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Experimental stroke protection induced by 4-hydroxybenzyl alcohol is cancelled by bacitracin

Elodie Descamps [a], Maud Petrault-Laprais [a], Pierre Maurois [b], Nicole Pages [b,c], Pierre Bac [b], Régis Bordet [a], Joseph Vamecq [a,d,*]

[a] EA 1046, Pharmacology, Faculty of Medicine, Research Branch, IMPRT, University of Lille North of France, Place de Verdun, 59045 Lille Cedex, France
[b] Faculty of Pharmacy, University Paris Sud 11, F-92296 Châtenay-Malabry and CNRS UMR 8162, IFR 13, Centre Chirurgical Marie Lannelongue, F-92350 Le Plessis Robinson, France
[c] Laboratoire de Toxicologie, Faculté de Pharmacie, Université Louis Pasteur, F-67401 Illkirch, France
[d] Inserm, Pharmacology, Faculty of Medicine, Research Branch, IMPRT, University of Lille North of France, Place de Verdun, 59045 Lille Cedex, France

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ABSTRACT

Induction of protein disulfide isomerase (PDI) is validated as a main mechanism by which 4-hydroxybenzyl alcohol (4-HBA), an active principle of *Gastrodia elata* Blume, reduces cerebral infarct volumes in a murine model of focal brain ischemia/reperfusion. In contrast to its position isomers, i.e. 3-hydroxybenzyl alcohol (3-HBA) and 2-hydroxybenzyl alcohol (2-HBA), and to aliphatic diols (1,4-butanediol and 1,5-pentanediol), 4-HBA administered intravenously at 25 mg/kg protected mice, significantly reducing total, cortical and sub-cortical infarct volumes by 42, 28 and 55%, respectively. All compounds, 4-HBA included, were devoid of antioedematous properties. Only the stroke protective 4-HBA, but neither 3-HBA nor 2-HBA, was capable of significantly inducing PDI in intact mouse brains. Stroke protection was fully prevented by bacitracin (500 mg/kg), a known inhibitor of PDI, which, without affecting basal brain PDI levels, altered the ability of 4-HBA to induce significantly PDI in intact brains. Taken as a whole, our data indicate that stroke protection induced by 4-HBA involves PDI as a key player, making this protein a valuable target to control brain injury disorders. The fact that 4-HBA, at doses up to 200 mg/kg, was devoid of neurotoxicity in the rotarod test is also a decisive element to promote the neuroprotective use of this plant compound.

1. Introduction

The majority (approximately 80%) of strokes are ischemic, and involve occlusion of arteries delivering essential nutrients and oxygen to the brain. There are now interventions for acute revascularisation, either pharmacologically or mechanically, and strategies based on developing pathophysiological models of stroke (Blakeley and Llinas, 2007). Major trials have evaluated thrombolytic agents in the treatment of acute ischemic stroke (Blakeley and Llinas, 2007). Beside emergency interventions alleviating cerebral vessel occlusion, general neuroprotective strategies also targeting the so-called penumbra lesions have been developed to limit the extension of the primarily injured core, permitting in practice some delayed intervention. Current and emerging neuroprotective strategies have been reviewed elsewhere (Mehta et al., 2007).

Animal models for the study of ischemic stroke include transient unilateral middle cerebral arterial occlusion (MCAO) which combines an hour of ischemia followed by 24 h of reperfusion (Bastide et al., 1999). In this model, Yu et al. (2005) have shown that preconditioning could be mimicked by 4-hydroxybenzyl alcohol (4-HBA), an inductor of protein disulfide isomerase (PDI) which notably lowers brain cortical infarct volumes. PDI catalyses the isomerisation of protein disulfide bonds (allowing re-arrangement of disulfide bridges within the protein structure), and, depending on the redox state, either oxidation of thiols or reduction of disulfides, assisting chaperones in protein folding and often being considered itself as a chaperone inhibiting protein aggregation (Wilkinson and Gilbert, 2004).

The amount of brain PDI may be increased by cerebral ischemia/reperfusion in rodents (Tanaka et al., 2000). As mentioned above, this increase, when amplified by a preconditioning administration of 4-HBA, is associated with a reduction of cerebral infarct volumes (Yu et al., 2005). Because PDI activity might protect proteins against oxidative damages, conformational changes (for instance, masking strategic sites of the protein during the lapse of time of oxidative injury), the induction of PDI might be a worthy means to

* Corresponding author at: Inserm, EA 1046, Pharmacology, University of Lille 2, Faculty of Medicine, Research Branch, 4th Floor, 1 Place de Verdun, F-59045 Lille Cedex, France. Tel.: +33 3 20 44 54 49; fax: +33 3 20 44 68 63.
E-mail address: joseph.vamecq@inserm.fr (J. Vamecq).

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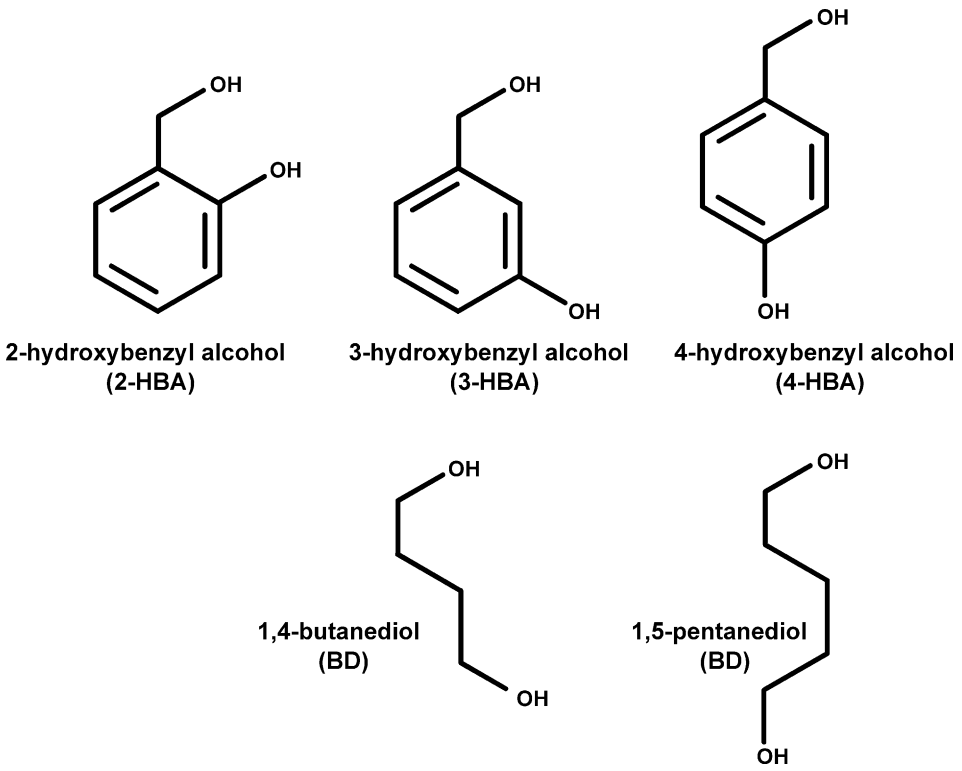

Fig. 1. 4-Hydroxybenzyl alcohol and structural analogues.

limit the extension of so-called penumbra lesions and hence be potentially suitable for either immediate or delayed intervention. Along these lines, the protective role of the PDI inducer 4-HBA was here studied in the MCAO model independently of any preconditioning protocol. Because many potentially protective (antioxidant, anti-excitotoxic, pro-PDI and GABAergic modulatory) activities are conveyed by 4-HBA (Kim et al., 2007; Yu et al., 2005; Liu and Mori, 1993), the accurate contribution of PDI to effects of 4-HBA on brain infarct volumes (and audiogenic seizure susceptibility) was assessed by inhibition experiments using bacitracin. Modulation of brain infarct volumes by several analogues of 4-HBA was also determined. These analogues were designed taking into account that 4-HBA is a diol aromatic compound combining phenol and a methylene unit substituted with an alcohol. Designed analogues included aromatic isomers represented by 2-hydroxybenzyl alcohol (2-HBA) and 3-hydroxybenzyl alcohol (3-HBA) and two aliphatic diol compounds, 1,4-butanediol (BD) and 1,5-pentanediol (PD) (Fig. 1). Compared to 4-HBA, the former two compounds were chosen in order to obtain a distinct pattern of the respective positioning of the two alcohol functions around the benzene ring; the latter two compounds were chosen to maintain the diol pattern but not the aromatic ring in their chemical formula. Compounds and vehicle were evaluated in the MCAO model as indicated in Fig. 2 and as mentioned above, independently of any preconditioning protocol.

2. Materials and methods

2.1. Chemicals

4-HBA, 3-HBA, 2-HBA, BD, PD and bacitracin were purchased from Sigma (Saint Quentin Fallavier, France). Mouse anti-PDI monoclonal antibody (SPA-891) was obtained from Assay Designs (Ann Arbor MI, USA).

2.2. Animals

C57 black J6 mice (28–30 g) were used (Janvier, Le Genest Saint Isle, France). Mice were housed six per cage, in an alternating 12:12 light/dark cycle with lights on at 07:00. All procedures involving animals and their care were performed in agreement with the local ethical committee for animal experimentation and in compliance with our institutional guidelines, which comply with current national and international laws and recommendations.

2.3. MCAO model

Anaesthesia was induced in mice by chloral hydrate administered via the intraperitoneal route at a dose of 300 mg/kg. The ostium of the right middle cerebral artery (MCA) was occluded intraluminally as described previously (Bastide et al., 1999). The right carotid arteries were exposed through a midline cervical incision and the common carotid and external carotid arteries were ligated with a silk suture. Aneurysm clip was placed across the internal carotid artery and an arteriotomy was made in the common carotid artery stump allowing the introduction of a 6/0 nylon suture monofilament with its tip rounded by flame heating. This was secured in place and the aneurysm clip on the internal carotid artery was removed. The suture was gently advanced into the internal carotid artery and passed into the intracranial circulation to lodge in the narrower lumen of the origin of the MCA. Mild resistance to this advancement indicated that the intraluminal occluder had entered the anterior cerebral artery and had been then placed beyond the ostium of MCA, ensuring obstruction of the latter. After 60 min, the monofilament was carefully removed, until its tip was blocked by ligature placed on common carotid artery, to permit reperfusion. The caudal artery catheter was removed and the artery was ligated to prevent bleeding. The animals were placed for 24 h in cage to recover from anaesthesia at room temperature and were allowed to eat and drink freely.

2.4. Administration of compounds in the MCAO model

Individual and combined administrations of compounds in the MCAO model were performed according to protocols appearing in Fig. 2 introduced above and commented in the figure legend.

2.5. Histology

Mice were sacrificed by the overdose of pentobarbital injected intraperitoneally 24 h after reperfusion. Brains were rapidly removed, frozen and coronally sectioned into 20-μm thick slices on a cryostat at 12 levels separated by 1-mm intervals according to stereotaxic section maps (Paxinos and Watson, 1986). Sections were stained with cresyl fast violet. The unstained area of the brain sections was defined as the infarct. Cortical and sub-cortical areas and total hemispheric areas of infarcts were calculated separately for each coronal slice by image analysis software (Color Image 1.32, National Institute of Mental Health, Bethesda, MD, USA) after digitisation by a scanner process. Total, cortical, sub-cortical infarct volumes,