CEREBRAL OEDEMA AND BLOOD-BRAIN AND BLOOD-CSF BARRIERS IN EXPERIMENTAL BRAIN TRAUMA : EFFECT OF INDOMETHACIN - A PROSTAGLANDIN SYNTHETASE INHIBITOR

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Summary : Cerebral oedema often occurs following trauma to the brain. Recently several biogenic amines have been suggested for their possible mediation in the pathophysiology of traumatic brain oedema. The present investigation indirectly indicates that prostaglandins of E series are also involved in the etiology of cerebral oedema, since administration of a potent PG synthetase inhibitor, Indomethacin significantly diminished oedematous swelling of traumatised rat brain.

Key words : brain trauma, cerebral oedema, blood-brain barrier, prostaglandin, indomethacin, inhibitor

INTRODUCTION

Recently, a series of investigations by several workers have provided the evidence that serotonin and catecholamines are involved in the pathophysiology of oedema following trauma to brain and cord (1,11,12,13,18).

Prostaglandins (PG) are also known biologically active and vasoactive substances like the monoamines. Their role in the dynamics of brain oedema production, however, have been scantly reported. The present paper reports the reduction of experimental traumatic oedema in rats by pretreatment with indomethacin, a potent PG synthetase inhibitor.

MATERIALS AND METHODS

All the experiments were carried out in albino rats (Charles Foster strain) of both sexes (120-200 g) under urethane (Riedel-de Haen AG, Seelze-Hannover) anaesthesia (1.8 *g/kg*, i p).

Evaluation of permeability of barriers and oedema following trauma

In a group of animals, the right cerebral cortex was exposed and stab injury was

made of 3 mm long and 2 mm deep over the cortical region located 3 mm posterior to bregma and 2 mm lateral from midline. Blood was allowed to clot and it was gently wiped out with saline soaked cotton. At the end of 40 min, following trauma, the permeability of blood-brain (BBB) and blood-csf (BCSF) was assessed by slowly injecting 0.4 *ml* of bromophenol blue dye (0.8%) through right common carotid artery whose external branch was ligated before injection of dye. Since this dye is normally impermeable to brain, therefore, if any blue staining of the cerebral cortical tissue and cerebroventricular wall occur, it would indicate the penetration of dye across cerebral capillaries into brain tissue as well as across choroid plexus into CSF respectively (2). Five minutes after dye injection, the whole brain was taken out and blue staining, if any, of the cerebral tissue and cerebroventricular wall were noted.

For the determination of oedematous swelling, the same brain was sharply incised along the midsagittal line into right and left half. The wet and dry weight of each half were noted and the percentage of water content for each half was calculated. The dry weight of the tissue was determined after repeatedly drying the sample in an oven at 50°C, until the weight became constant. The percentage of oedematous swelling was calculated from the following formula (16).

$$\frac{(\%\ \text{water in control}) + f}{100 + f} = \frac{\%\ \text{water in experiment}}{100}$$

where f is the % of swelling due to oedema.

In another group or animals, 4 *mg/kg* of indomethacin (Sigma Chemicals Co., U.S.A.) was administered intraperitoneally 20 min before traumatic injury was induced on right cerebral cortex following the procedures described above. Another dose of indomethacin, 2 *mg/kg* was injected ip 20 min after trauma was produced in order to sustain the PG inhibiting action of indomethacin. At the end of 40 min following stab injury, the permeability of brain barriers and oedema were determined following the same procedures already described. Students 't' test was used for statistical calculations.

RESULTS

In 5 rats, following a stab injury in the right carebral cortex, there occurred an increase in water content in the traumatised half of the brain as compared to that of left half of brain which acted as control in the same animal. Thus the Table I show that there is 2.61% increase in water in the traumatised brain (right half) as compared to the control. This increase in water in the injured brain led to an average of 10.81% swelling of the same as calculated from percentage of water in each half of the brain (16). This finding

indicates that a small increase of water in brain can result in a greater proportional increase in its volume which is in accordance with findings of other workers (16). Since the dry weight difference of each half of the brain in the same rat was found to be very small, as well as the mean difference of this dry weight between the control and indomethacin treated rats is almost negligible, therefore the percentage increase in water and oedematous swelling following trauma can be considered due to the result of actual accumulation of fluid in the traumatised brain.

TABLE I : Shows the various data obtained at the end of 40 min from left and right half of rat brain following stab injury on the right cerebral cortex.

Animal No.	*Dry weight difference between left and right half of brain (mg)*	**Difference of percentage of water content between left and right half of brain (mg)*	***Percentage of swelling*
1	4	5.80	19.73
2	8	1.70	10.2
3	6.1	1.13	5.10
4	1.0	2.02	9.29
5	14	2.35	9.91
Mean :	6.62±4.7	2.61±1.8	10.81±5.38

*Percentage water calculated from dry and wet weight of each half of brain.
**This has been calculated from the formula of Rapoport (16).

In 10 rats, PG synthetase inhibitor, indomethacin, 4 *mg/kg* was intraperitoneally administered 20 min before inducing trauma in right cerebral cortex, and another dose of 2 *mg/kg*, 20 min after trauma. The percentage increase in water and corresponding oedematous swelling in traumatised cortex, determined at the end of 40 min after injury, showed an average value of 0.92% and 3.73% as compared to control value of 2.61% and 10.81 respectively (Table II). Thus inhibition of PG synthesis in brain led to remarkable diminution of occurrence of oedema as a result of injury. Further increase in the dose of indomethacin (10 *mg* and 5 *mg/kg* before and after respectively) did not show further diminution of oedematous swelling as compared to lower dose.

Interestingly, indomethacin did not prevent or diminish the breakdown of BBB and BCSF barrier caused as a result of trauma (Table III).

TABLE II : Shows the various data obtained at the end of 40 min from left and right half of rat brain following stab injury on right cerebral cortex in indomethacin treated rats.

Animal No.	*Dry weight difference between left and right half of brain (mg)*	*Difference of percentage of water content between left and right half of brain (mg)*	*Percentage of swelling*
1	8	0.35	1.20
2	7	0.70	2.60
3	7	0.48	1.85
4	6	0.79	3.51
5	5	1.01	4.45
6	2	0.99	4.48
7	3	0.81	3.32
8	6	2.19	8.62
9	9	0.57	2.24
10	1	1.38	5.04
Mean ± SD	5.4±2.6	0.92±0.5 P* <0.05	3.73±2.11 P* <0.01

*Compared to control.

TABLE III : Shows the permeability of blood-brain and blood-csf barrier in the traumatised right cerebral cortex at the end of 40 min following stab injury in control and indomethacin treated rats.

Animal No.	*Control group*		*Indomethacin treated group*	
	Blood–brain	*Blood–csf*	*Blood–brain*	*Blood–csf*
1	+	—	+	+
2	+	—	—	—
3	—	—	+	—
4	—	—	+	+
5	+	—	+	+
6	+	+	+	+
7	+	+	+	—
8	+	+	+	+
9	—	—	+	+
10	+	+	+	+

The animal nos. 1 to 5 in control group correspond to those of Table-I and animals treated with indomethacin correspond to animal nos 1 to 10 of Table–II.

+Means opening of barrier; — means no opening of barrier

DISCUSSION

Glenn *et al.*(4) have shown that PGE_1 and PGE_2 produce oedema when injected into the hind paw of rats. And the oedema caused by injection of either histamine or serotonin was further aggravated when PG of E series are injected with histamine or serotonin. Higgs *et al.* (9) have shown that PG of E series are accumulated in carrageenin induced inflammatory exudate in rats and indomethacin inhibits such inflammatory swelling by 55%. Ferreira *et al.*(3) have shown that carrageenin induced inflammatory paw swelling in rats can be strikingly increased by PGE_1 or PGE_2 injection, and conversely, the swelling can be profoundly inhibited by prior administration of indomethacin. They have suggested that when a continuous generation of PG occurs, it can increase the generation of oedema throughout the development of the inflammatory reaction. There are now increasing evidence that PG of E series are released in inflammatory conditions like allergic reactions (14), ultra-violet induced inflammation (5) and in chronic rheumatoid arthritis (8). In patients with rheumatoid arthritis, indomethacin produces good symptomatic relief with decreased inflammatory swelling in affected joints (7,8). These findings strongly indicate that PG of E series play an important role in the genesis of inflammatory oedema (6).

Since oedema is a constant feature in traumatic brain injury, it is presumed that PG may also play a role as one of the chemical factors in the pathophysiology of oedema in brain trauma. The present result definitely support this view. Thus administration of indomethacin, a potent PG synthetase inhibitor, led to 66 percent diminution of oedematous swelling in acute traumatised rat brain and this finding envisages a role of PG in oedema formation. The release of prostaglandins in traumatised brain may occur from platelets. That is, in brain trauma, there occurs a severence of some blood vessel and blood platelets aggregate in the traumatised tissue. These platelets are known to be very rich in PG of E series (10,17) besides 5-HT. The contents of platelets have been shown to be always released following their aggregation. It has been shown by other workers that administration of indomethacin or aspirin in man produced inhibition of aggregation of platelets along with profound inhibition of PG synthesis in them (10). Thus disintegrated platelets in the traumatised zone may serve as potent source of prostaglandins. Besides platelets, prostaglandins can also be released from the neural tissue of traumatised zone since brain tissue also synthesis PG (15). The possible mechanisms by which the released prostaglandins are involved in brain oedema may be (i) through increased permeability of cerebral capillaries, (ii) producing ischemia by vasoconstrictor action (19) or (iii) through potentiating the actions of other chemical agents (5-HT or catecholamines) which may participate in oedema formation. The present results does not support the idea that PG may induce oedema by increased permeability of cerebral capillaries, because indomethacin, though it had remarkably diminished the oedema in experimental trauma, yet breakdown of blood-brain and blood-csf barriers were still observed in and around traumatised area of brain. The third possible mechanism is being considered now.

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