The sour side of vitamin C might mediate neuroprotective, anticonvulsive and antidepressant-like effects

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A R T I C L E   I N F O

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A B S T R A C T

In animal experiments, neuroprotective, anticonvulsive and antidepressant-like properties have been increasingly attributed to administrations of ascorbic acid (AA, vitamin C) in at least medium (low millimolar) doses, which however await validation in well controlled clinical studies. In mammalian cortical and subcortical neurons, small to modest acidification ($< 0.4$–$0.5$ pH-units) is belonging to the key strategies for controlling local excitability and is associated with neuroprotection, e.g. by limiting excitotoxicity. Such acidifications are furthermore involved in the mechanisms of some anticonvulsants and antidepressants. As AA-transport and regulation of intracellular pH (pHi) are closely interwoven, I suppose that the aforementioned beneficial AA-effects might be based upon a discrete “hormetic” acidification of cortical and or subcortical neurons via an AA-mediated weakening of their pHi-regulation. This assumption is supported by findings in non-neuronal cells suggesting both, intracellular acidification and inhibition of a core-element of the pHi-regulation apparatus by millimolar AA. In mammalian subcortical neurons, there is already first evidence of a modest acidification after adding low millimolar AA.

Introduction

Most mammals produce ascorbic acid (AA, vitamin C) from glucose in the liver. But humans, guinea pigs and some bats cannot synthesize AA and are dependent on dietary sources of this water-soluble vitamin \cite{1,2}. After oral intake of AA, fasting plasma concentrations are tightly controlled at $< 100 \mu M$ in humans \cite{3}. As single oral doses exceed $200$ mg, intestinal absorption decreases, urine excretion increases and ascorbate bioavailability is reduced \cite{3}. AA is essential for repair of every tissue in the body and is belonging to the physiologic key antioxidants to cope with reactive species (e.g. free radicals) originating from natural aerobic, xenobiotic or pathological metabolism, i.e. involving mitochondrial and non-mitochondrial sources, such as activation of monoamine oxidase, xanthine oxidase, nitric oxide synthase, cyclooxygenase, lipooxygenase, cytochrome p450 and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase \cite{4,5}. Reactive species are suggested to induce tissue damage and microvascular dysfunction but can also result from tissue damage and inflammation \cite{4,5}. Determining the cause-effect relationship between reactive species and tissue damage is rather complex if taken into account that reactive species must not be necessarily detrimental and are required to maintain normal cell functioning – e.g. cell signaling and apoptosis require special redox reactions \cite{4,5}. A balanced relationship between antioxidants such as AA and reactive species is assumed to obtain appropriate cell functioning \cite{4,5}. In case of preponderance of reactive species, oxidative stress should occur which has been brought into connection with aging and a multitude of chronic, often slowly progressing diseases including neurodegeneration, epileptic and affective disorders \cite{6,7}. However, this “oxidative stress theory of diseases” has not been proven as so far any antioxidant has met the criteria of efficacy for drug approval \cite{8}.

The brain is particularly vulnerable to oxidative damage owning to its high oxygen demand and wealth of mitochondria as well as its poor repair capacity \cite{7}. Especially hippocampus and neocortex neurons are known to accumulate AA in low mM-ranges even under restricted dietary conditions, which is largely driven by the sodium- and energy dependent transmembrane vitamin C-transporter (SVCT2, Fig. 1) \cite{1}. Some, but not all studies found evidence for an ascorbate-deficiency in neuropsychiatric disorders and there are still controversial results of clinical studies about the efficacy of adjunctive ascorbate with the best...
AA and neuroprotection effect

Most likely, SVCT2 serves as a key component of a powerful intracellular (and via a special transplasma membrane electron transport system also extracellular) defense system against oxidative stress [1,2]. In support, AA was shown to represent the major low molecular weight cytosolic reducing agent in neuron-rich brain areas such as hippocampus and neocortex (ascorbate/glutathione ratio is 4; in glia-rich areas, this ratio is found to be 0.24) suggesting a key strategic task in neuroprotection [12]. In this context, administration of AA reduced the formation of protein-aggregates involved in the pathogenesis of various neurodegenerative diseases and was demonstrated to protect neuroblastoma cells from β-amyloid apoptosis [13–16]. Parenteral AA decreased infarct sizes in both, primate and rodent models [14–16]. Also, this vitamin protected adult rat brain against pentylenetetrazol-induced apoptotic neurodegeneration [17] and reduced damage in the hippocampus during seizures [10,18]. AA-mediated neuroprotection deemed to overlap with neuromodulatory features, e.g. stimulation of catecholamine synthesis, dopamine D1- and D2 receptor-antagonism, inhibition of acetylcholinesterase and strengthening the resilience of neurons against glutamate-excitotoxicity [1,16].

AA and anticonvulsant effect

Low brain AA-levels promoted seizures in mouse models of decreased brain AA-transport and Alzheimer’s disease [11]. Of note, AA suppressed epileptiform activity in various experimental epilepsy models and may prevent seizure activity [19,20]. Most recently, a case-control study found significantly lower AA-levels in the blood of 40 generalized epileptic adult patients [21]. Therefore, AA is currently discussed as an auxiliary treatment of epilepsy [10] and clinical trials of AA as add-on anticonvulsive therapy are underway [22]. However, the mechanisms of the neuroprotective and anticonvulsive ascorbate effects are still obscure and primarily attributed to a consolidation of cell membranes by scavenging reactive species-activity that originates e.g. from lipid peroxidation or increased energy metabolism during pronounced seizure activity [10,21].
AA and antidepressant effect

The biological core hypothesis of depression disorder based upon monoamine deficiency in the central and most likely autonomous nervous system [23]. All approved antidepressants can stimulate intracellular pathways following postsynaptic activation of serotonin and catecholamine receptors to different extents through different mechanisms, the vast majority by slowing the cellular degeneration of monoamines [23]. Current research is underway to analyze the role of extra- and postsynaptic co-activation of glutamate non-NMDA receptors and the consecutive stimulation of cellular mTORC1-pathway being supposed to mediate more rapid clinical antidepressant response and adaptive resilience via more rapid generation of neurotrophins – needed for synaptic plasticity and hippocampal neurogenesis (neurotrophin hypothesis) [24–26]. At this point, it is interesting that AA-mediated neuroprotection deemed to overlap with neuromodulatory features, e.g. stimulation of catecholamine synthesis [1,16]. To precise, AA was found to strongly enhance neuronal synthesis of norepinephrine from dopamine [27]. Indeed, there is first evidence of an antidepressant-like effect of AA in animal experiments [28,29]. Intriguingly, this effect appeared to include increased mTORC1 signaling, which was shared with ketamine [28,29], a drug being at this juncture extensively under study due to its unique rapid antidepressant response in human treatment-resistant depression [26]. First small but well controlled proof-of-concept studies found a beneficial effect of medium-dosed AA (1000 mg/day) adjunctive to fluoxetine (10–20 mg/day) in pediatric major depression [30] and a lack of antidepressant efficacy in adult MDD when AA was used as an adjunct to up to 60 mg/day citalopram [31]. A previous open label study found fluoxetine and citalopram to be able to reverse markers of oxidative stress including decreased plasma AA-levels in MDD-patients [32].

Development of the hypothesis

All these beneficial-properties assigned to AA (the neuroprotective, the anticonvulsive and the antidepressant features) can be explained by mild to modest drops (< 0.04–0.5 pH-units) of the intracellular pH (pHi) of central neurons, too [34,35,42–44] as shown below in further detail. This is especially interesting, because AA serves as an enzyme cofactor in numerous metabolic reactions and this cofactor activity is based upon its ability to undergo redox reactions [5]. Technically, the redox potential of vitamin C directly depends on the pH (“redox buffer”); ascorbic acid ↔ ascorbyl free radical + e− ↔ dehydroascorbic acid + 2H+ + 2e−) and at physiological pH, 99.95% of vitamin C will be present as a mono-anion (ascorbate −, pKa1: 4.2) serving the cells as an excellent hydrogen donor antioxidant (2 ascorbate free radical− + H+ ↔ ascorbate− + dehydroascorbic acid) [1,2]. In fact, recent and previous experiments in non-neuronal tissues (human erythrocyte cells, rodent primary astrocyte cultures and rat colon cystic epithelial) indicated a modest AA-related acidification [39,40]. There is indirect evidence that this acidification is mediated by specific biological mechanisms, e.g. inhibiting transmembrane pH-regulators (i.e. subtype 1 of the sodium-proton exchanger-family (NHE) (Fig. 1)) by AA [39,40]. This is particularly interesting if looking at the physiological level, where transmembrane AA-transporters are closely related to bicarbonate and proton exchangers, the latter all elements of the cellular pH-regulation system [36,45]. In mammalian subcortical neurons, there is first evidence of an acidifying effect of low millimolar AA when administered to rodent hippocampal neurons (Fig. 2). Therefore, I assume that a mild cytoplasmatic acidiﬁcation of cortical and subcortical neurons resulting from at least medium doses of dietary AA contributes to its neuroprotective, anticonvulsive and antidepressant-like properties. In the following, some key results linking small to modest acidifications of cortical and subcortical neurons with beneficial effects are presented.

Intracellular acidification and neuroprotection effect

NMDA-induced superoxide production and neuronal death were prevented by intracellular acidification by as little as 0.2 pH units [46]. The generation of amyloid precursor protein could be reduced by intracellular acidification [47]. Additionally, a protective role of lactate had been related to modest intracellular acidification [38] which could to limit excitotoxicity [34,48].

Intracellular acidification and anticonvulsant effect

A mild lactate-mediated pH-drop in human neocortical pyramidal cells was found to reduce the epileptiform activity of these cells [38]. This corresponds with mild acidifications being found in rodent hippocampal neurons during the administration of some clinical and experimental anticonvulsants [34,44,49]. Furthermore, ketogenic acidification is associated with anticonvulsive activity [38].

Intracellular acidification and antidepressant effect

A couple of antidepressants have been shown to be associated with a mild acidification in cortical and subcortical neurons [34]. Furthermore, electroconvulsive therapy most likely acidifies regional neurons of interest transiently [38] and acute sleep deprivation – belonging also to the biological therapies for the treatment of depression [50] – is associated with further increases of brain lactate [51] which itself has the potency to acidify cortical and subcortical neurons mildly [38].

Evaluation of the hypothesis

In animal studies; AA and ketamine [26,52] should be compared with respect to their effects on neuronal pH and pH-regulation in neuron-rich areas, e.g. hippocampal pyramidal neurons. Moreover, activation of mTORC1 is worth to be studied on possible effects on neuronal pH because this pathway is assumed to be important for synaptic plasticity modulation in various neuropsychiatric disorders including mood disorders, epilepsy and neurodegeneration [53]. Conversely, intracellular acidification has been already found to reduce mTORC1-activity [54,55] what makes the cytoplasmic pH valuable to play the role of a negative feedback modulator of the mTORC1-activity.
In vitro ammonium prepulse technique [35] would be helpful to study the effect of AA and ketamine on pH-regulation. If pH-regulation is delayed by AA after an ammonium pulse, the rotational removal of sodium, bicarbonate or chloride from the bath medium can contribute to identify the affected elements of the pHi-regulation system [36,38].

On the clinical level, the efficacy of at least medium dosed AA (250–500 mg q.i.d given per os) should be studied in double-blind placebo-controlled studies in cohorts with patients suffering from major depression, probable Alzheimer’s dementia or certain epileptic disorders. In the first step, AA should be studied adjunctive to treatment as-usual in these disorders over 12 weeks. If this is beneficial, then in a second step, medium to high-dosed AA-monotherapy is recommended to be tested in these disorders, again under well placebo-controlled conditions—however, now using additional biomarkers, e.g. measurement of the parenchymal pH by special magnetic resonance imaging techniques [56,57] in neuron-rich vs. glia-rich brain regions of interest.

Discussion

Regarding intracellular acidifications, the “beneficial” hormesis zone [35,58] is assumed to be below 0.4–0.5 pH-units [35,59]. I suppose that AA-mediated pH-drops not exceeding this zone are advantageous to control increased excitability, aberrant synaptic plasticity and neuronal viability. Acidifications as well as alkaline shifts above 0.5 pH-units were associated with predominantly harmful effects on cell functioning and survival [33,38,60]. Typically, a weakening of the neuronal pH-regulation by different means was followed with acidifications within this “hormetic zone” [35,61]. Especially, an inhibition of the sodium proton subtype 1-transporter (NHE1)—a core element of pHi-regulation—was demonstrated to possess powerful anticonvulsive, neuro- and cardio-protection effects in vitro and in vivo [43,44,49,62]. Furthermore, in the forced swimming test and spontaneous alternation behavior test mouse-models, NHE1-attenuation via amiloride was observed to reduce behavioral depression and to improve memory [43]. There is first, though indirect evidence in experiments of non-neuronal cells that AA might be affect this NHE-driven pH-regulation [39,40] which is recommended here to be verified in studies on mammalian cortical and subcortical neurons. When found in its di-anionic form (pKa2 11.8) AA exerts even pro-oxidant effects, especially in the presence of catalytic metal ions [2]. The concentration of di-anionic ascorbate is known to increase by a factor 10 for every pH-unit increase [2], providing further evidence for a cytotoxic potential of a neuronal pH-drop, e.g. by AA itself.

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Declaration of Competing Interest


References