigen without demonstrable fibrinolytic activity could be due to insufficient plasminogen, inactive tPA antigen or the presence of plasminogen activator inhibitor. It seems likely that tPA is part of the body's normal response to blood clots in the CSF. Stressed neonates have plasma tPA levels ranging up to 58 µg/l but umbilical cord plasma from normal neonates has an upper limit for tPA of 8.8 µg/l [2]. Thus it is possible that diffusion across a damaged blood/brain or brain/CSF barrier, rather than local production, could have accounted for the presence of tPA in the CSF.

Acknowledgements This study was supported by research grants from the Faculty of Medicine of Oslo University, the Odd Fellow Foundation, and the Norwegian Heart Association. We are grateful to Dr Dominique Acolet, Hillingdon Hospital, England, for his clinical collaboration.

References

1. Brinker T, Seifert V, Dietz H (1992) Subacute hydrocephalus after experimental subarachnoid hemorrhage: its prevention by intrathecal fibrinolysis with recombinant tissue plasminogen activator. Neurosurgery 31: 306–312
2. Corrigan JJ, Jeter MA (1992) Tissue-type plasminogen activator, plasminogen activator inhibitor and histidine-rich glycoproteins in stressed human newborns. Pediatrics 89: 43–46
3. De Vries LS, Larroche JC, Levene MI (1988) Germinal matrix haemorrhage and intraventricular haemorrhage. In: Levene MI, Bennett M, Punt J (eds) Fetal and neonatal neurology and neurosurgery. Churchill Livingstone, Edinburgh, pp 312–325
4. Findlay JM, Weir B, Kassell NF, Disney LB, Grace MG (1991) Intracisternal recombinant tissue plasminogen activator after aneurysmal subarachnoid hemorrhage. J Neurosurg 75: 181–188
5. Findlay JM, Weir BKA, Stollery DE (1991) Lysis of intraventricular hematoma with tissue plasminogen activator. J Neurosurg 74: 803–807
6. Hill A, Shackleford GD, Volpe JJ (1984) A potential mechanism of pathogenesis for early post-hemorrhagic hydrocephalus in the premature newborn. Pediatrics 73: 19–21
7. Larroche JC (1972) Posthaemorrhagic hydrocephalus in infancy. Biologia Neonatorum 20: 287–299
8. Levene MI (1981) Measurement of the growth of the lateral ventricles in preterm infants with real time ultrasound. Arch Dis Child 56: 900–904
9. Mizoi, K, Yoshimoto T, Takahashi A, Fujiwara S, Koshu K, Sugawara T (1993) Prospective study on the prevention of cerebral vasospasm by intrathecal fibrinolytic therapy with tissue-type plasminogen activator. J Neurosurg 78: 430–437
10. Neuman T, Stephens RW, Salonen EM, Tinmusk T, Vaheri A (1989) Induction of morphological differentiation of human neuroblastoma cells is accompanied by induction of tissue type plasminogen activator. J Neurosci Res 23: 274–281
11. Öhman J, Servo A, Heiskanen O (1991) Effect of intrathecal fibrinolytic therapy on clot lysis and vasospasm in patients with aneurysmal subarchnoid hemorrhage. J Neurosurg 75: 197–201
12. Pang D, Sclabassi RJ, Horton JA (1986) Lysis of intraventricular clot with urokinase in a canine model. 3. Effects of intraventricular urokinase on clot lysis and posthaemorrhagic hydrocephalus. Neurosurgery 19: 553–572
13. Pittman RN, Ivins JK, Buettner HM (1989) Neuronal plasminogen activator: cell surface binding sites and involvement in neurite outgrowth. J Neurosci 9: 4269–4286
14. Qian Z, Gilbert ME, Colicos MA, Kandel E, Kuhl D (1993) Tissue-plasminogen activator is induced as an immediate-early gene during seizure, kindling and long-term potentiation. Nature 361: 453–457
15. Stolke D, Seifert V (1992) Single intracisternal bolus of recombinant tissue plasminogen activator in patients with aneurysmal subarchnoid hemorrhage: preliminary assessment of efficacy and safety in an open clinical study. Neurosurgery 30: 877–881
16. Ventriculomegaly Trial Group (1990) Randomised trial of early tapping v conservative management in infants with post-haemorrhagic hydrocephalus. Arch Dis Child 65: 3–10
17. Whitelaw A (1993) Endogenous fibrinolysis in neonatal cerebrospinal fluid. Eur J Pediatr 152: 928–930
18. Whitelaw A, Creighton L, Gaffney P (1991) Fibrinolytic activity in CSF after intraventricular haemorrhage. Arch Dis Child 66: 808–809
19. Zabramski JM, Spetzler RF, Lee S, Papodopoulos S, Bovill E, Zimmerman RS, Bederson J (1991) Phase 1 trial of tissue plasminogen activator for the prevention of vasospasm in patients with aneurysmal subarachnoid hemorrhage. J Neurosurg 75: 189–196