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Neuroprotective efficacy of ebselen, an anti-oxidant with anti-inflammatory actions, in a rodent model of permanent middle cerebral artery occlusion

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1 The aim of this study was to investigate whether delayed treatment with the anti-oxidant and anti-inflammatory agent ebselen reduces the volume of infarction in a rodent model of permanent focal cerebral ischaemia.

2 Ebselen (10 or 30 mg kg^{-1}) or vehicle was administered by gavage 30 min and 12 h after the induction of cerebral ischaemia by permanent occlusion of the left middle cerebral artery (MCA). Animals were killed 24 h following MCA occlusion, and the volumes of ischaemic damage in the ebselen and control groups were evaluated by quantitative histopathology.

3 Ebselen was quickly absorbed following oral (gavage) administration and reached peak levels in the plasma by 1 h post-administration (plasma selenium level of 0.68 ± 0.04 and 0.84 ± 0.1 μg ml^{-1} for 10 and 30 mg kg^{-1}, respectively, compared to control level of 0.51 ± 0.02 μg kg^{-1}).

4 Treatment with the lower dose of ebselen (10 mg kg^{-1}) significantly ($P<0.01$) reduced the volume of infarction in the cerebral hemisphere and cerebral cortex (by 31.8% and 36.7%, respectively compared with the placebo group).

5 The neuroprotective efficacy of the higher dose ebselen (30 mg kg^{-1}) was less than that of the lower dose ebselen (10 mg kg^{-1}). The volume of ischaemic damage in the cerebral hemisphere was reduced by 23.7% ($P<0.02$), and cerebral cortex by 27.5% ($P<0.01$).

6 Both doses of ebselen (10, 30 mg kg^{-1}) had no therapeutic efficacy on the caudate nucleus, where ischaemia was most severe, in this model.

7 Free radical-mediated injury is normally associated with reperfusion of ischaemic tissue. The present results suggest that oxidative injury is also a significant contributor to brain damage in models of maintained (permanent) ischaemia and that ebselen is effective in attenuating this free radical-induced damage.

Keywords: Ebselen; neuroprotection; focal cerebral ischaemia; anti-oxidant; free radicals; glutathione peroxidase mimic

Introduction

Free radicals are extremely reactive and have the capacity to attack all biological macromolecules including lipid membranes (peroxidation), proteins, carbohydrates and nucleic acids. The oxidative injury they produce is implicated in the development of brain damage following cerebral ischaemia (Kontos, 1989; Siesjö, 1992) and free radical mediated injury has been found to be potentiated by restoration of oxygen, on reperfusion of ischaemic tissue when levels of free radicals are markedly elevated (Sakamoto 1991, Phillis *et al*., 1994; Lancelot *et al*., 1995). In support of this, reperfusion-related brain damage is attenuated by free radical scavengers (Matsumiya *et al*, 1991; Xue *et al*., 1992) and exacerbated by increased oxygen levels during reperfusion (Mickel *et al*, 1987; Halsey *et al*., 1991).

Free radical toxicity *in vivo* is attenuated by the enzymes — superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) — and endogenous free radical scavengers such as vitamin C and E, α-lipoate and dihydrolipoate. SOD catalyzes the conversion of the superoxide radical to hydrogen peroxide, while catalase and the selenium-containing enzyme GPx with reduced glutathione, degrade hydrogen peroxide to water. GPx can also reduce lipid hydroperoxides generated by free radical attack on lipid membranes.

Ebselen (2-phenyl-1,2-benzisoselenazol-3 (2H)-one, Figure 1) a selenium-containing organic compound has been found to protect tissue against oxidative attack by mimicking GPx and phospholipid hydroperoxide glutathione peroxidase (PHGPx) (Sies, 1993). The reaction catalyzed is the reduction of a hydroperoxide at the expense of a thiol. GPx, PHGPx and ebselen all share the presence of a catalytically active selenium. The active site of GPx contains a selenocysteine which is converted during the catalytic cycle to a selenic acid derivative and glutathione selenylsulphide. Similarly, for ebselen, the analogous catalytic cycle involves the selenol form of ebselen, which after reaction with the hydroperoxide, is converted to ebselen selenic acid and via two further reactions with thiols, to ebselen selenodisulphide and back to ebselen selenol (Sies, 1993). Combined with suitable thiol compounds (e.g. reduced glutathione, dithioerythritol, N-acetylcysteine, dihydrolipoate, etc.) ebselen and certain of its metabolites can reduce hydrogen peroxide to water and attenuate lipid peroxidation by reducing organic-, cholesterol-, cholesterol ester- and phospholipid-hydroperoxides. In contrast to its reductive detoxification of hydroperoxides, ebselen is a surprisingly poor free radical scavenger and consequently this is not regarded as a significant component of its pharmacodynamic activity (Schewe, 1995).

To date, only a limited number of studies on the effects of ebselen in focal cerebral ischaemia have been published. Two pretreatment studies have demonstrated a significant reduction in ischaemic brain oedema (Matsui *et al*., 1990; Johshita *et al*.,

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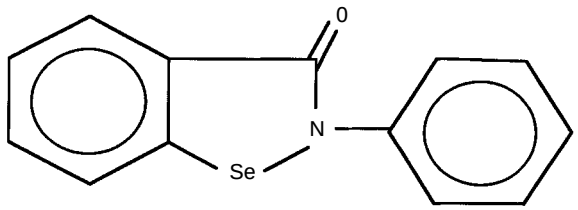

Figure 1 Structure of ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one).

1990). However, one must be careful in deriving any information on potential neuroprotection from these studies since it is possible to reduce oedema formation without influencing the volume of infarction. The best evidence for ebselen efficacy in ischaemia to date is a pretreatment study with endothelin-induced transient focal ischaemia from our own laboratory (Dawson *et al*., 1995). However, this study is open to criticism since the experiment was terminated at an early time point (4 h) when infarction had not fully matured and the lesion had not fully evolved. In addition the use of a pretreatment strategy and a model with a strong component of reperfusion-mediated injury gave ebselen the best possible opportunity of displaying efficacy.

In the present study much more stringent conditions have been used in the investigation of the neuroprotective efficacy of ebselen. Firstly, we have chosen a model of maintained focal ischaemia (permanent middle cerebral artery (MCA) occlusion) with a much more limited component of reperfusion injury. Secondly, a later end-point (24 h) has been chosen to allow maturation of tissue injury to infarction and the ischaemic lesion to reach its maximum size (Gill, 1992; Dawson *et al*., 1993), and finally, we have delayed treatment until 30 min after the ischaemic insult.

Methods

General surgical preparation

Male Fisher 344 rats weighing 277–340 g were anaesthetized with halothane in nitrous oxide/oxygen (70:30). Anaesthesia was induced with 5% halothane and subsequently maintained with 0.8–1.5% halothane. The rats were intubated transorally and artificially ventilated via a small animal respirator pump. The right femoral artery was cannulated and the cannula passed to the dorsal neck subcutaneously for chronic blood pressure monitoring and for sampling of arterial blood for gas tension, pH measurement and blood glucose measurement. Arterial blood pressure was monitored continuously while the animal was under anaesthesia and at 1 h and 24 h post-MCA occlusion. Arterial blood samples were taken at regular intervals from the anaesthetized animal and at 1 and 24 h after MCA occlusion, for assessment of respiratory status by use of a direct reading electrode system (Corning). Rats were maintained normotensive (MABP > 80 mmHg), normocapnic ($34 < PaCO_2 < 42$ mmHg), adequately oxygenated ($PaO_2 >$ 100 mmHg) and normothermic while under anaesthesia. Rectal temperature was maintained around 37°C by the use of heating lamps during the operation. A fine temperature probe was inserted into the temporalis muscle (ipsilateral to the exposed MCA) to assess brain temperature during MCA occlusion (Aronowski *et al*., 1994).

Induction of focal cerebral ischaemia

The left middle cerebral artery (MCA) was exposed by use of a modification of the method described by Tamura *et al*. (1981). Briefly, through a 2 cm skin incision, the temporalis muscle was incised and stripped sub-periosteally from the lateral and ventral aspects of the temporal bone to enter the infra-temporal fossa from the foramen opticum rostrally to the foramen ovale caudally. A small subtemporal craniectomy was made, centred 3 mm rostral to the foramen ovale, and the dura opened by a cruciate incision with a 25 gauge needle. Cerebral ischaemia was then induced by electrocoagulation of the MCA from the point where it crosses the inferior cerebral vein to a point proximal to the origin of the lenticulostriate branch. The MCA was then transected midway between the inferior cerebral vein and olfactory tract to ensure completeness of the occlusion. The time of transection was taken as the exact time of MCA occlusion. The craniectomy wound was then sutured and the animals allowed to recover from the anaesthetic. A subcutaneous injection of 2 ml of saline was given to prevent post-anaesthetic dehydration and body temperature was maintained with heating lamps until animals were fully conscious. Following recovery from anaesthesia, animals were returned to the animal house until the following day.

Ebselen treatment

Rats were randomly assigned into three groups of 15 to receive 10, 30 mg kg^{-1} ebselen or the placebo (carboxymethyl cellulose). The control group received placebo powder weighed out to match the lower of the two doses. Ebselen (10 or 30 mg kg^{-1}) or placebo was dissolved in distilled water and administered orally by gavage as a bolus (5 mg kg^{-1}) 30 min and 12 h after MCA occlusion.

In a separate group of rats, blood samples (1 ml) were taken 1, 2 and 4 h following gavage administration of ebselen ($n = 4$ per dose) in order to identify when drug levels peaked in plasma. Since the assay employed measures plasma selenium, blood samples were also collected from control rats and mean endogenous plasma selenium levels ($n = 5$) subtracted from the drug-treated samples to give the ebselen-derived selenium. All samples were immediately centrifuged, plasma removed and frozen at −20°C before analysis of plasma selenium (Smith, 1975). Blood volume was supplemented by an equivalent volume of saline after each sample was withdrawn.

Tissue processing and histological quantification of ischaemic damage

The rats were perfusion fixed for neuropathological analysis 24 h post-MCA occlusion with 40% formaldehyde, glacial acetic acid and absolute methanol (FAM; 1:1:8, v/v/v). Briefly, the rats were deeply anaesthetized with 4% halothane and placed in a supine position so that the thorax could be opened through bilateral incisions. A catheter was inserted into the left ventricle, the right atrium was incised, and heparin-treated saline was infused at a pressure equal to the MABP (95–120 mmHg) of the animal until the perfusate from the right atrium was bloodless. The saline was followed by approximately 300 ml of FAM. The rat was decapitated immediately after perfusion fixation, and the head stored in the fixative for at least 24 h. The brain was then removed. After the hindbrain had been detached, the forebrain was cut into four equally spaced coronal blocks that were processed, embedded in paraffin wax, and sectioned at multiple levels. The sections, stained with haematoxylin and eosin, were examined by light microscopy by one of us (D.I.G.) without knowledge of the experimental protocol.

Areas of cerebral infarction were then delineated at eight pre-selected coronal levels from 12.2 mm anterior to −0.5 mm posterior (Osborne *et al*., 1987), transcribed onto scale drawings of forebrain (Konig & Klippel, 1963) and measured on an image analyser (Cambridge Instruments Quantimet 970). These areas were then integrated, with the known distance between each coronal level, to determine the total volume of infarction in each specimen which was normalized to the mean hemisphere volume (570 mm^3) for Fisher 344 rats of the weights used.

Statistical analysis

All data are presented as mean ± s.e.mean. Physiological variables and the volumes of infarction were compared by one way ANOVA, with subsequent pairwise comparisons by two-tailed Student's unpaired *t* test with a Bonferroni correction factor for multiple comparisons. One rat of low dose ebselen group (10 mg kg^{-1}) was excluded from analysis because of failure to develop the characteristic caudate infarct (caudate lesion: 0.9 mm^3). This was thought to be the result of technical failure or a vascular anomaly in this particular animal.

Results

Plasma drug levels

Following gavage administration, ebselen was rapidly absorbed by the gastro-intestinal tract and reached peak plasma levels by 1 h (Figure 2). Since the assay employed measures plasma selenium, basal endogenous plasma selenium levels from controls (0.51 ± 0.02 μg ml^{-1}, $n = 5$) were first subtracted to reveal ebselen-derived plasma selenium. Plasma selenium following placebo administration was equivalent to control levels (0.50 ± 0.04 μg ml^{-1}, $n = 3$).

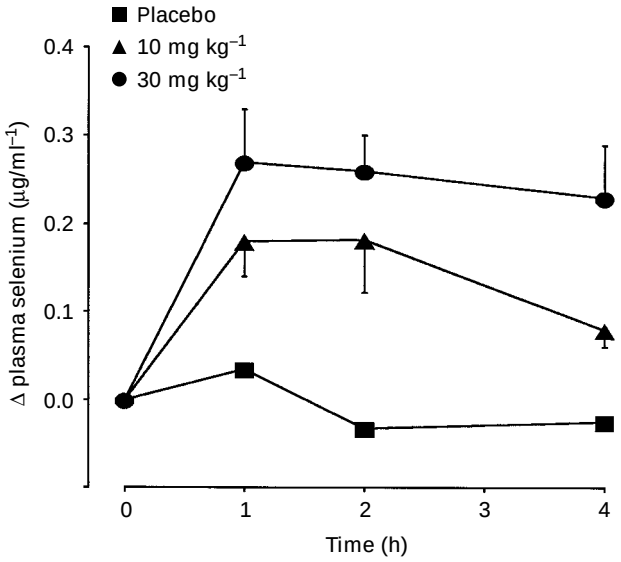

Figure 2 Ebselen levels in plasma, measured as plasma selenium, following gavage administration of placebo, 10 and 30 mg kg^{-1} ebselen. Data are presented as mean change in plasma selenium from basal endogenous plasma selenium (0.51 ± 0.018 μg ml^{-1}); vertical lines show s.e.mean.

Physiological variables

Cardiovascular and respiratory parameters at 20 min pre-MCA occlusion, MCA occlusion, 1 and 24 h post-MCA occlusion are shown in Table 1. All data are presented as mean ± s.e.mean. A significant increase in PaO_2 ($P < 0.05$) was identified with high dose ebselen (30 mg kg^{-1}) compared to the vehicle control group 1 h after the MCA occlusion. Mean arterial pressure, which was well matched between the three treatment groups, was not significantly affected by ebselen. No significant differences between the vehicle and drug-dosed experimental groups was found for any of the other parameters measured.

Analysis of the areas of ischaemic damage

Both doses of ebselen (10 mg kg^{-1}, 30 mg kg^{-1}) significantly reduced the volume of infarction in the cerebral hemisphere and in the cerebral cortex (Figures 3 and 4). Treatment with the lower dose of ebselen (10 mg kg^{-1}) significantly ($P < 0.01$) reduced the volume of ischaemic damage in the cerebral hemisphere and cerebral cortex (by 31.8% and 36.7%, respectively, compared with vehicle control group). The neuroprotective efficacy of the higher dose of ebselen (30 mg kg^{-1}) was less than that of the lower dose of ebselen (10 mg kg^{-1}). However, the higher dose of ebselen (30 mg kg^{-1}) significantly reduced the volume of ischaemic damage in the cerebral hemisphere by 23.7% ($P < 0.02$) and cerebral cortex by 27.5% ($P < 0.01$). Neither dose of ebselen (10, 30 mg kg^{-1}) had a significant therapeutic effect on the caudate nucleus in this model.

Discussion

Evidence for the involvement of free radicals in ischaemic brain injury is provided by the detection of free radicals during ischaemia, increased levels during reperfusion (Sakamoto *et al*., 1991; Phillis *et al*., 1994; Lancelot *et al*., 1995), reductions in the levels of endogenous radical scavengers during ischaemia and reperfusion (Bacon *et al*., 1996), resistance to reperfusion injury in transgenic mice overexpressing CuZn SOD (Yang *et al*., 1994) and the neuroprotective efficacy of antioxidants such as tirilazad (Clark *et al*., 1995), PBN (α-phenyl-

Table 1 Physiological variables before and following MCA occlusion

	MABP(mmHg)	*pH*	$PaCO_2$ (mmHg)	Pao_2 (mmHg)	*Glucose* (mM)	*Rectal temp.* (°C)	*TM temp.* (°C)
Vehicle							
Pre-MCAO	87.6 ± 3.9	7.42 ± 0.01	38.7 ± 0.7	159 ± 6	8.0 ± 0.3	37.2 ± 0.0	37.0 ± 0.03
MCAO	90.6 ± 3.3	7.39 ± 0.01	42.3 ± 0.9	147 ± 6	7.7 ± 0.3	37.0 ± 0.1	37.0 ± 0.03
1 h	116.1 ± 3.1	7.41 ± 0.00	40.2 ± 0.9	76 ± 2	6.4 ± 0.3	37.5 ± 0.1	
24 h	120.2 ± 1.8	7.49 ± 0.01	34.7 ± 0.6	97 ± 2	6.1 ± 0.3	38.1 ± 0.1	
Ebselen (10 mg kg^{-1})							
Pre-MCAO	92.0 ± 3.0	7.43 ± 0.01	38.8 ± 0.6	148 ± 4	7.8 ± 0.3	37.1 ± 0.1	37.0 ± 0.04
MCAO	91.7 ± 3.4	7.40 ± 0.01	40.2 ± 1.1	149 ± 4	7.8 ± 0.3	37.0 ± 0.1	37.0 ± 0.03
1h	118.9 ± 3.8	7.42 ± 0.01	41.4 ± 0.8	7 ± 2	7.0 ± 0.4	37.7 ± 0.1	
24 h	125.1 ± 2.8	7.47 ± 0.01	35.4 ± 0.8	94 ± 2	6.7 ± 0.2	38.2 ± 0.1	
Ebselen (30 mg kg^{-1})							
Pre-MCAO	88.5 ± 4.7	7.42 ± 0.01	39.2 ± 0.6	148 ± 7	8.0 ± 0.4	37.0 ± 0.0	37.0 ± 0.02
MCAO	94.1 ± 3.6	7.40 ± 0.01	40.7 ± 1.1	143 ± 6	7.7 ± 0.3	36.9 ± 0.1	37.0 ± 0.03
1 h	123.9 ± 2.1	7.40 ± 0.00	43.0 ± 0.9	83 ± 2*	6.6 ± 0.3	37.9 ± 0.1	
24 h	117.7 ± 3.2	7.48 ± 0.01	34.4 ± 1.0	100 ± 2	6.5 ± 0.4	38.0 ± 0.1	

MABP: mean arterial blood pressure; rectal temp.:rectal temperature; TM temp.: temporalis muscle temperature. Data are presented as mean ± s.e.mean ($n = 15$ in vehicle and ebselen 30 mg kg^{-1} groups, $n = 14$ in ebselen 10 mg kg^{-1} group). *$P < 0.05$ two-tailed *t* test with Bonferroni correction compared with vehicle.

N-*tert*-butyl nitrone) (Cao & Phillis, 1994; Zhao *et al*., 1994) and ebselen (Dawson *et al*., 1995; Knollema *et al*., 1996).

Ebselen, a selenium-containing organic compound with both anti-oxidant and anti-inflammatory properties is effective in attenuating free radical-induced damage both *in vitro* (Schewe, 1995) and *in vivo* (Dawson *et al*., 1995; Knollema *et al*., 1996). Its small size and lipophilicity provide efficient, rapid absorption following oral administration and, despite containing selenium, low toxicity because of metabolic retention of selenium within the molecule.

Anti-oxidant effects

Although a poor free radical scavenger, ebselen has the capacity to interact with an extensive variety of enzymes and substrates to ameliorate free radical induced damage (Schewe, 1995). While endogenous glutathione peroxidase has a specific requirement for reduced glutathione (GSH), ebselen is able to interact with any available thiol group to reduce hydroperoxides and also reacts with a variety of thiol-containing enzymes *in vitro*, including protein kinase C and NADPH oxidase (Schewe, 1995).

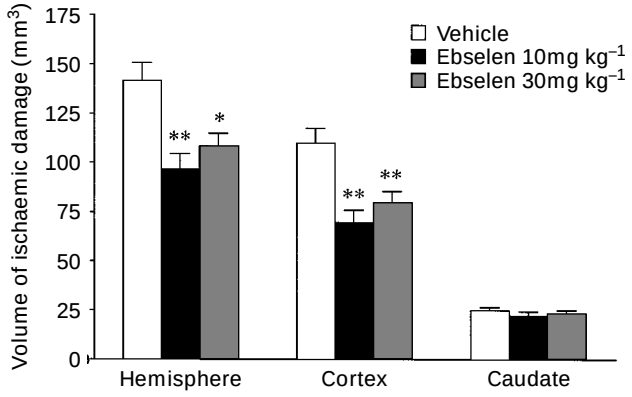

Figure 3 Volumes of cerebral infarction assessed 24 h after permanent MCA occlusion. Both 10 mg kg^{-1} and 30 mg kg^{-1} ebselen significantly reduced the volume of ischaemic damage in the cerebral hemisphere and cortex compared with the placebo control group. Data are presented as mean ± s.e.mean. $^{**}P<0.01$, $^{*}P<0.02$, Student's *t* test with Bonferroni correction.

As a glutathione peroxidase mimic, the specificity of ebselen for substrates ranges from hydrogen peroxide and small organic hydroperoxides to membrane-bound phospholipids, cholesterol hydroperoxides and the endoperoxide intermediates of arachidonic acid metabolism. It is important to remove hydroperoxides as well as free radicals since they can trigger the formation of further reactive oxygen species via lipid peroxidation, activate enzymes which generate deleterious products and promote initiation of apoptosis (Schewe, 1995). Cyclo-oxygenase and lipoxygenase, which generate prostaglandins, leukotrienes and thromboxanes from arachidonic acid, require a basal 'hydroperoxide tone' for their activity (Sies, 1993). By lowering peroxide levels, ebselen attenuates the activity of these enzymes, both of which release superoxide radicals as by-products. Prostaglandins, thromboxanes and leukotrienes have all been implicated in secondary brain injury following ischaemia. Collectively, they can promote vasoconstriction, platelet aggregation, thrombosis, leukocyte attraction, increased blood-brain barrier permeability and oedema (Kiwak *et al*., 1986). In addition to inhibiting their synthesis, ebselen can directly inactivate a number of these products thereby limiting vascular injury further (e.g. by converting 15-HPETE to inactive 15-HETE (Ochi *et al*., 1992) and by converting leukotriene B_4 to the biologically inactive 6-*trans* isomer (Kuhl *et al*., 1986)).

Anti-inflammatory effects

The anti-inflammatory effects of ebselen could also contribute to its neuroprotection. In addition to inhibition of arachidonic acid metabolism, it inhibits other free radical-generating enzymes, such as the inducible form of nitric oxide synthase and NADPH oxidase in inflammatory cells — possibly by blockade of thiol groups essential for enzyme structure and activity (Schewe, 1995). Ebselen also appears to inhibit selectively the protein kinase C responsible for NADPH oxidase activation and superoxide generation in activated leukocytes (Ichikawa *et al*., 1987), can inhibit lymphocyte mitogenesis, and inhibits leukocyte adhesion and transmigration across blood vessels *in vitro* (Issekutz & Lopes, 1992) and *in vivo* (Gao & Issekutz, 1993). The dual inhibition and inactivation of leukotriene B_4 will also prevent leukotriene-mediated chemotaxis and chemokinesis for leukocyte migration (Kuhl *et al*., 1986).

The mechanism of neuroprotection in both the current and the previous study (Dawson *et al*., 1995) is most likely due to ebselen mediated anti-oxidant effects rather than anti-inflam-

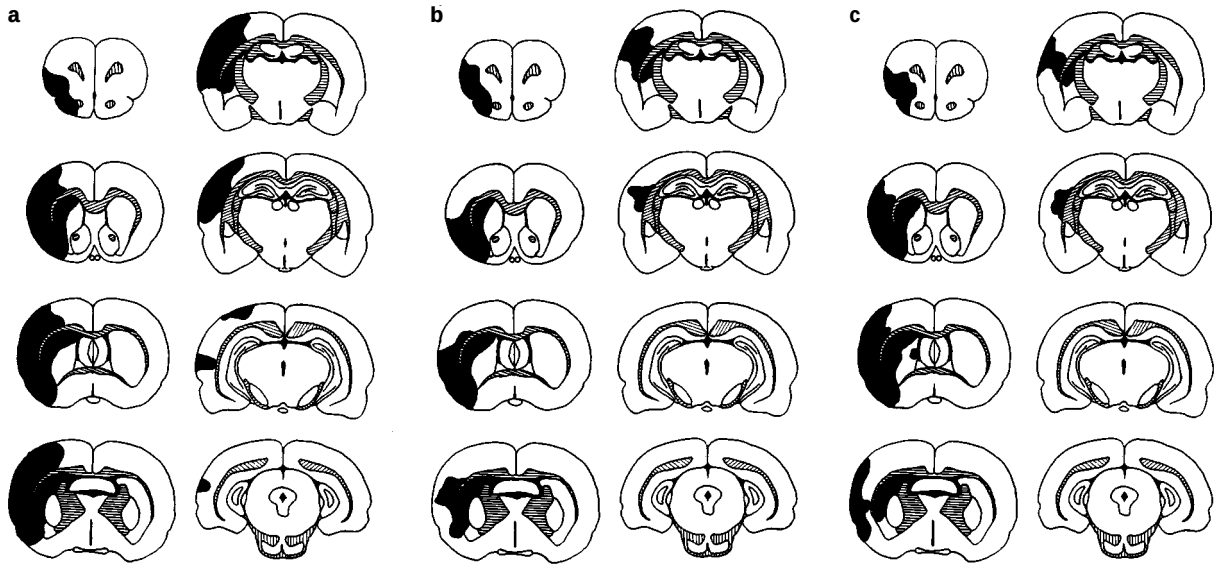

Figure 4 Representative line diagrams from (a) control (placebo) and ebselen ((b) 10 mg kg^{-1} and (c) 30 mg kg^{-1}) treated animals illustrating the topography of infarction throughout MCA territory. Cortical and caudate infarct size of illustrated examples are: placebo, 119 and 26 mm^3; ebselen 10 mg kg^{-1}, 63 and 23 mm^3; ebselen 30 mg kg^{-1} 73 and 24 mm^3, respectively.

matory effects, because of the models used and time points chosen. Inflammatory responses, particularly those involving invading inflammatory cells, are unlikely to have contributed significantly to brain injury by 4 h in transient MCA occlusion (Dawson *et al*., 1995) or by 24 h following permanent MCA occlusion (present study). The temporal profile for entry of inflammatory cells into the parenchyma is much faster when the ischaemic event is transient compared to permanent since reperfusion promotes delivery and earlier migration of inflammatory cells into the injured tissue. However, neutrophil numbers peak at about 48 h after transient focal ischaemia and after 72 h following permanent MCA occlusion (Chopp & Zhang, 1996), suggesting that ebselen neuroprotection demonstrated in the present and previous (Dawson *et al*., 1995) studies is more likely to be due to its multiple anti-oxidant effects. However, resident microglia are also activated in ischaemia and can contribute to inflammation-mediated brain injury (Nakajima & Kohsaka, 1993; Banati *et al*., 1993). Therefore, the possibility of ebselen-mediated inhibition of microglial activation cannot be ruled out. Further, if delayed inflammatory responses can contribute to infarct size, then the anti-inflammatory effects may become more pertinent at later time points.

In the present study, maintained focal ischaemia was induced by permanent occlusion and section of the MCA. In this situation, 'reperfusion' injury would be limited to penumbral regions of MCA territory, where blood supply can be supplemented by collateral blood vessels from the anterior and posterior cerebral arteries. Any reperfusion-type injury would commence with withdrawal of anaesthesia and the consequent increase in blood pressure. Cerebral autoregulation will be compromised in ischaemic brain tissue and consequently, CBF in these regions would increase in line with an increase in blood pressure, delivering both energy and O_2 for the production of free radicals in ischaemically compromised tissue. In contrast, the core of MCA territory (e.g. dorsolateral caudate), supplied by end arteries of the MCA would remain densely ischaemic, with no access to collateral flow.

Neuroprotection by ebselen administered post-ischaemia in this model is a significant finding, as it demonstrates, firstly, that following oral administration adequate drug levels reach the site of injury despite the preservation of MCA occlusion and, secondly, that free radical mediated injury represents a significant component of ischaemic injury even when ischaemia is maintained and reperfusion is restricted to increased collateral supply (Figure 4). The maximum level of protection achieved (infarct volume reduced by 37% in cortex) was substantial, although less than the 53% obtained in our previous study with transient focal ischaemia (Dawson *et al*., 1995). However, this is perhaps an inappropriate comparison since permanent MCA occlusion induces a more prolonged ischaemic insult with a greater core/penumbra ratio with respect to CBF. The reperfusion phase of transient ischaemia will restore flow throughout MCA territory raising blood flow above the critical threshold level for tissue infarction. This provides a window for drug intervention to attenuate reperfusion- and ongoing excitotoxic-injury, as well as improving access of drug to its site of action. In permanent MCA occlusion, a substantial volume of tissue has flow maintained below the ischaemic threshold and is destined to die, irrespective of any drug intervention. An alternative measure of drug efficacy which should also be considered is volume of tissue salvaged. In the present post-treatment study, 45.1 mm^3 and 33.6 mm^3 of brain tissue was salvaged after 10 and 30 mg kg^{-1} ebselen, respectively, with an infarct size in the placebo group of 142 mm^3. This compares with 19.2 mm^3 and 26.5 mm^3 salvaged after 10 and 30 mg kg^{-1} ebselen pre-treatment in our previous transient ischaemia study (Dawson *et al*., 1995), where infarct size in the placebo group was 55.7 mm^3.

Previously, the neuroprotective efficacy of anti-oxidants and free radical scavengers have been almost exclusively tested in models of reversible ischaemia which have a significant reperfusion phase. Efficacy in models of permanent MCA occlusion have been rare. One study exploring tirilazad treatment post-insult in the permanent MCA occlusion model has demonstrated significant neuroprotection (Park & Hall, 1994) but a second failed to provide any evidence of neuroprotection (Hellstrom *et al*., 1994). However, the spin-trap agent α-phenyl-tert-butyl-nitrone (PBN), examined following permanent MCA occlusion with ipsilateral common carotid artery occlusion was neuroprotective even with a delay of 12 h in dosing (Cao & Phillis, 1994).

In conclusion, ebselen treatment was well tolerated in animals and no significant cardiovascular or behavioural side-effects were recorded. The pattern of ebselen neuroprotection accords with that of blood supply within ischaemic MCA territory, in that significant neuroprotection was demonstrated in the cerebral cortex while the densely ischaemic caudate nucleus remained unprotected. In addition, the topographical pattern of neuroprotection (Figure 4) was consistent with that of collateral supply. Ebselen therefore appears a promising neuroprotective drug, because of the multiplicity of its mechanisms for inhibiting free radical-induced injury coupled with its lack of side-effects, good blood-brain penetrability and rapid absorption following oral administration.

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