Modulatory effect of curcumin on ketamine-induced toxicity in rat thymocytes: Involvement of reactive oxygen species (ROS) and the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway

Svetlana Pavlovic[1], Zorica Jovic[2], Radmila Karan[3], Dane Krtinic[2], Gorana Rankovic[2], Mladjan Golubovic[1], Jelena Lilic[1], Voja Pavlovic[4]*

[1]Department of Anesthesiology, Medical Faculty University of Nis, Nis, Serbia, [2]Department of Pharmacology, Medical Faculty University of Nis, Nis, Serbia, [3]Department of Anesthesiology, Clinical Centre of Serbia, Belgrade, Serbia, [4]Institute of Physiology, Medical Faculty University of Nis, Nis, Serbia

ABSTRACT

Ketamine is a widely used anesthetic in pediatric clinical practice. Previous studies have demonstrated that ketamine induces neurotoxicity and has a modulatory effect on the cells of the immune system. Here, we evaluated the potential protective effect and underlying mechanisms of natural phenolic compound curcumin against ketamine-induced toxicity in rat thymocytes. Rat thymocytes were exposed to 100 µM ketamine alone or combined with increasing concentrations of curcumin (0.3, 1, and 3 µM) for 24 hours. Cell viability was analyzed with CCK-8 assay kit. Apoptosis was analyzed using flow cytometry and propidium iodide as well as Z-VAD-FMK and Z-LEHD-FMK inhibitors. Reactive oxygen species (ROS) production and mitochondrial membrane potential [MMP] were measured by flow cytometry. Colorimetric assay with DEVD-pNA substrate was used for assessing caspase-3 activity. Involvement of phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway was tested with Wortmannin inhibitor. Ketamine induced toxicity in cells, increased the number of hypodiploid cells, caspase-3 activity and ROS production, and inhibited the MMP. Co-incubation of higher concentrations of curcumin (1 and 3 µM) with ketamine markedly decreased cytotoxicity, apoptosis rate, caspase-3 activity, and ROS production in rat thymocytes, and increased the MMP. Application of Z-VAD-FMK (a pan caspase inhibitor) or Z-LEHD-FMK (caspase-9 inhibitor) with ketamine effectively attenuated the ketamine-induced apoptosis in rat thymocytes. Administration of Wortmannin (a PI3K inhibitor) with curcumin and ketamine significantly decreased the protective effect of curcumin on rat thymocytes. Our results indicate that ketamine-induced toxicity in rat thymocytes mainly occurs through the mitochondria-mediated apoptotic pathway and that the PI3K/Akt signaling pathway is involved in the anti-apoptotic effect of curcumin.

KEY WORDS: Ketamine; curcumin; toxicity; thymocytes; PI3K/Akt signaling pathway; anti-apoptotic effect; protective effect; apoptosis; reactive oxygen species

INTRODUCTION

Ketamine, a noncompetitive N-methyl-D-aspartic acid (NMDA) receptor antagonist, is a widely used intravenous anesthetic in pediatric anesthesia, for sedation and/or analgesia of children during painful procedures. Due to its strong anesthetic and analgesic properties, a large number of children are exposed to ketamine worldwide [1]. Despite its accepted use in anesthesia, different *in vivo* studies showed the ability of ketamine to induce neurotoxic effects in the immature brain of primates and rodents [2,3]. Furthermore, the toxic effect of ketamine was confirmed in *in vitro* studies, demonstrating the pro-apoptotic potential of ketamine in neurons [4,5] and cells of the immune system [6]. These findings raised the concern whether similar toxicity occurs in the human brain or other developing organs. However, the precise mechanism of ketamine toxicity still remains unclear, even though most of the studies suggested apoptosis as a common mechanism involved in ketamine-induced toxicity [7].

Curcumin, the main component of turmeric powder extracted from the rhizomes of the plant *Curcuma longa*, is

commonly used in cooking. Different studies have shown that curcumin exerts a wide range of biological activities, including anticarcinogenic, antimicrobial and antiinflammatory effects [8]. The antioxidative activity of curcumin has also been reported, such as it could decrease the accumulation of reactive oxygen species (ROS), mitochondrial membrane potential (MMP) and release of cytochrome c [9,10], as well as increase the synthesis of antioxidative enzymes [11]. Moreover, growing evidence has indicated the immunomodulatory role of curcumin in the activation of the immune system cells, i.e., T and B cells, macrophages, natural killer cells and dendritic cells [12-14].

In the present study, we investigated the effect of curcumin on ketamine-induced toxicity in rat thymocytes and the possible molecular mechanisms underlying this phenomenon.

MATERIALS AND METHODS

Animals

Adult Wistar rats, weighing 190-220 g and aged 10-12 weeks, were maintained under conventional laboratory conditions and in accordance with the national animal protection guidelines. All animals were bred in the Vivarium of the Institute of Biomedical Research, Medical Faculty, Nis, Serbia (Ethics committee number: 13775).

Material

For the preparation of culture medium (CM), RPMI 1640 (Sigma-Aldrich, St. Louis, MO, USA) was used according to the manufacturer's instructions. The complete CM included 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum (FCS).

Cell Counting kit (CCK-8), 2',7'-Dichlorofluorescin diacetate (H2DCF-DA), Wortmannin, Z-VAD-FMK, Z-LEHD-FMK, curcumin, and rhodamine 123 were obtained from Sigma-Aldrich, St. Louis, MO, USA. Ketamine was purchased from Richter Pharma AG, Wels, Austria.

Preparation of thymocytes

Rat thymocytes were isolated as described previously [15]. The viability of the isolated cells, determined by trypan blue dye exclusion test, was over 96%. For further experiments, rat thymocytes were counted and adjusted to a density of 5×10^6 cells/ml of complete CM.

Cell culture and treatments

Isolated rat thymocytes were cultured in 96-well round-bottom plates (NUNC, Aarhus, Denmark). Each plate well contained 100 μl of cell suspension (5×10^5 cells), as we described previously [16]. The cells were treated with ketamine (100 μM) with or without increasing concentrations of curcumin (0.3, 1, and 3 μM), for 24 hours. Control cells were cultivated with appropriate amounts of vehicle alone, diluted in complete CM. All cell cultures were performed in triplicate and cultured for 24 hours in an incubator (Galaxy, Wolfe Laboratories, USA) with 5% CO_2 at 37 °C.

When indicated, rat thymocytes were stimulated in the presence or absence of a phosphoinositide 3-kinase (PI3K) inhibitor (Wortmannin) at final concentration of 10 μM [17], pan-caspase inhibitor (Z-VAD-FMK) at final concentration of 10 μM [18] or caspase-9 inhibitor (Z-LEHD-FMK) at final concentration of 20 μM [19], and incubated with 100 μM ketamine with or without curcumin (0.3, 1, and 3 μM). The number of apoptotic cells was evaluated 24 hours of incubation.

Based on our preliminary results and the results of previous studies where plasma levels of ketamine for anesthesia induction were as high as 100 μM [20] and the 100 μM concentration was demonstrated to be clinically relevant [21,22], we also used 100 μM of ketamine for inducing toxicity in rat thymocytes.

Curcumin was dissolved in dimethyl sulfoxide (DMSO) as a stock solution. The stock solution was stored at -20 °C and diluted in CM before use. The final DMSO concentration never exceeded 0.5% (v/v). Cultivation with the increasing concentrations of curcumin (0.3, 1, and 3 μM) was chosen based on our earlier reports [16,23], as well as based on another study which demonstrated that 3 μM was the highest concentration of curcumin that did not induce any cytotoxic effect in rat thymocytes [24].

Cell viability assay

Cell viability was analyzed by the CCK-8 assay kit, as reported earlier [25]. We added 10 μl of reaction mixture in each well and incubated for 2 hours. Soluble formazan product was quantified spectrophotometrically, by measuring the absorbance at 450 nm. For each sample, the basal values were subtracted from those obtained after different treatments. The absorbance was presented as the ratio of treated/control cells [26].

Apoptosis analysis

To identify cells undergoing apoptosis we evaluated their relative nuclear DNA content, as previously described [27]. The rate of apoptotic cells was determined based on the reduction in the fluorescence of a DNA-binding dye propidium iodide (Santa Cruz Biotechnology, Santa Cruz, CA, USA), using flow cytometry (Epics XL analyzer, Coulter, Krefeld, Germany). Rat

thymocytes with subdiploid DNA content (apoptotic cells) were determined and the results were presented as the ratio of control to treated cells.

Caspase-3 activity assay

The enzymatic activity of caspase-3 was determined using a colorimetric assay and chromogenic substrate DEVD-pNA (R&D Systems, Minneapolis, USA), according to the manufacturer's protocol. We detected the change in the absorbance at 405 nm and expressed the caspase-3 activity as the fold change of absorbance in the treated compared to non-treated cells. Before the calculation of the fold change in absorbance all background absorbance values were subtracted from the experimental results.

Determination of mitochondrial membrane potential (MMP)

Changes in the MMP of rat thymocytes were determined using a lipophilic cation rhodamine 123, as previously described [28]. Flow cytometric analysis was used to evaluate the fluorescence of intracellular rhodamine 123, as reported earlier [29]. For each sample, the basal values were subtracted from those obtained after different treatments and the results were presented as the ratio of the mean fluorescence intensity [26].

Measurement of intracellular reactive oxygen species (ROS) production

Changes in cellular ROS levels were determined using a redox-sensitive probe (H2DCF-DA) and flow cytometry, as

previously shown [29-31]. For each sample, the basal values were subtracted from those obtained after different treatments and results were presented as the ratio of the mean fluorescence intensity [26].

Statistical analysis

The results are presented as mean ± standard deviation (SD). Statistically significant differences between groups were determined using the analysis of variance (ANOVA) with Dunnett's post hoc test and student's *t*-test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

To determine the optimal dose of ketamine, rat thymocytes were cultured with increasing concentrations of ketamine (1, 10, 100, 500, and 1000 µM) for 24 hours. Our preliminary results showed that there was no difference in the viability of the cells treated with 1 and 10 µM of ketamine and non-treated control cells ($p > 0.05$). On the contrary, the exposure of thymocytes to the higher concentrations of ketamine (i.e., 100, 500, and 1000 µM) resulted in increased cytotoxicity ($p < 0.05$), as evaluated by the CCK-8 assay (Figure 1A). Based on these preliminary results as well as previous findings [20] we selected the concentration of 100 µM of ketamine for our experiments.

The effects of curcumin on ketamine-treated rat thymocytes were evaluated after co-incubation of the cells with increasing concentrations of curcumin (i.e. 0.3, 1, and 3 µM) and 100 µM ketamine, for 24 hours. Ketamine significantly increased cell toxicity compared to control cells [$p > 0.05$]
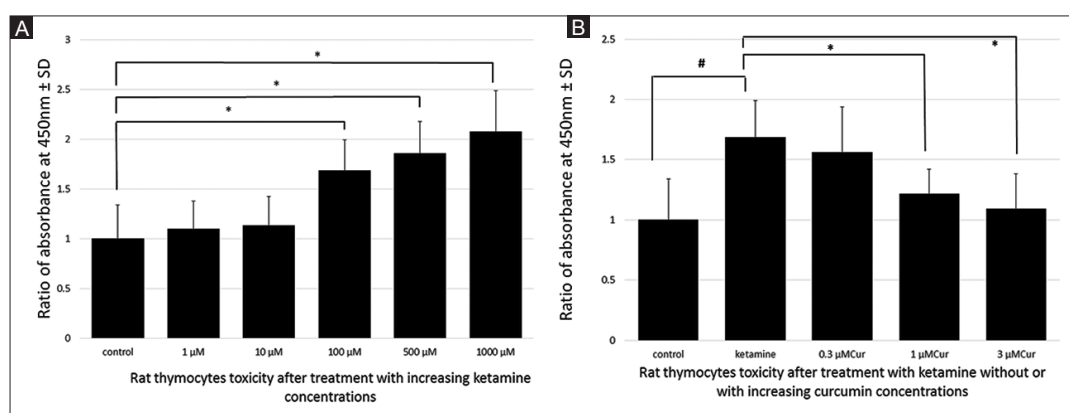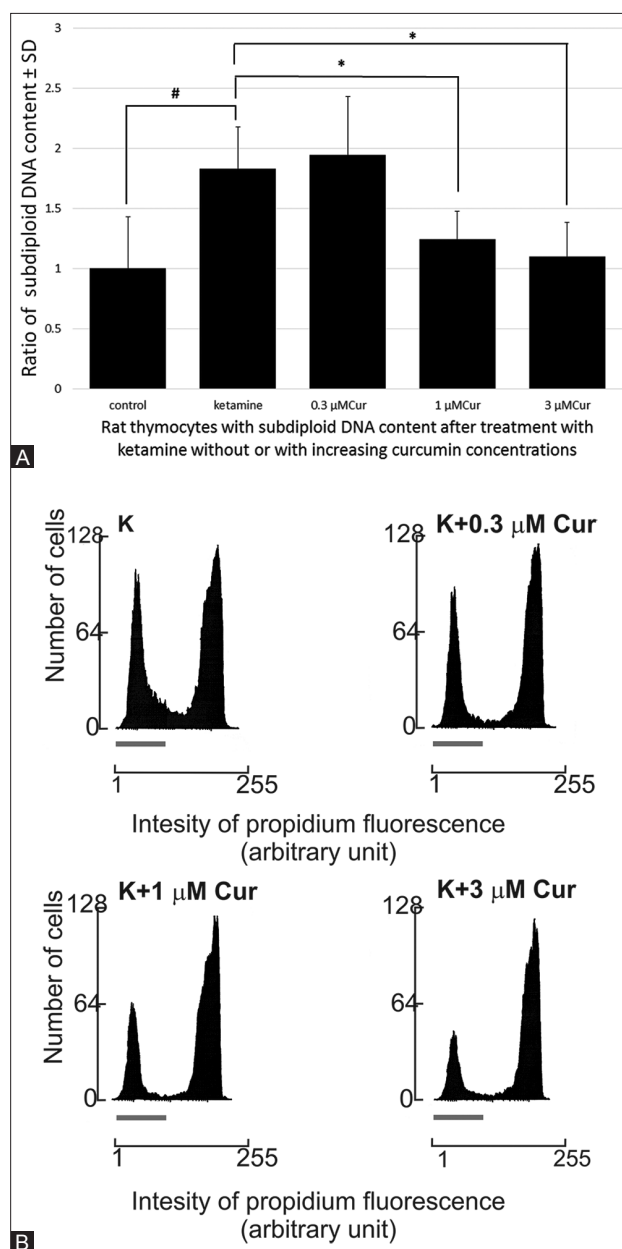

FIGURE 1. The effect of curcumin (Cur) on ketamine-treated rat thymocytes. (A) To determine the optimal concentration of ketamine, rat thymocytes were cultured with increasing concentrations of ketamine (1, 10, 100, 500, and 1000 µM) for 24 hours. There was no difference in the viability of the cells treated with 1 and 10 µM of ketamine and non-treated control cells ($p > 0.05$). On the contrary, the exposure of thymocytes to the higher concentrations of ketamine (i.e., 100, 500, and 1000 µM) resulted in increased cytotoxicity ($p < 0.05$). (B) The effect of increasing concentrations of curcumin (i.e., 0.3, 1, and 3 µM) combined with 100 µM ketamine on rat thymocytes incubated for 24 hours. Ketamine alone significantly increased cell toxicity compared to control cells ($p > 0.05$), while curcumin in the concentrations of 1 and 3 µM significantly decreased cell toxicity induced by ketamine ($p < 0.05$). The lowest dose of curcumin (0.3 µM) failed to protect rat thymocytes from ketamine-induced toxicity. Control cells were cultivated with appropriate amounts of vehicle. Data are presented as mean absorbance ratio (control to treated cells) ± SD. A graph: *$p < 0.05$ compared to control cells; B graph: #$p < 0.05$ compared to control cells; *$p < 0.05$ compared to ketamine-treated cells.

(Figure 1B). The CCK-8 assay showed that curcumin in the concentrations of 1 and 3 μM significantly decreased cell toxicity ($p < 0.05$), while the lowest dose of curcumin (0.3 μM) failed to protect rat thymocytes from ketamine-induced toxicity (Figure 1B).

To test whether the observed cytotoxic activity of ketamine is due to increased cell apoptosis, we analyzed the effect of ketamine as well as curcumin on apoptosis in rat thymocytes. As shown in Figure 2A, 100 μM of ketamine significantly induced apoptosis in rat thymocytes ($p < 0.05$), i.e., there was a significant increase in sub-G1 population, corresponding to apoptotic cells with hypodiploid DNA content [32]. In addition, administration of 1 and 3 μM curcumin significantly reduced the apoptosis rate ($p < 0.05$), while the lowest concentration of curcumin (0.3 μM) had no significant effect ($p > 0.05$) on apoptosis in ketamine-induced rat thymocytes (Figure 2A and B).

There are two major pathways of apoptosis, the extrinsic pathway is mediated by death receptors, while the intrinsic pathway is mediated by mitochondria. Caspase-3 is the final target of both pathways [33]. The extrinsic apoptotic pathway is triggered by the activation of death receptors and the subsequent cleavage of caspase-8 [34]. On the other hand, the intrinsic pathway is initiated by changes in the inner mitochondrial membrane which results in the release of apoptogenic factors from the mitochondria to cytosol and activation of caspase-9 [34]. To determine whether caspase activation was involved in the ketamine-induced apoptosis, rat thymocytes were cultured with ketamine in the presence of a caspase inhibitor and evaluated for DNA content. The treatment with ketamine and the pan caspase inhibitor Z-VAD-FMK significantly decreased ($p < 0.05$) the number of cells with subdiploid DNA content (Figure 3A), indicating that ketamine-induced apoptosis in rat thymocytes is caspase-dependent. To further investigate which signaling pathway is involved in apoptosis induced by ketamine, rat thymocytes were treated with ketamine and the caspase-9 inhibitor Z-LEHD-FMK, and the DNA content was analyzed. The apoptosis rate was significantly decreased ($p < 0.05$) in rat thymocytes after Z-LEHD-FMK treatment (Figure 3A), indicating the involvement of intrinsic pathway in the apoptosis induced by ketamine. The fact that the apoptotic activity was not completely inhibited by Z-LEHD-FMK suggests that the mitochondria-mediated apoptotic pathway has a major role in ketamine-induced apoptosis in rat thymocytes. This conclusion was later confirmed in the analysis of mitochondrial dysfunction in response to ketamine.

We next investigated which signaling pathway might be involved in the anti-apoptotic activities of curcumin in ketamine-induced rat thymocytes. We tested the PI3K signaling pathway, due to its crucial role in promoting survival in different cells [35]. As shown in Figure 3B, the pretreatment with the
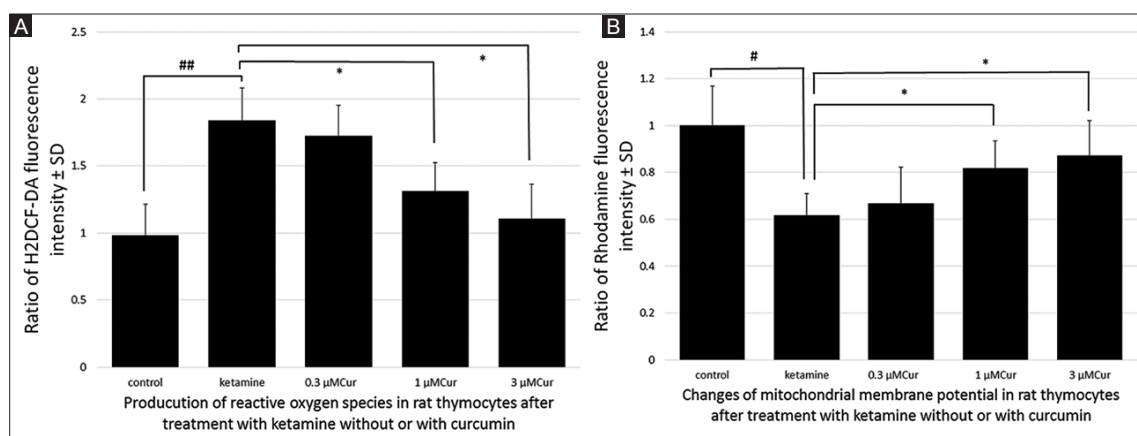
FIGURE 2. The effect of curcumin (Cur) on ketamine-induced apoptosis in rat thymocytes (A and B). Cells were cultured with 100 μM ketamine alone or in combination with increasing concentrations of curcumin (i.e. 0.3, 1, and 3 μM) for 24 hours. Ketamine significantly induced apoptosis in rat thymocytes ([#]$p < 0.05$ compared to control cells), i.e., there was a significant increase in sub-G1 population. The administration of 1 and 3 μM curcumin significantly reduced the apoptosis rate ($p < 0.05$), while the lowest concentration of curcumin (0.3 μM) had no significant effect ($p > 0.05$) on apoptosis in ketamine-induced rat thymocytes. Control cells were cultivated with appropriate amounts of vehicle. Data are presented as mean absorbance ratio (control to treated cells) ± SD. [#]$p < 0.05$ compared to control cells; [*]$p < 0.05$ compared to ketamine-treated cells. The bar under the histograms indicates the population of cells with subdiploid DNA content.

PI3K inhibitor Wortmannin significantly inhibited ($p < 0.05$) the protective effect of curcumin in ketamine-treated thymocytes. These findings suggest a possible role of the PI3K signaling pathway in curcumin-mediated protection of rat thymocytes exposed to ketamine.
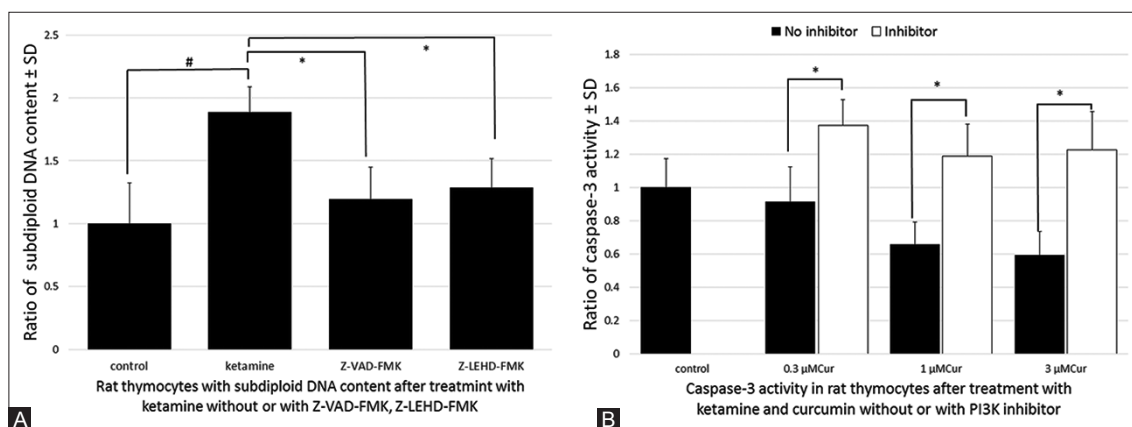
FIGURE 3. The effect of (A) pan caspase inhibitor Z-VAD-FMK and caspase-9 inhibitor Z-LEHD-FMK on subdiploid DNA content in rat thymocytes and (B) a PI3K inhibitor (Wortmannin) on caspase-3 activity in rat thymocytes treated with ketamine and increasing curcumin (Cur) concentrations. The cells were cultured with 100 μM ketamine alone or combined with pretreatment of 10 μM Z-VAD-FMK or 20 μM Z-LEHD-FMK. Z-VAD-FMK significantly decreased ($p < 0.05$) the number of cells with subdiploid DNA content in ketamine-induced rat thymocytes. Similarly, the apoptosis rate was significantly decreased ($p < 0.05$) in rat thymocytes after Z-LEHD-FMK + ketamine treatment. Next, rat thymocytes were treated with 10 μM Wortmannin, 100 μM ketamine, and increasing concentrations of curcumin (i.e., 0.3, 1, and 3 μM). The treatment with the PI3K inhibitor Wortmannin significantly inhibited ($p < 0.05$) the protective effect of curcumin in ketamine-induced thymocytes. Control cells were cultivated with appropriate amounts of vehicle. Data are presented as mean absorbance ratio (control to treated cells) ± SD. In A graph: #$p < 0.05$ compared to control cells; *$p < 0.05$ compared to ketamine-treated cells; In B graph: *$p < 0.05$ compared to cells treated without Wortmannin. The black bars represent cells treated without Wortmannin, while the white bars represent cells treated with Wortmannin.

FIGURE 4. The effect of ketamine and curcumin (Cur) on (A) reactive oxygen species (ROS) production and (B) mitochondrial membrane potential (MMP) in rat thymocytes. The cells were treated with 100 μM ketamine alone or combined with increasing curcumin concentrations (0.3, 1, and 3 μM) for 24 hours. Control cells were cultivated with appropriate amounts of vehicle. Data are presented as mean absorbance ratio (control to treated cells) ± SD. Ketamine significantly increased the ROS production ($p < 0.01$) and decreased MMP ($p < 0.05$) in rat thymocytes. The simultaneous application of curcumin (1 or 3 μM) and 100 μM ketamine significantly inhibited the ROS production ($p < 0.05$) and restored the MMP ($p < 0.05$) in thymocytes, compared to controls. Moreover, at the concentration of 0.3 μM, curcumin failed to induce any significant changes in the ROS production and MMP. ##$p < 0.01$ compared to control cells; #$p < 0.05$ compared to control cells; *$p < 0.05$ compared to ketamine-treated cells.

Considering the above-described findings and the critical role of MMP and ROS in cells undergoing apoptosis [36], we next analyzed whether ketamine and curcumin had any effect on the MMP and ROS levels in rat thymocytes. Our results showed that ketamine significantly increased the ROS production ($p < 0.01$) and decreased MMP ($p < 0.05$) in rat thymocytes (Figure 4A and B). The application of curcumin (1 or 3 μM) with 100 μM ketamine significantly inhibited the ROS production ($p < 0.05$) and restored the MMP ($p < 0.05$) in thymocytes, compared to controls (Figure 4A and B). Moreover, at the concentration of 0.3 μM, curcumin failed to induce any significant changes in the ROS production and MMP (Figure 4A and B).

DISCUSSION

Curcumin is a natural substance commonly used in cooking. It displays a number of pharmacological properties, including antioxidant, antiinflammatory and antitumor activities [8,37]. Immunomodulatory effects of curcumin have also been shown, demonstrating that ability of curcumin to modulate the activation of different cells of the immune system [12].

In our experiments, ketamine induced cytotoxicity in rat thymocytes after 24 hours of incubation. The observed cytotoxicity was due to an increased apoptosis rate, as indicated by the number of cells with subdiploid DNA content. These findings are in line with previous reports demonstrating the pro-apoptotic potential of ketamine in different immune cells, such as lymphocytes [6], macrophages [38] and dendritic cells [22]. The pretreatment with the pan caspase inhibitor Z-VAD-FMK effectively decreased the apoptosis rate in ketamine-induced rat thymocytes, indicating that the ketamine-induced apoptosis mainly occurred in a caspase-dependent manner. Furthermore, the ketamine-induced apoptosis was significantly attenuated by the caspase-9 inhibitor Z-LEHD-FMK. These results indicate that the intrinsic apoptosis pathway plays a major role in apoptosis induced by ketamine in rat thymocytes. The observed mitochondrial dysfunction in ketamine-treated rat thymocytes further confirmed this conclusion. However, considering that the caspase inhibitors used in this study did not completely suppress ketamine-induced apoptosis, additional studies should test if ketamine triggers apoptosis through caspase-independent pathways.

Taking into account previous reports on the association between cytotoxicity and DNA damage and increased ROS production [39], we also evaluated these mechanisms in ketamine-treated rat thymocytes. The flow cytometric analysis showed an increased ROS production and decreased MMP in rat thymocytes after ketamine treatment, which corresponded to the decreased cell viability and increased apoptotic rate in these cells demonstrated in the previous experiments. Similarly, other studies showed that ketamine induced ROS production and changes in the MMP in immune [6] and non-immune cells [1,40]. Mitochondrial dysfunction, including the loss of the MMP, is critical in cells undergoing apoptosis and is highly associated with the accumulation of ROS [36]. A decreased MMP and the release of cytochrome c from mitochondria are key steps in the intrinsic apoptotic pathway. The cells of the immune system are sensitive to oxidative stress primarily because of a high content of polyunsaturated fatty acids in their plasma membrane [41]. The accumulation of ROS may induce apoptosis through oxidative stress or direct damage of ROS to various cellular components, including membrane lipids, proteins and DNA [42]. All these findings suggest the possibility that increased ROS production and mitochondrial dysfunction have an important role in ketamine-induced apoptosis in rat thymocytes, with a potential impact on the cell growth.

We further tested whether curcumin modulates the ketamine-induced toxicity in rat thymocytes. We found that the co-incubation of thymocytes with 1 or 3 μM curcumin and 100 μM ketamine resulted in markedly decreased ketamine-induced toxicity and apoptosis rate. These findings are

in line with previous reports indicating the protective role of curcumin in lymphocytes [43,44]. On the other hand, the minimal concentration of curcumin (0.3 μM) used in this study did not affect ketamine-induced toxicity in rat thymocytes, which is also in accordance with a previous report suggesting that curcumin mainly exerts its protective effect at micromolar concentrations [24]. In addition, we demonstrated that 1 and 3 μM of curcumin effectively inhibited the ROS production and restored the MMP in ketamine-treated rat thymocytes, indicating that increased ROS production and disruption of MMP were associated with ketamine-induced cytotoxicity. Consistently with our findings, other studies showed that curcumin was able to restore the MMP and decrease ROS production in rat thymocytes [16] as well as in other cells [45,46]. An important characteristic of mitochondrial dysfunction is the loss of MMP. In the intrinsic apoptotic pathway MMP is regulated by different signals, resulting in the release of cytochrome c, followed by the activation of caspase-9 and caspase-3 [37]. B-cell lymphoma 2 (Bcl-2), a member of the Bcl-2 family of proteins with anti-apoptotic action, regulates the intrinsic apoptotic pathway by increasing the stability of MMP and inhibiting cytochrome c release from mitochondria. On the other hand, Bcl-2-like protein 4 (Bax), another member of the Bcl-2 protein family, increases mitochondrial permeability allowing cytochrome c to pass into the cytosol [47]. The ability of curcumin to upregulate Bcl-2 expression and downregulate Bax expression, with the resulting changes in the Bcl-2/Bax ratio that affects the cell susceptibility to apoptosis, has been demonstrated in rat thymocytes [16] and in non-immune cells [46,48]. Similarly, a protective role of curcumin by inhibiting the intrinsic apoptotic pathway has been showed in different cells [16,46,49]. All these findings are in agreement with the decreased cytotoxicity, apoptosis rate, ROS production, and caspase-3 activity as well as restored MMP observed in our experiments in rat thymocytes after co-treatment with ketamine and curcumin.

The PI3K/Akt signaling pathway has the major role in cell survival and apoptosis and alterations in the PI3K/Akt signaling cascade may activate different downstream molecules that regulate cell apoptosis [50]. Our results showed that the inhibitory effect of curcumin on caspase-3 activity was significantly suppressed by the PI3K/Akt inhibitor Wortmannin, suggesting that the PI3K/Akt pathway is required for the anti-apoptotic effects of curcumin in ketamine-induced rat thymocytes. PI3K may activate protein kinase B (Akt), one of the key downstream kinases, which, in return, prevents the release of cytochrome c from mitochondria and inhibits caspase-9 activation [51]. In addition, the activated PI3K/Akt pathway suppresses Bax and promotes Bcl-2 protein expression [52]. In support of these observations, previous studies also demonstrated that the effect of curcumin on the

mitochondrial pathway of apoptosis is mediated through the PI3K signaling pathway [48,53]. All this suggests that the signaling through the PI3K/Akt cascade may be important for the protective role of curcumin in ketamine-induced apoptosis in rat thymocytes.

CONCLUSION

In summary, we showed that ketamine induced apoptosis in rat thymocytes mainly via the mitochondrial cell death pathway, induction of oxidative stress, and mitochondrial dysfunction. Furthermore, higher concentrations of curcumin decreased the ketamine-induced toxicity in rat thymocytes, apoptosis rate, caspase-3 activity, via decreased ROS production and prevention of mitochondrial dysfunction associated with the PI3K/Akt signaling pathway. Additional studies are necessary to investigate the role of other signaling pathways in mediating the protective effect of curcumin, which may further be used in preventing the negative effects of ketamine on the immune system.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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