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COMMENTARY

Quercetin Might Promote Autophagy in a Middle Cerebral Artery Occlusion-Mediated Ischemia Model: Comments on Fawad-Ali Shah et al.

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Dear Editor,

The recent paper by Fawad-Ali Shah on this journal describes the role of the flavonoid quercetin in a cerebral ischemia model induced in adult Sprague–Dawley male rats by middle cerebral artery occlusion (MCAO) [1]. The authors reported that quercetin in MCAO-induced ischemia modulated the expression of the enzymes isocitrate dehydrogenase [NAD^+] (which is present in two isoforms, EC. 1.1.1.41 and EC 1.1.1.42), adenosyl-homocysteinase (EC, 3.3.1.1), pyruvate kinase (EC 2.7.1.40), ubiquitin carboxy terminal hydrolase L1 (EC, 3.1.2.15), besides to the proteins heat shock protein 60 (hsp60) and collapsin response mediator protein 2 (CRMP2) [1]. Actually, many further important proteins are upregulated (and some of them down-regulated) by quercetin in the reported MCAO model [1].

Following Fawad-Ali Shah et al.'s paper, we hypothesized that the up- or down-regulation of the many specific proteins by quercetin might deal with the occurrence of an autophagic mechanism, directly or indirectly promoted by the flavonoid itself in the MCAO-induced ischemia [1]. Autophagy is a regular, physiological process adopted by cells to disassembly dysfuncional, damaged or unnecessary components, including organelles such as mitochondria (mitophagy). It usually involves a cascade of events starting with the phosphorylation of beclin-1 (the mammalian homologue of Atg6) [2]. The cell self-digesting mechanism that is responsible for the removal of long-lived proteins and damaged organelles by lysosomes, which form vacuolar autophagosomes, is called macro-autophagy and is crucial for cell survival [3]. Despite this, autophagy can also rule a complex interplay with apoptosis, triggering with caspases a death signal and contributing in the pathogenesis of several diseases [4]. Therefore, a complex cross-talk between autophagy and apoptosis is finely tuned in order to ensure cell survival or promote its death when cell function is damaged or compromised [5].

Quercetin has a fundamental role both in autophagy and apoptosis.

The flavonol attenuates that neuronal autophagy leading to apoptosis in brain injury, and further papers reported that the flavonol, as well as isoflavones, induced neuronal autophagy after brain injury, in order to prevent neurotoxicity and cell death [6–8]. However, the role of quercetin in the autophagy-mediated mechanisms of cell survival is particularly complex, due to the numerous targeted molecules involved in the process. Depending on the level of damage caused by ischemia or oxidative injury, quercetin inhibits autophagy if the autophagic response is not addressed as a survival but as a pro-apoptotic signal [9]. Actually, because of the existence of the many different targeted molecules from quercetin as a pro-survival molecule, the flavonol can prevent or reduce a cellular damage acting on the autophagic machinery. Evidence was reported showing that in oxidative damage and in ischemia-induced models quercetin may trigger a protective action by promoting autophagy [10]. Therefore, during brain injury following ischemia, quercetin may induce pro-survival mechanisms leading to autophagy as occurring for other cell damaging events [11–14]. Quercetin exerts a protective role on MCAO-induced apoptosis in neuronal cells and induces autophagy-mediated cell survival in neuronal PC12 cells [15, 16].

The reported role of quercetin on autophagy-mediated cell survival, in order to counteract brain injury-induced apoptosis, suggested us to comment the reported data by

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Fawad-Ali Shah et al. as we retrieved further insights on the role of quercetin in MCAO. We realized that some of the up-regulated proteins reported by the authors are also involved in the modulation of autophagy [17–20].

The authors reported that the ability of quercetin to restore the physiological levels of isocitrate dehydrogenase [NAD^+], which decreased with MCAO-induced ischemia, improved energy preservation in both damaged neuronal and glial cells, promoting their survival response [1]. They gave to their result an explanation based on metabolism.

We observed that the cross-talk between glial cells and neurons is of the utmost importance, during brain injury. A recent paper reported that in the ischemic stroke, inflammatory cells in the glia play a fundamental role in the exacerbation of ischemia causing neuronal cell apoptosis, if cells of myeloid lineage lack of an autophagic response [21]. Autophagy in this case has a protective role in preventing ischemia-induced damage of neuronal cells and interestingly quercetin, as many other polyphenols, is also an autophagy inducer [22].

In this perspective, we suggested an alternative hypothesis. We hypothesized a possible alternative to the energetic hypothesis forwarded by the authors, also accounting on the survival role exerted by quercetin via the activation of an autophagic response in brain myeloid cell lineages [23].

Some of the upregulated proteins found by the authors gave us this suggestion.

Isocitrate dehydrogenase [NAD^+] (IDH) might play a major role in autophagy, as emerging from research in IDH mutations and IDH-derived oncometabolites (2-hydroxyglutarate), associated with some forms of aggressive tumors, such as acute myeloid leukemia or glioblastomas, where autophagy is particularly compromised [18, 24–26]. Depletion of IDH following MCAO-induced ischemia affects the ability of cytosolic acetyl-CoA to regulate autophagy [25]. During ischemia, the metabolic adaptation to the abrupt lack of oxygen was controlled by the activation of AMPK. This was sustained also by an up-regulation of adenosyl-homocysteinase and acetyl-CoA synthetase, in order to support survival mechanisms via an autophagic response [25].

Furthermore, in MCAO-induced ischemia, quercetin triggers the upregulation of the ubiquitin carboxy terminal hydrolase L1, an enzyme that regulates autophagy, by about twofolds, modulating the relationship apoptosis/autophagy and its association with the NF-κB-mediated signaling [1, 18, 26, 27]. In the paper by Fawad-Ali Shah some listed proteins are activated during the astrocytic-neuronal signaling and regulation of autophagy, for example PEA-15 (phosphoprotein enriched in astrocytes 15 kDa), which regulates autophagy via the activation of a JNK-mediated signaling or also alpha-synuclein [28, 29]. This evidence suggested us that a possible autophagic response induced by quercetin caused those proteins upregulation.

Fawad-Ali Shah et al. reported also that quercetin down-regulated the expression of hsp60 and CRMP2 [1].

Hsp60 is involved in brain injury caused by ischemic conditions and is crucial for regulating endoplasmic reticulum stress via an autophagic response. However, the down-regulation of hsp60 observed by the authors may not assess the occurrence of a pre-activation of autophagy by ischemic preconditioning, in order to boost further endogenous defense mechanisms [30]. Furthermore, although CRMP2 is particularly involved in autophagy, as it exerts a role in the efficient traffic of LC3-II containing autophagic vesicles, its involvement cannot be fully explained with the action exerted by quercetin on autophagy [21].

Despite our hypothesis, some controversial issues should be further addressed.

It is well known that the efficient activity of CRMP2 (inhibition of calpain or CRMP2 overexpression) is protective towards neuronal or axonal damage [31]. Nevertheless, Fawad-Ali Shah et al. pointed to the fact that CRMP2 is cleaved by calpain into two (62 and 55 kDa) proteins, an evidence that could indicate that quercetin did not inhibit calpain in inducing CRMP2 degradation, contrarily to the reported evidence [1]. Therefore, it's reasonable to suggest that, if quercetin promotes autophagy in the MCAO model, this process should not be CRMP2-mediated, at least observing the data from Fawad-Ali Shah et al. While the reduction of hsp60 may be discussed as the promoting action of the flavonoid towards a protective stress response, the inhibitory action on CRMP2 overexpression appears much more difficult to explain in an autophagic model. This evidence may be particularly controversial, as the injury caused by MCAO has a dramatic consequence on neuronal microtubules. It is possible that in this context quercetin may inhibit calpain, which notoriously has a cleavage activity towards CRMP2 [32–36]. This encourages to deepen the evidence reported by the authors with more insightful data.

Limitations to our hypotheses are to be further discussed.

The role of quercetin on the regulation of cell survival via an autophagic response as been widely reported in the literature but a pro-apoptotic signal, also via autophagy, can be elicited when cell damage is particularly important. The regulation of autophagy by a flavonoid is particularly intriguing to assess the fine tuning exerted by a plant-derived molecule on the complex mechanisms leading to cell survival. Therefore, the level of ischemic damage on the interplay autophagy-apoptosis in neuronal cells, the dose of quercetin and its treatment protocol, are of major importance to elucidate the role of quercetin in MCAO-ischemic model. Our suggestion is to progress the outstanding research study reported by Fawad-Ali Shah investigating the role of quercetin in the autophagy mechanisms underlying MCAO.

Finally, according to our opinion, the activity of quercetin might not simply account on its pro-survival potential

simply due to its anti-oxidant property. The ability of plant polyphenols to induce a pro-survival response encompasses also many further mechanisms beside the upregulation of stressors scavengers, such as mitophagy, autophagy and modulated apoptosis. Besides the reported conclusion that a possible mechanism of quercetin-mediated prevention of MCAO-induced ischemia involves an energetic balance, the paper by Fawad-Ali Shah sheds also an intriguing light on the possibility that quercetin may act on the ischemic damage, e.g. during a stroke, by, even indirectly rescuing the autophagic potential of neurons and of their associated glial cells.

Compliance with Ethical Standards

Conflict of interest The Authors state they have no conflict of interest.

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