Dichotomous effects of autophagy on infarct volume in experimental permanent/transient ischemic stroke model: a systematic review and meta-analysis

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According to the recent findings, autophagy modulation is being a potential therapeutic target in the management of ischemic stroke in a pre-clinical setting. However, the pros and cons of autophagic response strongly depend on the activation time of autophagy after injury. In this systematic review, we aimed to explore the impacts of pharmacological modulation of autophagy on infarct size in experimental ischemic stroke models. Based on our preliminary search, 3551 publications were identified. Of twenty-nine publications that met the inclusion criteria, twenty studies reported infarct volume reduction by percentage (%) with no evidence of any publication bias while nine studies reported by mm³, which had publication bias (39.25 units, standardized mean differences (SMD) = 41.92, 95% confidence interval (CI): 30.33 to 53.51). Based on a meta-analysis, the point estimate (pooled mean difference) for improvement of infarct volume during autophagy modulation according to the mm³ and percentage were 35.64 (mean differences (MD) = 35.64, 95% CI: 26.43 to 44.85, z-value = 7.58, p-value < 0.001) and 14.38 (MD = 14.38, 95% CI = 10.50 to 18.26, z-value = 7.26, p < 0.001) units, respectively. Despite the undeniable role of autophagy in ischemic stroke, the dichotomous effects of autophagy regarding infarct volume reduction should be taken into account. Based on our findings, the studies included in this meta-analysis mostly reported a negative relation between autophagy induction and stroke volume development due to over-activity of autophagy upon the severe ischemic stroke; therefore, further pre-clinical studies are also recommended to establish adjusted autophagy with considering a time-dependent effect as a promising therapeutic target.

Keywords

Autophagy; Dual effects; Ischemic stroke; Pre-clinical setting

1. Introduction

Ischemic stroke (IS), one of the devastating disorders, is the intended second leading cause of mortality and disability followed by vascular occlusion and irreversible damage of the brain tissue [1, 2]. Despite the rising aged population, the incidence of stroke is expected to grow thereby a demand to accede a novel and more effective therapeutic approach is increasing, particularly for patients suffering from acute cerebral ischemia [3, 4]. It has been proved that long-lasting autophagy besides a variety of other neurologic conditions plays a crucial role in cerebral ischemic injury. However, growing pieces of evidence demonstrated that autophagy has the potential to exert controversial effects (either detrimental or beneficial) in cerebral IS [5]. In better words, regulated and moderate autophagy may provide a neuroprotection effect while an excessive or inappropriate activation of autophagy could trigger deleterious effects to develop cell death [6, 7]. Autophagy, a catabolic-conserved process through the breakdown and subsequent recycling of cellular constituents, is an essential physiological intracellular process for maintaining cellular homeostasis and simultaneously participates in bio-energetic procedures under various stress conditions [8]. This phenomenon is highly regulated by numerous molecules such as microtubule-associated protein 1A light chain 3 (LC3), Beclin-1, and P62 (a scaffold protein) that have a necessary role in the regulation of the autophagy signaling pathway [9]. Of note, the excessive activation of autophagy and related effectors in neural cells have been firmly established in a variety of focal ischemic stroke conditions.
models such as experimental middle cerebral artery occlusion (MCAO). Moreover, recent evidence demonstrated that the over-activity of neuronal autophagy through persistent stress, such as cerebral ischemia, results in cell damage, especially in the border area of lesion sites [10, 11]. Therefore, autophagy regulation could be considered a potential target for IS treatment [12]. In contrast, it has also been reported that pre-activation of autophagy in the brain tissue could enhance brain ischemic tolerance, facilitate cellular energy production, and prevent neuronal apoptosis during subsequent exposure to the ischemic conditions [13]. For instance, rapamycin, as a well-known autophagy inducer has a palliative effect on pre-clinical IS damage through the activation of mitophagy, suggesting that autophagy has a beneficial effect on ischemia/reperfusion injury. Although there is no debate regarding autophagy participation in cerebral ischemia, the accurate function of autophagy in IS remains controversial. In hence, the main purpose of this systematic review refers to uncover a total pattern of infarct volume evolution after autophagy modulation quantitatively via meta-analysis in the experimental models of stroke.

2. Methods

2.1 Search strategy

For the primary systematic search strategy, Embase, Medline (via PubMed, Ovid) databases were used. Notably, all considered studies were published in English and the inception date of each database was qualified for inclusion in this review (from 1980-Jan till 2021-May). In addition, the search strategy aimed to explore both published and unpublished studies with the combination of Mesh and free keywords such as autophagy, macroautophagy, cerebrovascular accident, ischemic stroke, and autophagy biomarkers. A complete search strategy in the PubMed database is brought in the supplementary material (Appendix Table 3).

2.2 Inclusion and exclusion criteria

This quantitative study was deliberated to include all studies calculated infarct size following the assessment of autophagy detrimental and/or protective effects as the primary outcome in the IS model of rodents who underwent experimental transient/prominent ischemia induced by MCAO as well as focal cerebral ischemia. There was not any exclusion based on the route of drug administration, divergent medications used for anesthesia, and the duration of treatment. The full text of selected studies that did not meet the inclusion criteria such as clinical trials, in vitro experiments, non-English written articles, the published conference abstracts, and the articles without standard quality, such as not mentioned quantitative changes in case of the infarct size with percentage or mm$^3$, were ultimately excluded.

2.3 Data extraction

To retrieve quantitative article selection, two reviewers (AR and NV) independently screened the relevant titles and abstracts. After eligible articles inclusion, to determine the risk of bias, the full-texts of all included articles were also precisely screened by two reviewers (AR and NV), independently. Meanwhile, any discrepancies were arbitrated by a third reviewer (FS). Endnote X9 as a reference management software (Thomson Corporation Inc., USA) was used to organize titles and abstracts of studies as well as duplicated identification. It should be noted that corresponding authors of primary studies were contacted for any missing or clarifying unclear data, where required. Finally, required data extraction from the articles was summarized in the extraction diagrams (Table 1, Ref. [10, 14–21] and Table 2, Ref. [22–41]) and intended study designs including first author’s name, year of publication, study location, type of animals (species, sex), sample size, name of therapeutic agents, related-dose, route of administration, experimental model of ischemic stroke, and infarct size alternation (% or mm$^3$) were prepared.

2.4 Statistical meta-analysis

The numbers of animals and average stroke volume (mean ± SD) in each group were extracted from the included articles. Next, the differentiation of the stroke volume for each study was calculated, and then the pooled mean differences were achieved by meta-analysis. To combine mean differences, the random effect model was used whereas the heterogeneity between studies was assessed by Cochran statistics (Q) and I$^2$ test, which demonstrate the percentage of the variance between studies. For data analysis, CMA software was applied. To assess the publication bias, Egger’s regression test and the Funnel Plot were used. Besides, to further evaluation of possible publication bias, the Trim and Fill method was performed. Effect sizes were also expressed as pool mean differences (for continuous data) and their 95% confidence interval (CI) was calculated for further analysis. Regarding the subgroup analysis, it could be calculated when there is adequate data. Finally, these findings were presented in a description form to assist in data presentation where statistical pooling is not possible. The $p$-value of less than 0.05 was considered statistically significant.

3. Results

3.1 Advanced search features

Following the systemic search using the database, 3551 articles were identified. 2363 duplicated and 933 irrelevant articles were excluded after a preliminary evaluation of the articles according to the title and abstract. Following the full-text assessment for article eligibility, of a total number of 256, 227 articles were also excluded. Ultimately, 29 articles supporting the inclusion criteria were included in the current meta-analysis. The relevant flow chart of determined and included articles was outlined in Appendix Fig. 5. According to the obtained data from the included articles, the animals were assigned to the control group without any intervention, the stroke group induced by permanent/transient MAOC manner, and treatment groups received autophagy modulators.

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Fig. 1. Effect of autophagy process on stroke volume based on mm³ measurement. (A) Forest panel analysis represented by mean differences and 95% CIs following the search strategy till 2021. (B) Subgroups analysis according to the cell death/protective role of the autophagy) represented by mean differences and 95% CIs, showing that the autophagy process mainly involves in the stroke volume progression and subsequently promotes the cell death.
Table 1. Designed characteristics of included studies based on mm$^3$.

<table>
<thead>
<tr>
<th>Publication</th>
<th>Year</th>
<th>Country</th>
<th>Species &amp; Gender</th>
<th>Sample size (n)</th>
<th>(Dose, route of delivery)</th>
<th>Time course of autophagy assessment (h)</th>
<th>Levels of LC3 after Treatment</th>
<th>Temp/Perm Infarct (mm$^3$)</th>
<th>Outcome (autophagy effect)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li J et al. [14]</td>
<td>2015</td>
<td>China</td>
<td>Female SD rats</td>
<td>5</td>
<td>17-AGG (80 mg/kg), i.p.</td>
<td>24</td>
<td>Decreased</td>
<td>Temp 96.7 ± 12.23</td>
<td>Cell death</td>
</tr>
<tr>
<td>Li W-L et al. [15]</td>
<td>2013</td>
<td>USA</td>
<td>Male wild-type (B6, 129PF2) and p50 knockout (p50, B6, 129P-Nfkb1) mice</td>
<td>5</td>
<td>NF-kB</td>
<td>12, 24</td>
<td>Decreased</td>
<td>Perm -9.4 ± 3.65</td>
<td>Cell death</td>
</tr>
<tr>
<td>Li H et al. [16]</td>
<td>2015</td>
<td>China</td>
<td>Male SD rats</td>
<td>3</td>
<td>002C-3 (10 g/kg), i.v.</td>
<td>24</td>
<td>Decreased</td>
<td>Temp 100 ± 13.24</td>
<td>Cell death</td>
</tr>
<tr>
<td>Shu S et al. [17]</td>
<td>2016</td>
<td>China</td>
<td>Male SD rats</td>
<td>15</td>
<td>EA 24 h</td>
<td>6, 24, 72</td>
<td>Decreased</td>
<td>Temp 18.2 ± 3.27</td>
<td>Cell death</td>
</tr>
<tr>
<td>Feng D et al. [10]</td>
<td>2016</td>
<td>China-USA</td>
<td>Male C57BL/6 mice</td>
<td>KN</td>
<td>E24 h</td>
<td>6, 12, 24</td>
<td>Decreased</td>
<td>Temp 21.00 ± 3.18</td>
<td>Cell death</td>
</tr>
<tr>
<td>Liu N et al. [18]</td>
<td>2011</td>
<td>Japan</td>
<td>Male C57BL/6 mice</td>
<td>5</td>
<td>Edaravone A, 9 mg/kg i.v.</td>
<td>48</td>
<td>Decreased</td>
<td>Temp 23.7 ± 5.47</td>
<td>Cell death</td>
</tr>
<tr>
<td>Liu N et al. [18]</td>
<td>2011</td>
<td>Japan</td>
<td>Male C57BL/6 mice</td>
<td>5</td>
<td>Edaravone B, 9 mg/kg i.v.</td>
<td>48</td>
<td>Decreased</td>
<td>Temp 25.3 ± 5.15</td>
<td>Cell death</td>
</tr>
<tr>
<td>Liu Y.Y. et al. [19]</td>
<td>2017</td>
<td>China</td>
<td>Male SD rats</td>
<td>4</td>
<td>PF11 (6, mg/kg), i.v.</td>
<td>24</td>
<td>Decreased</td>
<td>Perm 7.6 ± 1.09</td>
<td>Cell death</td>
</tr>
<tr>
<td>Liu Y.Y. et al. [19]</td>
<td>2017</td>
<td>China</td>
<td>Male SD rats</td>
<td>4</td>
<td>PF11 (6, mg/kg), i.v.</td>
<td>24</td>
<td>Decreased</td>
<td>Perm 7.88 ± 0.79</td>
<td>Cell death</td>
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<tr>
<td>Jiang Zh et al. [20]</td>
<td>2015</td>
<td>China and USA</td>
<td>Male SD rats</td>
<td>5</td>
<td>MB, 1 mg/kg, i.p.</td>
<td>24</td>
<td>-</td>
<td>Temp 36.00 ± 8.24</td>
<td>Protective</td>
</tr>
<tr>
<td>Shen PP et al. [21]</td>
<td>2016</td>
<td>China and USA</td>
<td>Male Wistar rats</td>
<td>5</td>
<td>CSD Preconditioning</td>
<td>6, 12, 24</td>
<td>Increased</td>
<td>Temp 10.62 ± 1.5</td>
<td>Protective</td>
</tr>
</tbody>
</table>

17-AGG, 17-allylamo-17-demethoxygeldanamycin; CSD, Cortical Spreading Depression; MB, Methylene blue; Mel, Melatonin; NF-κB, Nuclear factor kappa B.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Country</th>
<th>Species &amp; Gender</th>
<th>Sample size (n)</th>
<th>Dose &amp; route of delivery of therapeutic agents</th>
<th>Time course of autophagy assessment (h)</th>
<th>Level of LC3 after Treatment</th>
<th>Temp/Perm</th>
<th>Infarct size reduction (%)</th>
<th>Outcome (autophagy effect)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li Q et al. [22]</td>
<td>2014</td>
<td>China</td>
<td>Male wild-type ICR mice</td>
<td>16–20</td>
<td>Rap 8 ng/2 micro DMSO 0.1%, i.c.v.</td>
<td>6, 24, 48, and 72</td>
<td>Increased Perm</td>
<td>11.86 ± 2.16</td>
<td>11.86</td>
<td>Protective</td>
</tr>
<tr>
<td>Bu Q et al. [23]</td>
<td>2014</td>
<td>China</td>
<td>Male Wild-type ICR mice + SD rats</td>
<td>10</td>
<td>w007B10 mg/kg, i.v.</td>
<td>24</td>
<td>Decreased Temp</td>
<td>16.8 ± 1.44</td>
<td>16.8</td>
<td>Cell death</td>
</tr>
<tr>
<td>Bu Q et al. [23]</td>
<td>2014</td>
<td>China</td>
<td>Male Wild-type ICR mice + SD rats</td>
<td>10</td>
<td>w007B 50 mg/kg, i.v.</td>
<td>24</td>
<td>Decreased Temp</td>
<td>35.7 ± 1.16</td>
<td>35.7</td>
<td>Cell death</td>
</tr>
<tr>
<td>Fu L et al. [24]</td>
<td>2016</td>
<td>China</td>
<td>Male Balb/c mice</td>
<td>6</td>
<td>CC (20 mg/kg), i.p.</td>
<td>24</td>
<td>Increased Perm</td>
<td>22.43 ± 0.56</td>
<td>22.43</td>
<td>Cell death</td>
</tr>
<tr>
<td>Li Y et al. [25]</td>
<td>2015</td>
<td>China</td>
<td>Male SD rats</td>
<td>12</td>
<td>Ebselen, gavage</td>
<td>14 day</td>
<td>Decreased Temp</td>
<td>18.2 ± 3.27</td>
<td>18.2</td>
<td>Cell death</td>
</tr>
<tr>
<td>Chi O.Z. et al. [26]</td>
<td>2016</td>
<td>USA</td>
<td>Male Fischer Rat</td>
<td>8</td>
<td>Rap, 20 mg/kg, i.p.</td>
<td>24</td>
<td>Decreased Temp</td>
<td>16.4 ± 3.2</td>
<td>16.4</td>
<td>Cell death</td>
</tr>
<tr>
<td>Lu T et al. [27]</td>
<td>2011</td>
<td>China</td>
<td>Male SD rats</td>
<td>3</td>
<td>GRβ1, 1.25 mg/kg intra nasal</td>
<td>24</td>
<td>Decreased Temp</td>
<td>23.14 ± 1.23</td>
<td>23.1</td>
<td>Cell death</td>
</tr>
<tr>
<td>Lu T et al. [27]</td>
<td>2011</td>
<td>China</td>
<td>Male SD rats</td>
<td>3</td>
<td>GRβ1, 12.5 mg/kg intra nasal</td>
<td>24</td>
<td>Decreased Temp</td>
<td>29.81 ± 1.13</td>
<td>29.8</td>
<td>Cell death</td>
</tr>
<tr>
<td>Wu M et al. [28]</td>
<td>2017</td>
<td>China</td>
<td>Male SD rats</td>
<td>6</td>
<td>Pre- Rap (3.0 mg/kg.), i.p.</td>
<td>24 h, 7 days</td>
<td>Increased Temp</td>
<td>12.6 ± 1.73</td>
<td>12.6</td>
<td>Cell death</td>
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<tr>
<td>Wu M et al. [28]</td>
<td>2017</td>
<td>China</td>
<td>Male SD rats</td>
<td>6</td>
<td>Post-Rap (3.0 mg/kg.), i.p.</td>
<td>24 h, 7 days</td>
<td>Increased Temp</td>
<td>8.3 ± 1.46</td>
<td>8.3</td>
<td>Cell death</td>
</tr>
<tr>
<td>Qi Zh et al. [29]</td>
<td>2012</td>
<td>China</td>
<td>Male SD rats</td>
<td>4</td>
<td>IPOC 10</td>
<td>24</td>
<td>Increased Temp</td>
<td>22.00 ± 2.75</td>
<td>22.0</td>
<td>Cell death</td>
</tr>
<tr>
<td>Qi Zh et al. [29]</td>
<td>2012</td>
<td>China</td>
<td>Male SD rats</td>
<td>4</td>
<td>IPOC 50</td>
<td>24</td>
<td>Increased Temp</td>
<td>18.00 ± 2.4</td>
<td>18.0</td>
<td>Cell death</td>
</tr>
<tr>
<td>Qi Zh et al. [30]</td>
<td>2015</td>
<td>China and USA</td>
<td>Male SD rats</td>
<td>4</td>
<td>RIC</td>
<td>24</td>
<td>Increased Perm</td>
<td>10.62 ± 1.5</td>
<td>10.6</td>
<td>Protective</td>
</tr>
<tr>
<td>Wang R et al. [31]</td>
<td>2014</td>
<td>China</td>
<td>Male Wistar rats</td>
<td>6</td>
<td>Res 30 mg/kg, i.p.</td>
<td>24</td>
<td>Increased Temp</td>
<td>9.29 ± 3.97</td>
<td>9.29</td>
<td>Protective</td>
</tr>
<tr>
<td>Jeong J.H. et al. [32]</td>
<td>2016</td>
<td>Korea</td>
<td>Male SD rats</td>
<td>5</td>
<td>IF</td>
<td>24</td>
<td>Increased Perm</td>
<td>38.64 ± 0.98</td>
<td>38.6</td>
<td>Protective</td>
</tr>
<tr>
<td>Li L et al. [33]</td>
<td>2017</td>
<td>China and USA</td>
<td>Male SD rats</td>
<td>6</td>
<td>GM1 25 mg/kg, i.p.</td>
<td>24</td>
<td>Decreased Perm</td>
<td>6.8 ± 1.57</td>
<td>6.8</td>
<td>Cell death</td>
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<tr>
<td>Li L et al. [33]</td>
<td>2017</td>
<td>China and USA</td>
<td>Male SD rats</td>
<td>6</td>
<td>GM1 50 mg/kg, i.p.</td>
<td>24</td>
<td>Decreased Perm</td>
<td>1.6 ± 1.91</td>
<td>1.6</td>
<td>Cell death</td>
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<tr>
<td>Lu K.M. et al. [34]</td>
<td>2019</td>
<td>China</td>
<td>Male SD rats</td>
<td>3</td>
<td>HBO</td>
<td>3, 6, 12, 24, and 48</td>
<td>Decreased Perm</td>
<td>5.7 ± 0.016</td>
<td>5.7</td>
<td>Cell death</td>
</tr>
<tr>
<td>Li G et al. [35]</td>
<td>2012</td>
<td>China</td>
<td>Male Sprague-Dawley (SD) rats</td>
<td>5</td>
<td>IPOC</td>
<td>24</td>
<td>Decreased Perm</td>
<td>17.48 ± 1.59</td>
<td>17.4</td>
<td>Cell death</td>
</tr>
<tr>
<td>Qi Zh E et al. [36]</td>
<td>2014</td>
<td>China and USA</td>
<td>Male SD rats</td>
<td>3–4</td>
<td>HSYA (2 mg/kg), i.v.</td>
<td>24, 48, and 72</td>
<td>Increased Temp</td>
<td>10.62 ± 2.26</td>
<td>10.6</td>
<td>Protective</td>
</tr>
<tr>
<td>Chen et al. [37]</td>
<td>2020</td>
<td>China</td>
<td>Male ICR Mice</td>
<td>6</td>
<td>TAT-SKP2 (1 mg/kg/day), i.p.</td>
<td>1, 3, 6, 12, and 24</td>
<td>Increased Temp</td>
<td>49.7 ± 7.2</td>
<td>49.7</td>
<td>Protective</td>
</tr>
<tr>
<td>Chen et al. [37]</td>
<td>2020</td>
<td>China</td>
<td>Male ICR Mice</td>
<td>6</td>
<td>TAT-SKP2 (2 mg/kg/day), i.p.</td>
<td>1, 3, 6, 12, and 24</td>
<td>Increased Temp</td>
<td>41.9 ± 11.2</td>
<td>41.9</td>
<td>Protective</td>
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<tr>
<td>Chen et al. [37]</td>
<td>2020</td>
<td>China</td>
<td>Male ICR Mice</td>
<td>6</td>
<td>TAT-SKP2 (4 mg/kg/day), i.p.</td>
<td>1, 3, 6, 12, and 24</td>
<td>Increased Temp</td>
<td>34.2 ± 8.3</td>
<td>34.2</td>
<td>Protective</td>
</tr>
<tr>
<td>Li et al. [38]</td>
<td>2020</td>
<td>USA</td>
<td>Male C57/BL6j mice</td>
<td>6</td>
<td>28% (2.8 g/kg/d) Ethanol, Gavage</td>
<td>24</td>
<td>Decreased Temp</td>
<td>–20%</td>
<td>–20%</td>
<td>Protective</td>
</tr>
<tr>
<td>Pan et al. [39]</td>
<td>2020</td>
<td>China</td>
<td>Male Sprague-Dawley rats</td>
<td>16 and 32</td>
<td>Treadmill</td>
<td>3 and 7 days</td>
<td>Decreases Temp</td>
<td>20.72 ± 2.62</td>
<td>20.7</td>
<td>Cell death</td>
</tr>
<tr>
<td>Wang et al. [40]</td>
<td>2020</td>
<td>China</td>
<td>Male C57/BL6j mice</td>
<td>8</td>
<td>STS, 10 mg/kg, i.p.</td>
<td>1 and 3</td>
<td>Decreases Temp</td>
<td>29.81 ± 3.35</td>
<td>29.8</td>
<td>Cell death</td>
</tr>
<tr>
<td>Wang et al. [40]</td>
<td>2020</td>
<td>China</td>
<td>Male C57/BL6j mice</td>
<td>8</td>
<td>STS, 20 mg/kg, i.p.</td>
<td>1 and 3</td>
<td>Decreases Temp</td>
<td>22.71 ± 3.55</td>
<td>22.7</td>
<td>Cell death</td>
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<tr>
<td>Wang et al. [40]</td>
<td>2020</td>
<td>China</td>
<td>Male C57/BL6j mice</td>
<td>8</td>
<td>STS, 40 mg/kg, i.p.</td>
<td>1 and 3</td>
<td>Decreases Temp</td>
<td>21.59 ± 2.95</td>
<td>21.6</td>
<td>Cell death</td>
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<tr>
<td>Wang et al. [41]</td>
<td>2021</td>
<td>China</td>
<td>Male Sprague-Dawley rats</td>
<td>6</td>
<td>HBO 100% oxygen and 1.5 atmosphere absolute pressure</td>
<td>72</td>
<td>Decreases Temp</td>
<td>20.12 ± 2.940</td>
<td>20.1</td>
<td>Cell death</td>
</tr>
</tbody>
</table>

CC, C compound; EA, Electroacupuncture; GM,1 Ganglioside; HBO, Hyperbaric Oxygen Therapy; HSYA, Hydroxysafflor yellow A; IPOC, Ischemic Post conditioning; Mel, Melatonin; Rap, Rapamycin; Res, Resveratrol; STS, Sodium tanshinone IIA sulfonate; TAT-SKP2, Sphingosine Kinase 2-mimicking TAT-peptide;
3.2 Differences in mean of stroke volume based on mm$^3$

In 13 studies, the mean of stroke volume has been calculated while the heterogeneity between included studies was significant (Q-value = 59.83, df = 12, p < 0.001, I$^2$ = 79.94%). According to the meta-analysis results, the pooled mean difference of stroke volume between stroke and treatment groups was 35.65 units (MD = 35.65, 95% CI = 26.43 to 44.85, z-value = 7.58, p < 0.001). Additionally, in studies with the protective role of autophagy (n = 2), the pooled mean difference of stroke volume between the two groups was estimated 39.25 units (MD = 39.25, 95% CI = 35.06 to 43.44, z-value = 7.08, p < 0.001). In all, the forest plot analysis showed that autophagy activation in 11 and 2 studies contributed to cell death and protection, respectively. The Forest plot of the subgroup analysis was also shown in Fig. 1B. Based on the obtained results, in studies that reported the autophagy negative effect (n = 11), the results of subgroup analysis showed that pooled mean difference in terms of stroke volume between stroke and treatment groups was 35.06 units (MD = 35.06, 95% CI = 25.35 to 44.77, z-value = 7.08, p < 0.001). Additionally, in studies with the protective role of autophagy (n = 2), the pooled mean difference of stroke volume between the two groups was estimated 39.25 units (MD = 39.25, 95% CI = 35.06 to 43.44, z-value = 7.08, p < 0.001).

3.2.1 Publication bias

The relevant publication bias for the funnel plot has been shown in Fig. 2. According to the consequence of the stroke volume mean difference, egger’s regression test revealed that publication bias was practically significant between studied groups (t-value = 3.24, df = 11, p-value = 0.007). Moreover, the Trim and Fill method was performed for publication bias modifying, which added one study for missed study modulation. The results of this analysis also showed that the adjusted pooled mean difference for stroke volume between the two groups was 39.25 units (AMD = 41.92, 95% CI = 30.33 to 53.51).

3.2.2 Sensitivity analysis

According to the results shown in Fig. 1, studies conducted by Li J et al. [14], and Li H et al. [16], could be considered as a source of heterogeneity among studies. Therefore, the sensitivity analysis was performed regardless of these studies. Based on the results of sensitivity analysis, it has been clarified that pooled mean difference for stroke volume between stroke and treatment groups was 15.09 (MD = 15.09, 95% CI: 10.12 to 20.04, z-value = 5.95, p-value < 0.001), while for studies with the detrimental effect of autophagy the sensitivity analysis was estimated 12.98 (MD = 12.98, 95% CI: 8.21 to 17.75, z-value = 5.33, p-value < 0.001).

3.3 Differences in mean of stroke volume based on the percentage (%)

Based on the percentage of the infarct volume mean, which has been reported in 29 studies, the heterogeneity between the studies was also statistically significant (Q-value = 4830.82, df = 28, p < 0.001, I$^2$ = 99.4%). In Fig. 3A, the forest plot of combined results has been depicted in detail, which showed that the pooled mean difference for stroke volume between stroke and treatment groups was 14.38% (MD = 14.38, 95% CI = 10.50 to 18.26, z-value = 7.26, p < 0.001) (Fig. 3A). The related forest plot of subgroup analysis has been summarized in Fig. 3B. As shown in Fig. 3B, autophagy exhibited a cell death effect in 17 studies while 12 studies reported the protective role of autophagy. In this respect, the subgroup analysis by considering the autophagy controversial effects showed that the pooled mean difference for stroke volume between stroke and treatment groups regarding the cell death outcome was 12.52% (MD = 12.52, 95% CI: 7.91 to 17.14, z-value = 5.33, p < 0.001). In addition, the studies in which the protective role of autophagy towards the infarct volume progression were proved indicated that the pooled mean difference for stroke volume between two groups was 17.12% (MD = 17.12, 95% CI: 9.08 to 25.15, z-value = 4.18, p < 0.001).

Publication bias

Publication bias assessment of the mean differences of the stroke volume has been shown in the funnel plot (Fig. 4). According to egger’s regression test, there was no significant publication bias between different groups (t-value = 1.96, df = 27, p = 0.06).

4. Discussion

To the best of our knowledge, IS, as a more common type of stroke and a devastating disease, is mainly characterized by the major lack of regional cerebral blood supply in a distinct area of the cerebral tissue [42]. IS could be defined as one of the major leading causes of a corresponding loss of neurologic function, particularly in the aging population [43, 44]. Besides the dysregulated autophagy, it has been also documented that other pathological conditions such as mitochondrial dysfunction, oxidative stress, acidosis, calcium overload, and inflammatory response are associated with the pathogenesis of cerebral ischemia-reperfusion injury (IRI) [45]. The current systematic review and meta-analysis aimed to clarify autophagy modulation (either inhibition or induction) and its possible effects on the histological and infarcted volume restoration in animal models of ischemic stroke. As men-
Fig. 3. Effect of autophagy modulation on stroke volume based on percentage (%). (A) Forest panel analysis according to included studies and represented by mean differences and 95% CIs, following the search strategy till 2021, (B) Subgroups analysis according to the cell death/protective role of the autophagy represented by mean differences and 95% CIs, showing that the autophagy process mainly involves in the stroke volume progression and subsequently promotes the cell death.
Infarct volume reduction and ongoing neuroprotection effects of different agents or conditions mostly mediated by autophagy inhibition resulted in infarcted volume reduction. This outcome strongly implicated that prolonged stimulation, as well as the overexpression of autophagy, plays a major role in infarct size progression in stroke subjects, which negatively could exhibit in the high level of rapamycin (20 mg/kg), as well. In a study conducted by Chi et al. [26], it has been shown that mTOR, as a main target of rapamycin, exerts an imperative role not only in the maintenance of the cellular survival also governs the oxygen balance following the cerebral IRI likely through AKT and S6K1 phosphorylation in the cerebral cortex. Therefore, the high dose of rapamycin can increase infarct volume via mTOR inhibition as well as limitation of O2 consumption during reperfusion [26]. Notably, the protective effects in neural cells induced by autophagy, are predominately mediated using mTOR1 inhibitors such as rapamycin and metformin preconditioning as well as mTOR2 activation [55]. While utilizing rapamycin in low doses may also have enhanced autophagy activity enough in a non-mTORC2 manner to maintain neuronal survival following ischemia. Another protective activity of autophagy intercedes by scavenging accumulated misfolded proteins and cytoplasmic worn-out components in response to acute IS [56, 57]. To interrogate the exact role of the multi-phase autophagy process, a primary clinical trial to clarify the autophagy inhibitory/induction effect on mTOR2 is highly recommended in the context of IS. Another critical issue refers to the MCAO-induced IS leading to neuronal death by autophagosome accumulation and blocks autophagy flux in which increases the intracellular LC3, Beclin-1 (well-known autophagic biomarkers), and P62 (an adaptor protein) that conversely shows autophagy flux [19, 54]. The effect size of both autophagy modulations for infarct volume reduction was approximately equal. Together, there is no significant publication bias regarding the mean infarct volume percentage while publication bias was observed in mean differences of infarction volume amount (mm³) between studied groups.

Regarding the latent underlying mechanisms of action involved in autophagy regulation, Zhang et al. [58] indicated that chloride channel-3, as a signal molecule, exerted a neuroprotective role, which can directly activate autophagy machinery through the interaction between Beclin1 and Vps34 in a self-protective manner to impede infarct volume progression following acute IS (AIS), in vivo. In contrast, it has been reported that FK506 binding protein 5 (FKBPS), as a novel prognostic and diagnostic value, is up-regulated in subjects with AIS and participates in disease severity. FKBPS by autophagy induction through the downstream AKT/FOXO33 blocking could promote AIS exacerbation [59]. Another target signaling pathway to suppress dysregulated autophagy refers to the AKT/mTOR axis stimuli as well as autophagy-related gene 7 (Atg 7) downregulation emerging by dichloromethane therapy against IS in rats [60]. Notably, the results of a recent study conducted by Cai et al. [61] also showed that one of the substantial mechanisms in-

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**Fig. 4. Funnel plot of publication bias between studied groups calculated by egger’s regression test.** Pooled mean difference (CI: 95%).
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<th>Search Query</th>
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volved in the neuroprotective role of tissue-type plasminogen activator (tPA), a well-known thrombolytic medication in the clinical treatment of cerebral IRI, e.g., IS, is mainly related to the activation of FUN14 domain-containing 1 (FUNDC1)-mediated mitophagy to retrieve mitochondrial dysfunction following the AMPK phosphorylation and subsequent apoptotic cell reduction. Previously, it has been reported that the elevated level of inflammatory mediators, such as annexin A1 and monomeric C-reactive protein, can worsen the prognosis of the post-ischemic aged brain, in vivo [62, 63]. Interestingly, a cross-talk between autophagy and inflammation has also been delineated, which corroborated the benefits of moderate autophagy in facing post-stroke inflammatory response through the mTOR/AMPK pathway and subsequent inflammosome inhibitions [64]. Collectively, beyond the existing conventional therapies, novel therapeutic approaches such as hypothermia-induced infarct size reduction and autophagy modulation are of great significance, recently [50, 62]. Even so, as a limitation of the current study, the possible effect of some critical risk factors including aging, co-morbidities, and raised inflammatory mediators should be considered in upcoming studies, as well.

5. Conclusions

Given the conflict effects of autophagy regarding the infarct volume reduction, the studies included in this meta-analysis mostly reported a negative relation between autophagy induction and stroke volume development due to excessive autophagy activity following severe IS; in hence, it seems that further studies are also required to explore the underlying mechanisms to clarify the exact intervention role of autophagy modulation during cerebral ischemia for translating the potential therapeutic target in stroke patients.

Abbreviations

AIS, Acute Ischemic stroke; CI, confidence intervals; CMA, Comprehensive Meta-analysis; FKBPS, FK506 binding protein 5; IS, Ischemic stroke; IRI, ischemia-reperfusion injury; LC3, microtubule-associated protein 1A light chain 3; MCAO, middle cerebral artery occlusion; mTOR, mammalian target of rapamycin; SD, standard deviation.

Author contributions

AR—Designed the study; NV and FS—Performed search strategy; HH—Performed the methodological analysis; RR—Revised the final draft; YS—Contributed to writing the manuscript; SS—Interpreted the analyzed Data.
Ethics approval and consent to participate
The ethic number approved by Ethics Committee of Tabriz University of Medical Sciences for this study is IR.TBZMED.REC.1398.294.

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Conflict of interest
The authors declared no conflict of interest.

Appendix
See Table 3, Fig. 5.

Fig. 5. Search and selection process of systematic review.

References


L. to mitigate ischemic stroke by activating the AKT/mTOR signaling pathway to suppress autophagy. Brain Research. 2020; 1749: 147047.


