ISCHAEMIA REPERFUSION ENDOTHELIAL INJURY IN THE RABBIT BRAIN

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SUMMARY:

The distal internal carotid artery (DICA), middle cerebral artery (MCA) and Azygous anterior cerebral artery (AzACA) were isolated via the transorbital approach in anaesthetized rabbits. Cerebral blood flow (CBF) was measured using radiolabelled microspheres before, during and 15 minutes after one hour's unilateral occlusion of DICA, MCA and AzACA. At 50 minutes of ischaemia, animals received either a placebo (control) or one of four drugs; superoxide dismutase (SOD) (25 mg/kg), catalase (10 mg/kg), methimazole (MMI) (5 mg/kg) or indomethacin (10 mg/kg). A sixth group was maintained on a tungsten-supplement diet for 14 days prior to the induction of ischaemia. A seventh group was sham operated.

Microvascular integrity within the brain was assessed by leakage of albumin-dye complex (ADC) across the blood-endothelial barrier (BEB), as quantified by microspectrofluorometry. In brain regions with substantial ADC leakage, CBF was reduced by 65% during ischaemia as compared with a 23% reduction in brain regions maintaining normal vascular integrity. CBF values returned to or exceeded baseline levels during reperfusion.

In the control group, ADC leakage across the BEB was increased 50% within the occluded hemisphere (OH), and extensive ADC extravasation into the interstitial space was seen. All treatment groups (SOD, catalase, indomethacin, MMI, and tungsten diet) afforded protection of cerebral endothelium during reperfusion as evidenced by minimal ADC leakage into the interstitial space.

The present study shows that the increased leakage of ADC into the brain parenchyma associated with ischemia/reperfusion (I/R) is increased by O2 radical formation, produced by arachidonic acid (AA) catabolism and by the conversion of hypoxanthine to xanthine by xanthine oxidase (XO). In addition, it appears that neutrophils are also involved in the process since MMI protected the endothelial damage associated with I/R.

KEY WORDS:

Cerebral Ischaemia, SOD, MMI, Tungsten, Catalase, Indomethacin, Free Radicals.

Abbreviations:

AzACA: Azygous anterior cerebral artery,
DICA: Distal internal carotid artery,
MCA: Middle cerebral artery,
CBF: Cerebral blood flow,
SOD: Superoxide dismutase,
MMI: Methimazole,
ADC: Albumin-dye complex,
BEB: Brain endothelial barrier,
I/R: Ischemia/reperfusion,
AA: Arachidonic acid,
XO: Xanthine oxidase,

CSF: Cerebrospinal fluid,
MABP: Mean arterial blood pressure,
EB: Evan's blue,
RBF: Renal blood flow,
OH: Occluded hemisphere,
UH: Unoccluded hemisphere,
nm: Nanometer,
CNS: Central nervous system,
SAH: Subarachnoid hemorrhage,
PGH synthase: Prostaglandin H synthase,
EDRF: Endothelium derived relaxing factor,
XD: Xanthine dehydrogenase,
PGs: Prostaglandins,
DMTU: Dimethylthiourea

INTRODUCTION:

Immediate restoration of blood flow to ischaemic brain tissue may improve the clinical outcome. However, reperfusion of ischaemic neural tissue has been correlated with a breakdown of the BEB with a subsequent increase in microvascular permeability leading to cerebral aedema (16). The precise mechanisms of breakdown of the BEB leading to the cellular damage and associated with increases in microvascular permeability are not well defined. However, the roles of oxygen-derived free radicals have been proposed (4,8,21).

In many organ systems, oxygen-derived free radicals have been implicated in the vascular disruption associated with reperfusion of ischaemic tissue (4,21). Evidence indicates that, with reperfusion and restoration of oxygen to the tissue, there is rapid production of superoxide anion ($\cdot O_2^-$) and hydrogen peroxide (H_2O_2) (24). In the presence of iron or other transition metals there follows production of the hydroxyl radical ($\cdot OH$) which subsequently causes extensive tissue damage (24). CSF contains enough free catalytic iron to support this reaction (13). In addition, tissue damage will activate the neutrophil system which in turn can result in further production of oxygen radicals and additional tissue damage (11).

In this study, using free radical scavengers such as SOD, catalase, MMI, indomethacin and pretreatment with tungsten we have shown significant protection of the BEB following reperfusion injury. That was established by a significant decrease of the dye leakage within the ischemic hemisphere.

METHODS:

General Protocol: Seventy-four adult New Zealand white rabbits of either sex, weighing between 2 and 3.5 kg. were used in these experiments. All surgical procedures were approved by the Institution Animal Care Committee and conform with guidelines set forth in the NIH "Guide for Care and Use of Laboratory Animals". The animals were premedicated with 25 mg/kg of chlorpormazine (Sigma) and 50 mg/kg of ketamine HCl (Ketalar, Parke-Davis) intramuscularly. A marginal ear vein was canulated and anaesthesia was induced with pentobarbital sodium (Butler; 10 mg/kg/hour). A tracheostomy was then performed and the animal was mechanically respirated with room air. Neuromuscular blockade was achieved by intravenous administration of pancuronium bromide (Elkins-Sinn; 05 mg/kg/hour). A catheter was introduced into the aortic arch via a femoral arteriotomy and was connected to a pressure transducer (Statham-Gould P23d), for continuous measurement of mean arterial blood pressure (MABP) (Grass Model 7D polygraph, Quincy, MA). A left atrial line was inserted via left thoracotomy for injection of radiolabelled microspheres (see Blood Flow Measurements). Frequent arterial samples were obtained for blood gas determination (Instrumentation Laboratories Model 513 blood gas analyzer). Blood gases were maintained within normal limits by appropriate adjustments of the mechanical ventilator. Body temperature was measured with a rectal probe and maintained at 37°C with a heating pad.

Ten animals were used for the sham operated group. Two different types of sham operation were performed, as follows; Sham I. without transorbital approach (4 animals), and Sham II. transorbital approach, and dissection of cerebral arteries without clippage (6 animals).

For induction of focal cerebral ischaemia the right globe was decompressed and the orbital

content removed to expose the optic foramen. Using a dissecting microscope and dental drill, a retroorbital craniectomy was performed superior and lateral to the optic foramen to expose the dura mater overlying the MCA, AzACA, and DICA. The dura mater was opened and the arachnoid membrane dissected to isolate these vessels (Figure 1).

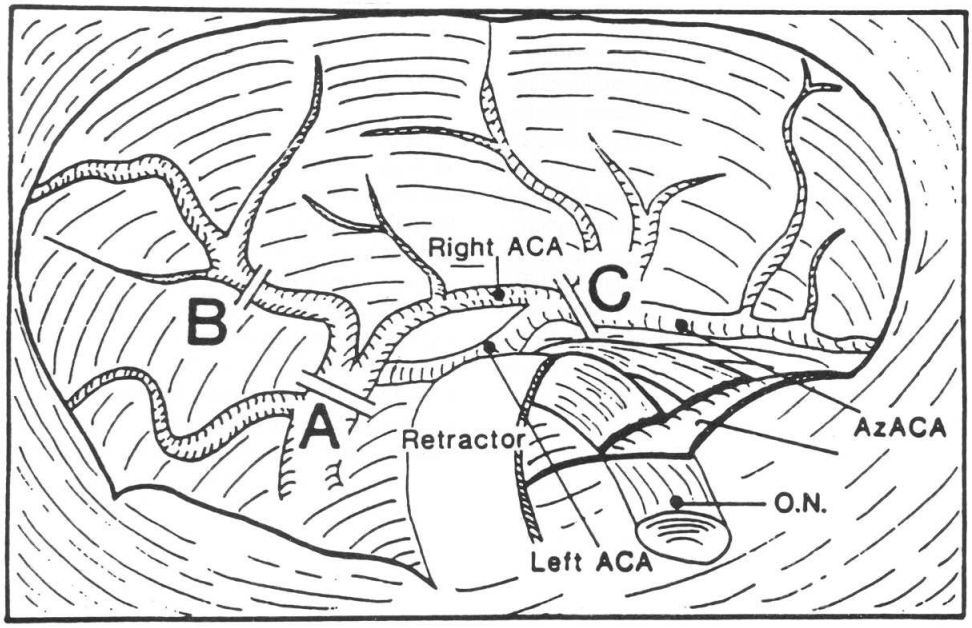

Fig 1 : View of the ACA, AzACA, and ON following unilateral transorbital approach. ON:Optic nerve. Letters A to C indicate each intracranial artery. A: DICA, B: MCA, C:AzACA. Bars (=) next to each letter represent the site of clip application.

After isolation of these vessels preocclusion CBF was determined by radiolabelled microsphere injection (see Blood Flow Measurements). Sixty-four animals were then assigned at random to 1 of 5 experimental groups. In group 1 (n=22) the animals underwent the clippage of AzACA, MCA, and DICA for one hour at the points indicated in Figure 1. Forty minutes after clippage CBF was measured during the ischaemic period. Ten minutes later (after 50 minutes of ischaemia and 10 prior to reperfusion) the animals received a bolus injection of 10 ml normal saline. After 60 minutes of ischaemia, the microaneurysm clips were removed and reperfusion was allowed. After 15 minutes of reperfusion a final CBF determination was made. In the animals in groups 2 to 5 the clips were applied as in group 1; however, after 50 minutes of ischaemia (10 minutes prior to reperfusion), these groups were administered one of the four following agents dissolved in 10 ml saline; Group 2 (n=9) 25 mg/kg of bovine copper-zinc SOD (Peroxinom, Pharmacia Pharmaceuticals); group 3 (n=8) 10 mg/kg of catalase (Sigma); group 4 (n=9) 5 mg/kg MMI (Sigma); and group 5 (n=6) 10 mg/kg indomethacin. For group 6 (n=10), 14 days prior to the terminal experiment, animals were maintained on a tungsten-supplemented diet (0.7 gr sodium tungstate/kg food, ICM Biomedicals). For group 6, the terminal experiment utilized the protocol as described for group 1.

After the final microsphere injection, Evan's Blue (EB) was infused intravenously (30 mg/kg). At this dose EB is completely bound to serum albumin and in the normal state the BEB presents an impermeable barrier to this ADC (30). The animals were sacrificed 60 minutes later by pentobarbital overdose (65 mg/kg) and the brain and kidneys were harvested. Brains

were fixed for 48 hours in 10% formaldehyde and 30% sucrose. They were then frozen and cut in a rostral to caudal manner in alternating 500u and 50u thick sections (slicing block microtome). The 500u brain slices were used for CBF determinations. The 50u slices were viewed using epifluorescent and light microscopy to assess microvascular integrity (see Microvascular integrity).

Blood Flow Measurements: Separate determinations of CBF and RBF measurements were made using radiolabelled microspheres (15um diameter; 50dpm/microsphere) during preocclusion, at 40 minutes of ischaemia and at 15 minutes of reperfusion. Microspheres (Ce-141, Sr-85, Sc-46, Nen-Tract, Du Pont, Wilmington, DE, $2x10^5$/ml) were vigorously agitated for 5 minutes before being injected through the left atrial catheter at 0.5 cc volume over a 10 second period followed by 0.5 cc saline flush. Reference blood samples were collected using a Harvard pump (Harvard Apparatus) from the arterial (aortic) catheter at a rate of 1.36 ml/minute starting 10 seconds before injection. The order of microsphere injections was randomized for each individual experiment. Blood withdrawal continued for 2 minutes after injection with equal volume replacement of blood using normal saline. After sacrifice and subsequent serial sectioning of the rabbit brain the 500u sections were first divided into hemispheres that had been subjected to focal ischemia (OH) and contralateral hemispheres (unoccluded hemisphere-UH). These 500u slices were than grouped into rostral, middle and caudal sections for assessing regional changes in CBF during control, ischaemia and reperfusion time periods. In 42 of the animals, these slices were additionally subdivided, based on macroscopic examination of the OH, into regions with extensive ADC leakage (blue tint) or sections with minimal ADC leakage (normal). Corresponding sections were taken from the contralateral hemisphere within each 500u tissue slice for paired comparisons. Tissue (Brain and kidney) and blood samples were then placed in a LKB gamma counter and radioactivity in each tissue sample and from each of the three arterial blood withdrawals was determined and corrected for energy overlap and background. Blood flow to various tissue samples (Q_T) was then calculated for each microsphere injection on a per gram wet tissue basis according to the equation

$$Q_T = (R_T \times Q_{ref}/R_{ref})/wt$$

where wt is the tissue weight, RT and Rref are radioactive counts in the tissue and reference blood flow samples, and Qref is the withdrawal rate of the arterial blood sample, ie the reference organ (23).

Microvascular Integrity: The 50u sections were examined by both light microscopy and microspectrofluoroscopy (Lietz MPV-3 Microfluorometer) to determine the amount of ADC leakage across the BEB. The brain tissue sections were exposed to light of wavelength of 530-560 nm, which excites the dye complex and the dye complex in turn, fluoresces the light of a wavelenght of 580 nm. The amount of emitted light (580 nm) was quantified fluorometrically. The amount of epifluorescence is directly proportional to the amount of dye present in the tissue section (12). Paired observations were made between the ratio of epifluorescence in the OH/ epifluorescence in the UH to determine relative ADC leakage into the cerebral interstitial space. At least 16 paired measurements were made at random points within each mounted section.

Statistical Analysis: Mean responses (+SE) were determined for each variable (MABP, CBF, RBF [renal blood flow], arterial blood gases, CNS [central nervous system] tissue epifluorescence, and body temperature) during the control, ischaemic and reperfusion time periods. Within-group comparisons between control, ischaemic and reperfusion periods were analyzed by correlated analysis of variance. Between-group effects at each time period (control, ischaemia, and reperfusion) were likewise analyzed by analysis of variance. Whenever a significant F ratio was achieved, a protected t-test (least squared difference) was applied to identify mean differences reaching statistical significance.

RESULTS:

Physiological parameters are given in Table 1. In all groups MABP, RBF, pO_2, pCO_2 or temperature were not significantly different among preocclusion, ischaemia or reperfusion time periods. However, there were small but significant decreases in pH and bicarbonate measurements between the preocclusion and both ischaemia and reperfusion time periods.

Table 1 : Physiological parameters of the animals.

	Pre-occlusion	Ischaemia	Reperfusion
MABP(mm/Hg)	108±3	96±4	103±3
RBF(ml/min/gr)	2.3±0.8	2.4±0.9	2.3±0.8
pH	7.42± .02	7.40± .02*	7.40±.02*
pO2(mm/Hg)	117.1±2.5	118.7±2.9	119.5±3.0
pCO2(mm/Hg)	26.7±0.7	25.9±0.6	25.7±0.7
HCO3-(mEq/L)	17.4±0.6	16.2±0.7*	16.1±0.7*
Temperature(C°)	37.1±0.1	37.1±0.1	37.1±0.1

MABP: Mean arterial blood pressure.
RBF: Renal blood flow.
Asterisks (*) indicate significant difference ($p<0.05$) from preocclusion levels.

Both groups of the sham operated animals showed minimal difference of CBF measurements among pre-occlusion, ischaemia and reperfusion time periods.

Data of the mean CBF (+SE) from group 1 (control group) showed that within the UH (Figure 2, Panel A), there were no significant changes in CBF among preocclusion, ischaemia and reperfusion time periods. Each hemisphere was further subdivided into rostral, middle and caudal sections. The overall patterns in CBF were similar for all 6 experimental groups during ischaemia. In contrast, in the OH a significant drop in CBF was noted at 40 minutes of ischaemia (Figure 2, Panel B, shaded bars). With clip removal, CBF returned to preocclusion levels throughout the entire brain.

Since patterns of CBF changes during ischemia in rostral, middle and caudal brain sections were similar, data was pooled based solely on OH versus UH. Within the OH, blood flows at 40 minutes of ischaemia were significantly

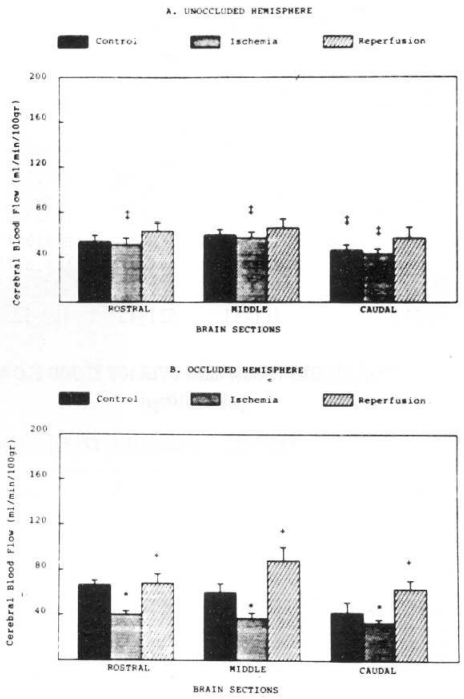

Fig. 2 : Cerebral blood flow values in the rostral, middle and caudal sections of the unoccluded (Panel A), and the occluded (panel B) hemispheres of the control group animals (n=24). Asterisks ( ) indicate significant differences ($p<0.05$) of cerebral blood flow values between preocclusion (filled bars) and ischaemia (shaded bars) or reperfusion (cross-hatched bars) time points; crosses (+) indicate significant difference between ischaemia and reperfusion time points; double crosses (++) indicate significant differences at each time point between occluded and unoccluded hemispheres.

less than the corresponding levels in the UH for all groups (Table 2). Hyperaemia, relative to both pre-occlusion and ischaemia time points were observed at 15 minutes of reperfusion in every group with the exception of the control group. Within the UH, blood flows were unaffected during ischaemia, but hyperaemia was noted in the groups treated with SOD (group 2) and MMI (group 4) at 15 minutes of reperfusion.

Focal cerebral ischaemia produced heterogeneous damage throughout the OH of

Table 2 : Cerebral blood flow values during each experimental protocol.

OCCLUDED HEMISPHERE AVERAGE BLOOD FLOW (ml/min/100gr)

TREATMENT	PRE-OCCL	ISCHAEMIA	REPERFUSION
CONTROL	51.3±6.2	34.6±3.5*	55.6±5.6^{+}
SOD	36.5±3.1	27.9±3.2*	49.8±3.4*$^{+}$
CATALASE	34.2±3.4	22.6±1.2*	57.2±5.2^{+}
METHIMAZOLE	42.4±3.6	28.7±3.8*	56.5±4.3*$^{+}$
INDOMETHACIN	43.7±4.9	34.6±5.0	62.9±6.4*$^{+}$
TUNGSTEN	42.9±3.6	32.2±7.0	64.2±9.4*$^{+}$
SHAM I	48.1±3.0	50.3±3.0	49.2±3.0
SHAM II	51.1±3.1	52.1±4.1	51.2±3.2

UNOCCLUDED HEMISPHERE AVERAGE BLOOD FLOW (ml/min/100gr)

	PRE-OCCL	ISCHAEMIA	REPERFUSION
CONTROL	52.3±6.9	49.5±5.9#	54.6±6.5
SOD	41.7±4.7	37.4±4.6#	49.1±4.4^{+}
CATALASE	36.8±3.2	31.4±1.9#	36.7±0.9#
METHIMAZOLE	47.2±5.9	42.1±5.9#	56.1±6.8^{+}
INDOMETHACIN	42.9±3.4	46.0±5.4#	59.7±9.9
TUNGSTEN	41.5±3.0	42.9±10.4#	42.6±6.2#
SHAM I	49.3±3.0	48.2±3.1	49.2±1.1
SHAM II	52.8±4.3	52.3±4.2	52.2±4.1

(continued)
Continuation of Table 2.
Asterisks (*) indicate significant difference from preocclusion levels.
Crosses (+) indicate significant difference between ischaemia and reperfusion.
Pound signs (#) indicate significant difference between occluded and unoccluded hemispheres.

the rabbit brain. Analysis of the relative changes of CBF in the rostral, middle and caudal segments of both hemispheres, were further subdivided based on macroscopic evaluation of relative microvascular damage of the OH. Within the OH, brain tissue was subdivided into segments with substantial blue tint (indicative of ADC leakage into the interstitial space) (Figure 3) and normal sections that were white (indicative of minimal ADC leakage from intravascular space). Corresponding regions were taken from the UH for paired comparisons (Figure 4). In the UH (Figure 5, Panel A) minimal changes in CBF were encountered. In contrast,

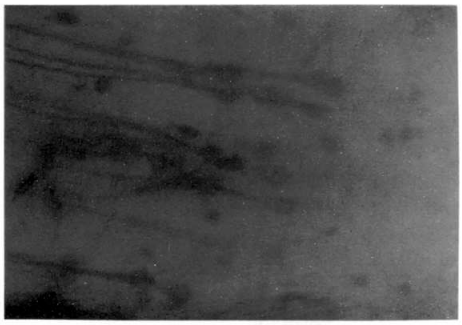

Fig. 3 : Extensive distribution of ADC throughout the interstitial space of the blue tint tissue of OH. Moreover the extent of the ADC leakage into the interstitial space was sufficient for neuronal uptake.

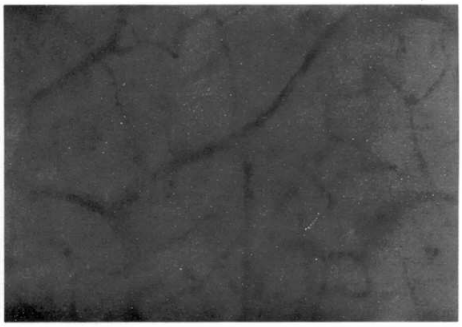

Figure 4. Shows that the ADC was primarily restricted in the intravascular space of UH.

a significant decrease of CBF was measured within the regions of the brain showing blue tint compared to the normal white tissue segments within the OH (Figure 5, Panel B).

Table 3 illustrates the average changes in CBF for all groups based on relative flow changes in normal versus blue tint tissues of the OH compared to the matched regions within the UH. Overall, the one-hour unilateral occlusion of three cerebral arteries produced substantial ADC leakage in approximately 18% of the brain mass within the OH. There was no significant difference in blood flow during the preocclusion time period in any cerebral region. During ischaemia, blood flow was reduced throughout the OH, but especially within the blue tint tissues where it was reduced by 65%.

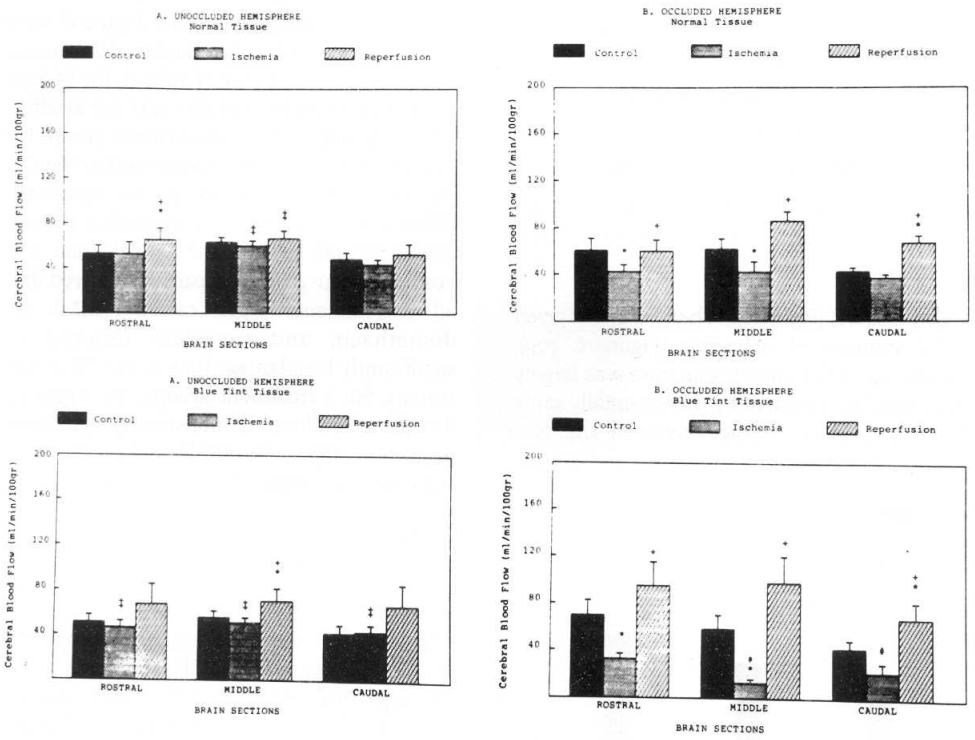

Fig. 5 : Cerebral blood flow values in the rostral, middle and caudal sections of the unoccluded (panel A) and occluded (panel B) hemispheres of the control group animals (n=22). While bars on the left side of the graph (Panel A) show blood flow measurements from the UH with normal and white appearance tissues on macroscopic examination; bars on the right side represent blood flow values from brain tissue with significant blue appearance on macroscopic examination of the OH (panel B). Panel A indicates the corresponding regions of the UH. Asterisks (*) indicate significant differences ($p<0.05$) between preocclusion (filled bars) and ischaemia (shaded bars) or reperfusion (cross-hatched bars) time points; crosses(+) indicate significant difference between ischaemia and reperfusion time points; double crosses (++) indicate significant differences at each time point between OH and UH; pound signs (#) indicate significant difference between normal and blue tint tissues.

Table 3. rCBF values between hemispheres and normal and blue tint tissue samples.

	NORMAL TISSUE BLOOD FLOW (ml/min/100gr)		
	PRE-OCCL	ISCHAEMIA	REPERFUSION
OH	44.5∓2.4	34.3∓2.4*	61.1∓2.9*
UH	45.3∓2.1	43.4∓3.0	51.9∓3.3*

	BLUE TINT TISSUE BLOOD FLOW (ml/min/100gr)			%BLUE TINT
	PRE-OCCL	ISCHAEMIA	REPERFUSION	TISSUE
OH	42.9∓3.0	14.9∓1.8*+	75.6∓6.7*+	17.1∓1.1
UH	41.6∓2.5+	39.9∓3.4+	49.1∓5.1	

OH: Occluded Hemisphere, UH: Unoccluded hemisphere.
Asterisks (*) indicate significant difference from preocclusion levels.
Crosses (+) indicate significant difference between normal and blue tint tissues.

In contrast, blood flow was reduced only 23% in the OH in CNS regions which exhibited minimal ADC leakage. In addition, the hyperaemia associated with reperfusion was greater within the blue tint tissues compared to normal tissue.

Using microfluorometric techniques, the location and extent of ADC leakage into the interstitial space can be compared between OH and UH. For each brain section (rostral, middle or caudal), the epifluorescence of the occluded hemisphere was normalized (% change) to the corresponding areas of the UH. While both sham operated group animals did not show any ADC leakage on either hemisphere (Table 4), I/R injury showed a 50% greater dye leakage in the

Table 4 : % Fluorescent dye leakage between hemispheres of the sham operated group animals.

	% LEAKAGE OF FLUORESCENCE (OCCLUDED/UNOCCLUDED HEMISPHERE)			
	ROSTRAL	MIDDLE	CAUDAL	TOTAL
SHAM I	1.01∓0.03	1.01∓0.02	1.01∓0.01	1.01∓0.01
SHAM II	1.00∓0.02	1.00∓0.01	1.00∓0.01	1.00∓0.01

OH versus UH (Figure 6). When SOD was given at 50 minutes of ischaemia (Figure 6, top), disruption of the microvasculature was largely prevented as is evident by the essentially same amount of ADC leakage between the two hemispheres. Similar results were noted when catalase, indomethacin or MMI (Figure 6) were given intravenously at 50 minutes of ischaemia. In contrast, maintenance of animals for 14 days on tungsten-supplement diet was not as effective as the antioxidant administered groups for preventing excess ADC leakage within the OH (Figure 6, top). For all groups, no significant differences were noted in relative dye leakage among rostral, middle and caudal brain sections. Between- group comparisons showed that all 5 treatments (SOD, catalase, MMI, indomethacin and tungsten) resulted in significantly less damage than in the I/R group. Among the 5 treatment groups, the tungsten diet protected least, demonstrating significant protection only in the middle and caudal brain segments as compared to I/R group.

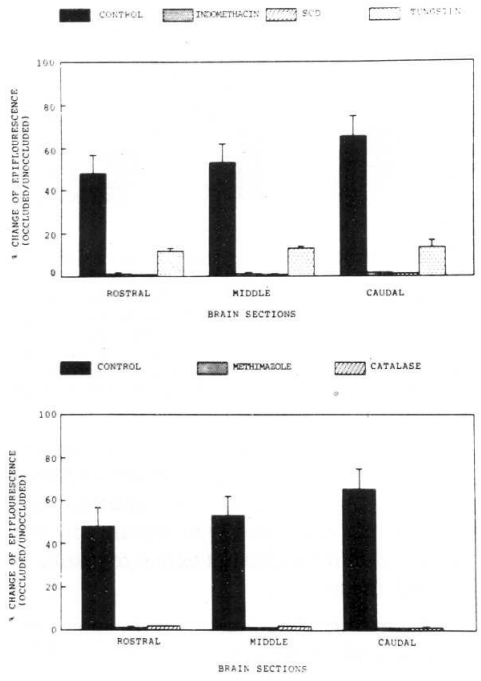

Fig. 6 : Comparison of the amount of ADC leakage following the BEB disruption was determined by microfluorometric assay. Paired observations were taken between the occluded and unoccluded hemispheres. Values calculated as % of ADC leakage OH/ % of ADC leakage UH. ADC leakage in all treatment groups (2 to 6) are significantly less than the controls.

DISCUSSION:

In this study, we have quantified the extent and location of ADC extravasation within the rabbit brain based on the fluorescent properties of EB by measuring its fluorescence using microspectrofluorometry (12). These data indicate a 50% increase in overall ADC leakage across the BEB one hour after focal cerebral I/R. This damage is evident throughout the rostral, middle and caudal sections of the OH. We have also demonstrated that during ischaemia, while the regional CBF of the blue tint tissue of the OH decreased to 65% of the preocclusion levels, normal tissue blood flow of the OH decreased 23% of the preocclusion levels.

We have demonstrated that antioxidant administration or XO+XD depletion by tungsten supplement diet, showed significant protection of cerebral microvascular integrity. Disruption of the BEB following focal cerebral ischaemia I/R injury is related to production of free oxygen radicals.

In our model we choose EB-albumin complex as the marker to evaluate the integrity of the cerebral microvasculature when EB is complexed with albumin, it has a hydrated diameter of 78A° and can not cross the BEB in normal cerebral tissue (30). However, in response to CNS damage, ADC leaks into the interstitial

space as was evident from both macroscopic and microscopic examination of brain tissue (28,30).

Disruption of the BEB occurs in various types of pathology, including the administration of hyperosmotic solutions, acute arterial hypertension, inflammation, head injury, SAH, seizures, and after transient episodes of cerebral ischaemia (16,17,20,21,22,30,34). In conjunction with disruption of the BEB, substantial leakage of fluid and protein into the interstitial space occurs and cerebral aedema develops (16). The mechanisms for the increase in microvascular permeability and subsequent extravasation of the proteins into the cerebral interstitial space after these type insults are presently not known. Oxygen-derived free radicals cause endothelial damage in many organ systems in which ischemic tissue has been reperfused (21,24). The cerebral vascular effects of oxygen radicals include increased vascular protein permeability, decreased O2 consumption of the vessel wall, endothelial and smooth muscle injury, increased platelet aggregation, and phagocyte accumulation in tissues (21). Oxygen radicals cause pronounced cellular effects such as lipid peroxidation, increased membrane permeability, enzyme inhibition, DNA damage, release of Ca^{+2} stores, mitochondrial destruction, and cytoskeleton disruption (21). The primary event in oxygen radical-mediated reperfusion toxicity is the development of aedema associated with damaged vascular endothelium.

Studies have shown a significant protective effect following ischaemia/reperfusion utilizing various free radical scavengers such as SOD, catalase, allopurinol, dimethyl sulfoxide (DMSO), vitamin E, mannitol, vitamin C, aminosteroid U-74006F and deferoxamine in ischaemic brain injury (9,14,26,32,35,36).

Recent evidence suggests that oxygen radicals are involved in the pathogenesis of the vascular and parenchymal damage associated with cerebral I/R (21). Potential sources of oxygen radicals of ischaemic brain associated with reperfusion following ischaemia include the mitochondria, XO, myelin, unsaturated fatty acid metabolism via prostaglandin H (PGH) synthase (cyclo-oxygenase pathway), autooxidation of haemoglobin and polymorphonuclear leucocytes (21) and, SOD, catalase, glutathione peroxidase and methionine sulphoxide reductase are intracellular factors that can protect cells against free radical damage (24).

In the present study bovine-copper-zinc SOD or catalase caused significant protection of the cerebral microvasculature in I/R. In addition to their protective effect a marked hyperaemia occurred throughout the brain during reperfusion in all SOD and catalase treated animals. Free oxygen radicals are known to produce vasoconstriction in the cerebral microvasculature, either by inhibition of the vasodilatory effect of EDRF or by direct vasoconstriction related to superoxide anion ($\cdot O_2$) (25,31). SOD and catalase are the scavengers of superoxide anion and H_2O_2 respectively (24).

In our study the marked hyperaemia observed during reperfusion in the presence of SOD or catalase was likely due to either elimination of the oxygen radical constrictor effect or protection of EDRF(s) from being inactivated by oxygen radicals (25,31).

Molecular O_2 is a necessary factor in the XO-dependent conversion of hypoxanthine to xanthine. This reaction produces either $\cdot O_2$ by one electron reduction or H_2O_2 by two electron reduction of O_2 (27).

Three distinct molybdenum-containing enzyme systems have been described in animals, XO and XD, aldehyde oxidase and sulfite oxidase (18). Inhibition of molybdenum utilization either by dietary or intraperitoneally administered tungsten decreases the XO and XD in many tissues. Also XO appears to be concentrated within endothelial cells and can generate O_2 metabolites, particularly H_2O_2.

In our study, the tungsten-treated group animals showed some protection against I/R injury. However, the fact that the tungsten supplement diet did not totally protect, suggests that the time period of the tungsten supplied diet used to inactivate xanthine oxidase+xanthine dehydrogenase (XO+XD) was not sufficient to decrease XO and XD

activity or rabbits may not have small amounts of cerebral XO and XD activity. In addition, the tungsten diet is known to increase vascular permeability in the lung (1). This is the likely reason why only partial protection occurred. However, the partial depletion indicates that XO is required to produce the maximal damage. Depletion of cerebral XO and XD in tungsten-treated gerbils subjected to cerebral ischaemia, decreased brain aedema and brain H_2O_2 levels (29). However, tungsten treatment did not decrease neurological deficits occurring during hte ischaemic period (29).

Cerebral I/R causes release of free fatty acids that can lead to the synthesis of highly active eicosanoids (19), particularly the cycloxygenase-derived products such as PGs and thromboxane and the lipoxygenase-derived products such as leukotrienes, and hydroxyacids (2). Clinical studies have measured elevated levels of both AA and prostaglandins (PGs) in the cerebrospinal fluid (CSF) of humans after ischaemic stroke and subarahcnoid haemorrhage (SAH) (5), and blocking the cyclo-oxygenase enzyme pathway by indomethacin prevented endothelial damage associated with I/R. Indomethacin given before reperfusion significantly improved postischaemic perfusion and ameliorated the decrease in cortical specific gravity (6). However, no beneficial effect was exerted on nutritive perfusion or the electrical recovery of the cerebral cortex (6). Moreover in prolonged brain ischaemia, brain aedema was worsened by administration of indomethacin during reperfusion (2,5).

In our study, indomethacin was given after 50 minutes of ischaemia, and during the reperfusion period blood flow of the occluded hemisphere increased significantly and the BEB was protected from the I/R damage.

One potential cellular source of free radicals is oxygenation of unsaturated fatty acids by the lipoxygenase and cyclo-oxygenase pathways (21). It has also been shown that AA-induced brain aedema is associated with the release of free radicals (7).Indomethacin not only blocks the cyclo-oxygenase pathway but also stops the production of ambient free radicals during synthesis of PGs (2). However, inhibition of cyclo-oxygenase may increase the lipoxygenase pathway which could produce potentially toxic products.

Other antioxidants used in our study had similar effects to that of indomethacin with respect to protecting the BEB integrity and improving postischaemic blood flow. Since those antioxidants do not prevent PG synthesis, the deleterious effect of reperfusion on the BEB integrity is associated not only with an increased synthesis of AA products but also by the generation of free oxygen radicals.

MMI is the active metabolite of carbimazole which inhibits thyroid hormone biosynthesis by preventing organification of iodide in the thyroid.

In our study the i.v. administration of MMI at 50 minutes of ischaemia, prevented acute microvascular damage and brain aedema associated with I/R. MMI is a potent scavenger of ·OH radical (3,33) and has been shown to protect against ·OH damage of sympathetic nerve terminals in vitro; presumably by scavenging O_2 radicals (10).

During the first few hours of the reperfusion period, granulocytes accumulate in regions of the brain that are subjected to ischaemia (15). Neutrophils and macrophages secrete both $\cdot O_2^-$ and H_2O_2 (11). MMI also inhibits production of oxygen radicals by monocytes and neutrophils via peroxidase catalysed reaction (3). Since tissue damage activates neutrophils, protection of the endothelium from I/R by MMI administration is not surprising.

In the presence of iron or other transitional metals, oxygen radicals cause lipid peroxidation which produces extensive tissue damage (13). MMI is also an iron and copper chelator (10).

Dimethylthiourea (DMTU) administered just before reperfusion, decreased brain aedema and brain H_2O_2 levels and improved survival in symptomatic gerbils subjected to I/R injury (29). Moreover DMTU decreased the infarct size following focal cerebral ischaemia in rats (26). DMTU is also an antithyroid drug and its therapeutic effect on hyperthyroidism is similar to that of MMI.

These data indicate that MMI treatment protects the cerebral microvasculature, attenuates cerebral aedema formation, and minimizes secondary neuronal damage.

We evaluated the protection of the cerebral endothelial barrier by free radical scavengers during I/R. However, cerebral parenchymal function after ischaemia in all groups was not evaluated and was probably not protected, but any breakdown in the BEB can lead to cerebral dysfunction.

The present study shows that antioxidant administration, decreases or inhibits free radical generation and indomethacin protects the cerebral endothelium from damage associated with I/R.

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