K_{ATP} channel openers may protect MOG-G-UVW cells from hypoxia mimetic insult induced by $CoCl_2$

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Abstract: This study is going to find out whether K_{ATP} channel is playing a protective role or not under neuronal insult model. A human astrocytoma cell line, MOG-G-UVW, was used in this study. RT-PCR was performed to find out whether K_{ATP} channel exists or not at mRNA level and which subunits are the main compositions. Cell proliferation MTS assays were used to test the response of the cells to the insults and/or K_{ATP} modulators. Hypoxia mimetic induced by $CoCl_2$ was applied as a cell insult model. Diazoxide, nicorandil and cromakalim were used as K_{ATP} openers, and tolbutamide and glibenclamide were used as K_{ATP} blockers. K_{ATP} channel exists in MOG-G-UVW cell line at mRNA level and the main composition is $K_{ir}6.2$ and SUR2A. 65 μM is the EC_{50} of $CoCl_2$ insult to MOG-G-UVW cells and 125 μM can further induce cell death. K_{ATP} openers cannot protect the cells from 65 μM $CoCl_2$ insult, but diazoxide and nicorandil can reduce the cell death under 125 μM $CoCl_2$. K_{ATP} blockers, tolbutamide and glibenclamide, cannot protect the cells at all, and tolbutamide may further reduce the cell numbers from the same dose of $CoCl_2$ insult. K_{ATP} activation may protect the cells from hypoxia mimetic insult induced by $CoCl_2$ at a high dose, but K_{ATP} blockers may further induce cell death.
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1. Introduction

Cellular neurodegeneration is a hallmark of many central nervous diseases including stroke, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). The primary insults leading to this degeneration are no doubt different but what may possibly prevent the neuronal death or protect the cells from neuronal insults are widely studied. Amongst these common features is surely the homeostasis of intracellular contents, such as calcium (Ca^{2+}), potassium (K^+) and adenosine-5'-triphosphate (ATP), and the functions of mitochondrial at a physiological level are essential to keep the neurones alive. For example, one hypothesis for AD asserts that β-amyloid (Aβ) peptides actually form ion channels in neuronal membranes transporting ions such as calcium (Ca^{2+}) into the cell hence mediating cytotoxicity (Kagan et al., 2002, Arispe et al., 2007).

K^+ channels are purported to be involved in the life and death decisions that cells, and in particular neurones, make. The role of these ion channels in the cascade of events which leads to cell death is confusing. On the one hand it is argued that the activation of potassium channels can be neuroprotective (Coles et al., 2008, Henney et al., 2009) by virtue of the hyperpolarisation and resultant decrease in Ca^{2+} entry into the cell. On the other hand there is a view that activation of K^+ channels can lead to K^+ efflux, water loss, cells shrinkage and apoptosis (Burg et al., 2006). Regarding to the ATP-sensitive K^+ channel (K_{ATP}), two opposite opinions were raised previously for its possible role in neuronal death.

One in vivo study on the $K_{ir}6.2$ knocked out mouse model has found that the neurones were severely damaged after focal cerebral ischemia induced by middle cerebral artery occlusion, whereas few injured neurones were found in the wild type counterparts (Sun et al., 2006). Another study on a $K_{ir}6.2$ mutant mouse model has also found that the activity of substantia nigra pars reticulate neurones, which plays an important role in the control of seizures, was enhanced during hypoxia, while the activity of the same neurones in normal mice was inactivated by the opening of the postsynaptic K_{ATP} channel (Yamada et al., 2001). Similar neuronal activity has been found in the normal mice when tolbutamide, which is a K_{ATP} channel blocker, was applied (Yamada et al., 2005). In addition, another study on rat hippocampal cells has found that diazoxide (a K_{ATP} opener) treatment of neurones reduces neuronal death from insults of $A\beta_{25-35}$ or hypoxia induced by ferrous sulphate ($FeSO_4$) (Goodman & Mattson, 1996). Also, data from mice administrated diazoxide has shown that the infarct volume in the brain under cerebral ischemia was reduced (Liu et al., 2002).

In the contrast, other studies have claimed the opposite. One study on mammalian central neurones has shown cell apoptosis induced by

cromakalim, a K_{ATP} channel opener, in electron micrographs (Yu et al., 1997), and another study in rat hippocampal neurones has found that 5-hydroxydecanoate, a mitochondrial K_{ATP} blocker, can increase cell survival from glutamate or staurosporine insult (Liu et al., 2003). In addition to neurones, another study on rat preadipocytes has found that diazoxide can reduce the relative cell number, while the K_{ATP} channel blocker glibenclamide can increase cell number, in MTT assays (Wang et al., 2007). For a possible mechanism, it was believed that K^+ efflux from mitochondria can cause mitochondrial membrane depolarization, cyt c release, and induce subsequent extrusion of cytosolic K^+ via a K^+ channel on the cell membrane.

Since it is still unclear what the role of K_{ATP} channel is in neuronal death, it would be useful to further investigate its possible effects in cell death in this study. A functional K_{ATP} channel is constructed from four inward rectifier (K_{ir}) subunits and four sulphonylurea receptor (SUR) subunits (Burg et al., 2006). Although the ion-conducting structure of K_{ATP} channel exists in the K_{ir} subunits (Ho et al., 1993), SUR subunits are also essential for K_{ATP} channel function, since SUR subunits represent the ATP binding area. There are several inward rectifier K^+ channels found so far ($K_{ir}1$ to $K_{ir}7$), but only $K_{ir}6$ is found to be ATP sensitive (Alexander et al., 2009), hence it is named the K_{ATP} channel. There are two subtypes of K_{ATP} channel, $K_{ir}6.1$ and $K_{ir}6.2$, and there are three different SUR thus far, SUR1, SUR2A and SUR2B (Alexander et al., 2009).

A human brain astrocytoma cell line, MOG-G-UVW, was applied in this study since astrocytes were used to study anti-inflammation function and there relevant role in neuronal protection previousely (Spillantini, 2011). Reverse transcription (RT)-polymerase chain react (PCR) was applied to find out whether K_{ATP} channel message exists in this cell line and which subunits are the main composition. The, K_{ATP} modulators were applied in combination with a hypoxia mimetic insult model to find out the possible effect, if any, of this channel.

In this study, hypoxia mimetic insult induced by cobalt chloride ($CoCl_2$) was applied to induce the cell death since it has been widely used in previous studies (Gasperi et al., 2010, Bautista et al., 2009). Particularly, $CoCl_2$ can increase the generation of Aβ by up-regulating the expression of amyloid precursor protein (APP) as well as the expression of β-secretase and γ-secretase (Zhu et al., 2009). Then, K_{ATP} channel activators, diazoxide, nicorandil and cromakalim, as well as K_{ATP} channel blockers, glibenclamide and tolbutamide, were tested in combination with $CoCl_2$ insult on MOG-G-UVW cells.

In previous studies, 10 μM levcromakalim evoked a hyperpolarisation under current clamp, and this effect of levcromakalim can be inhibited by glibenclamide with an EC_{50} of 55 nM. 10 μM glibenclamide alone depolarises the neurones (Hogg et al., 2001). For diazoxide, 200 – 500 μM can increase the channel opening probability by 480 ± 120% (Allen & Brown, 2003). For the K_{ATP} blocker, it has been found in previous studies that the K_{ATP} channel current (I_{KATP}) can be inhibited reversibly by tolbutamide with an EC_{50} at 34.1 μM, and can be inhibited irreversibly by 0.3 – 3 nM glibenclamide (Allen & Brown, 2003). The inhibitory effect of tolbutamide was confirmed in a current clamp experiment suggesting that 100 μM tolbutamide depolarises the neurones (Hogg et al., 2001). An irreversible effect of glibenclamide was argued by another study which suggested that the dose of glibenclamide to induce half-maximal inhibitory effect was increased from 9.1 μM to only 10.6 μM by 1 mM nicorandil (Obata & Yamanaka, 2000).

2. Methods and materials

2.1 Methods

MOG-G-UVW is a human astrocytoma cell line (from Sigma-Aldrich). It is cultured in a sterilised incubator at 37 centigrade (°C) with 5% of carbon dioxide (CO_2, p/p). The composition for the culture medium was Dulbeceo's modified Eagle medium (DMEM) and nutrient mixture F10 ham (F10) (1:1, v/v) with 10% (v/v) of foetal bovine serum (FBS) and 100 units (u) of penicillin-streptomycin mixture (P/S). PBS and P/S were kept at -20 °C and all other mediums were kept at 4 °C.

Ribonucleic acid (RNA) was extracted from a confluent flask (75 square centimetre, cm^2) of cells with TRIzol reagent (from Life Technology, stored at 4 °C). DNAse inactivation was performed to remove all the remaining deoxyribonucleic acid (DNA) with a DNAse 1 kit (from Ambion, stored at -20 °C). The RNA concentration (w/v) and quality (260/280) in the final product was determined with a spectrophotometer (DU730, from Beckmam Coulter). The RNA product was kept at -80 °C. Thereafter, RT was performed to transcript message RNA (mRNA) into DNA with a reverse transcriptase kit (from Promega, stored at -20 °C). 1 microgram (μg) of RNA was added in each RT reaction and the RT programme was: 25 °C × 5 minutes (min) + 42 °C × 1 h + 70 °C °C × 15 min. The DNA product was kept at -20 °C.

PCR was then applied to find out whether K_{ATP} channel exists at the mRNA level in MOG-G-UVW cells. The primers of K_{ATP} subunits were designed on Primer III website (http://frodo.wi.mit.edu/primer3) and the sequences of primers are listed in Table 1. PCR was performed

with a Go Taq DNA polymerase ket (from Promega, stored at -20 °C) and the PCR programme was: 95 °C × 2 min + [95 °C × 30 seconds (s) + annealing temperature × 45 s + 72 °C × 1 min] × 30 – 40 cycles + 72 °C × 5 min. Two different PCR conditions were applied: 55 °C as the annealing temperature for 30 cycles and 58 °C as the annealing temperature for 40 cycles. Primers were from Life Technology and kept at -20 °C. PCR thermocycler is from Biometra. PCR results were tested with electrophoresis (100 mV). The gel was 1% agaorse and the buffer was 1 × TAE containing 40 milli mole (mM) Tris [$(HOCH_2)_3CNH_2$] base, 20 mM acetic acid (CH_3COOH) and 1 mM ethylenediaminetetraacetic acid (EDTA). All these drugs were from Fisher Scientific.

Cell proliferation MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt, from Promega] assays were applied to test the possible effects, if any, of K_{ATP} modulators in MOG-G-UVW cells. Unless specific information was given, cells were seeded as 3,000 per well in 100 micro litre (μL) per well of medium in 96 well plates, and were insulted with $CoCl_2$ and/or K_{ATP} modulator for 24 hours (h) after being incubated overnight. To insult the cells, all drugs were dissolved in insult medium (culture medium without FBS). Then, 20 μL of MTS and phenazine methosulfate (PMS, from Sigma-Aldrich) mixture (20 : 1, v/v) was added into each well and the cells were incubated again for another 2 – 4 h. Finally the absorbance of each well was read at 490 nanometre (nm) with a laser plate reader (from Tecan). In each experiment there were blank wells prepared for each sample. The blank wells were the wells with the same solution mix but with no cells present. The absorbance of the blank was subtracted from the absorbance of each relevant sample for data analysis. The net absorbance (where the absorbance of the relevant blank was subtracted) of each sample was thereafter compared with that of control, where the relative cell number was set at 1, and final ratio was the relative cell number of each sample. Both MTS and PMS were light sensitive and kept at -20 °C.

2.2 Drug solutions

$CoCl_2$, cromakalim, diazoxide, nicorandil and tolbutamide are from Sigma-Aldrich, and glibenclamide is from Tocris. $CoCl_2$ was dissolved in water (H_2O) at 10 mM for stock. Cromakalim, diazoxide and glibenclamide were dissolved in DMSO at 10 mM for stock. Nicorandil and tolbutamide were dissolved in C2H5OH at 10 mM for stock. Diazoxide and nicorandil stocks were stored at -20 °C. $CoCl_2$, cromakalim, glibenclamide and tolbutamide stocks were stored at 4 °C. $CoCl_2$ is light sensitive.

3. Results

3.1 Primer design

The primers for K_{ATP} channel are listed in Table 1:

Table 1 Primers for K_{ATP} subunits

Primer		Sequence	Location
Kir6.1	Forward	5'-CATCTTTACCATGTCCTTCC-3'	560 – 893 bp in total 3,281 bp
NM_004982	Reverse	5'-GTGAGCCTGAGCTGTTTTCA-3'	
Kir6.2	Forward	5'-ACTCCAAGTTTGGCAACACC-3'	1,197 – 1,550 bp in total 1,635 bp
D50582	Reverse	5'-CTGCTGAGGCCAGAAATAGC-3'	
SUR1	Forward	5'-ATGAGGAAGAGGAGGAAGAG-3'	2,941 – 3433 bp in total 4,892 bp
L78207	Reverse	5'-GCGATGGTGTTACAGTCAGA-3'	
SUR2A	Forward	5'-GGCCTTTGCTTCACTGTCTC-3'	1,730 – 1,939 bp in total 4,670 bp
NM_005691.2	Reverse	5'-TTTGGCTGAACTCCAGTGTG-3'	
SUR2B	Forward	5'-TGGGAACACATTTTCTGCAA-3'	1,528 – 1,677 bp in total 4,650 bp
NM_020297	Reverse	5'-CGCATGGGTCACAAATGTAC-3'	

3.2 RT-PCR

Two annealing temperatures, 55 °C and 58 °C, have been trialled on the amplification of K_{ATP} subunits in MOG-G-UVW cell line. The figures are shown in Figure 1 and 2.

From the two figures, it can be seen that higher annealing temperature with more amplification cycles make the bands for $K_{ir}6.1$, SUR1 and SUR2B visible and make the SUR2A band clearer, but this condition also causes the bands to be unspecific. From Figure 1, it may also be noticed that $K_{ir}6.2$ with SUR2A might be the main subunit composition of K_{ATP} channel in MOG-G-UVW cells, but the existence of $K_{ir}6.1$, SUR1 and SUR2B cannot be eliminated.

3.3 K_{ATP} activators effect against $CoCl_2$ insult

Firstly, the possible effect, if any, of diazoxide alone on the cell proliferation was tested in MOG-G-UVW cells. The results are shown in Figure 3.

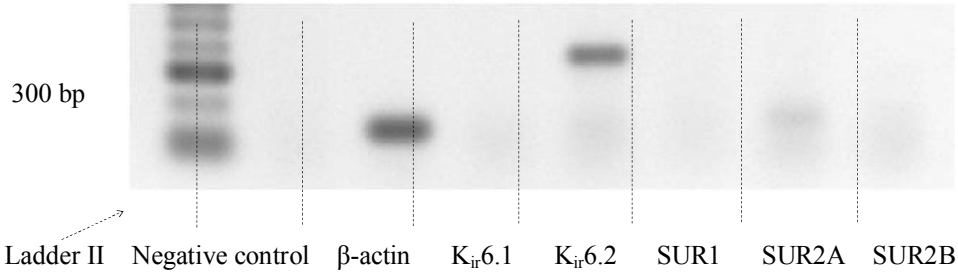

Figure 1. K_{ATP} subunits in MOG-G-UVW cells, 55 °C, 30 cycles.

Figure 2. K_{ATP} subunits in MOG-G-UVW cells, 58 °C, 40 cycles. The bands were unspecific.

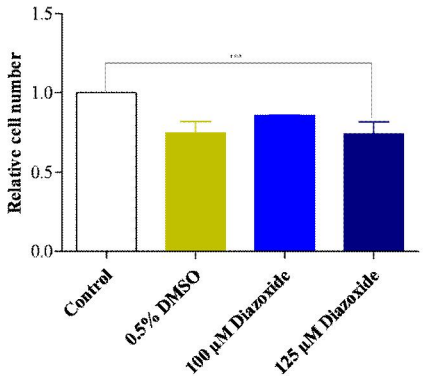

Figure 3. The effect of diazoxide alone in MOG-G-UVW cells. The results were the means of 9 for 125 µM diazoxide and the control, and 3 for DMSO vehicle and 100 µM diazoxide.

A one way ANOVA test for all the groups in Figure 3 suggested that the difference amongst those groups was significant ($p < 0.05$), and the Dunnett's post test (comparing each group with the control) indicated that 125 µM diazoxide caused a significant reduction in relative cell number from the control ($p < 0.01$); the vehicle, DMSO and 100 µM diazoxide did not have any significant effect on the relative cell number. It can be concluded that diazoxide cannot induce cell death in MOG-G-UVW cells at concentrations of 100 µM or lower.

Diazoxide was then tested against two different doses of $CoCl_2$ in MOG-G-UVW cells. The results are shown in Figure 4.

For the results shown in Figure 4A, a one way ANOVA test for all the groups showed that the difference amongst those groups was significant ($p < 0.001$), and the Tukey's post test (comparing every two groups) indicated that 65 µM $CoCl_2$ caused a significant reduction in the relative cell number from the control ($p < 0.001$). A one tail t test suggested that 100 µM diazoxide in combination with 65 µM $CoCl_2$ had a significant effect on the relative cell number from the same dose of $CoCl_2$ alone ($p < 0.05$).

Thus, it can be concluded that diazoxide cannot protect MOG-G-UVW cells from a low dose of $CoCl_2$ (65 µM), and may even further reduce the relative cell number from $CoCl_2$ alone. However, in the presence of a high dose of $CoCl_2$ (125 µM), 10 µM diazoxide can paradoxically protect the cells.

Besides diazoxide, another K_{ATP} activator, nicorandil was also tested on the $CoCl_2$ insult in MOG-G-UVW cells. The results are shown in Figure 5.

For the results shown in Figure 5A, a one way ANOVA test for all the groups showed that the difference amongst all the groups was significant ($p < 0.001$), and the Tukey's post test (comparing every two groups) indicated that 65 µM $CoCl_2$ caused a significant reduction in the relative cell number from the control. However the difference in the relative cell numbers between $CoCl_2$ + nicorandil and $CoCl_2$ alone was not significant.

For the results shown in Figure 5B, a one way ANOVA test for all the groups suggested that the difference amongst those groups was significant ($p < 0.001$) and the Tukey's post test (comparing every two groups) indicated that 125 µM $CoCl_2$ caused a significant reduction in the relative cell number from the control, and 10 µM or 100 µM nicorandil in

combination with 125 µM $CoCl_2$ also had a significant effect on the relative cell number in comparison with the same dose of $CoCl_2$ alone.

Thus, it can be concluded that nicorandil cannot protect MOG-G-UVW cells against a low dose of $CoCl_2$ (65 µM), but protects the cells against a high dose of $CoCl_2$ (125 µM).

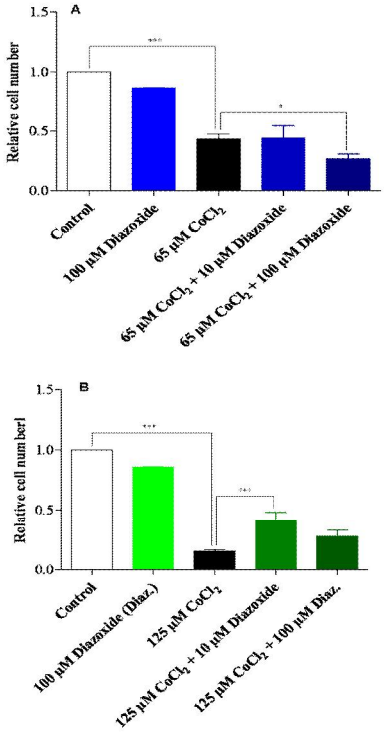

Figure 4. The effect of diazoxide (Diaz.) in combination with $CoCl_2$ insult in MOG-G-UVW cells. A: 65 µM $CoCl_2$, the results are the means of 30 for $CoCl_2$ alone and the control, 9 for $CoCl_2$ + 10 µM diazoxide, 6 for $CoCl_2$ + 100 µM diazoxide, and 3 for diazoxide alone. And B: 125 µM $CoCl_2$, the results are the means of 12 for $CoCl_2$ alone and the control, 9 for $CoCl_2$ + 10 µM diazoxide, 6 for $CoCl_2$ + 100 µM diazoxide, and 3 for diazoxide alone seen with a high dose of $CoCl_2$. From the results shown in Figure 4B, a one way ANOVA test for all the groups suggested that the difference amongst those groups was significant ($p < 0.001$), and the Tukey's post test (comparing every two groups) indicated that 125 µM $CoCl_2$ alone caused a significant reduction in the relative cell number from the control ($p < 0.001$), and 10 µM diazoxide in combination with 125 µM $CoCl_2$ also had a significant effect on the relative cell number in comparison with the same dose of $CoCl_2$ alone ($p < 0.001$).

In addition to diazoxide and nicorandil, the third K_{ATP} activator, cromakalim, was also tested against the same doses of $CoCl_2$ in MOG-G-UVW cells. The results are shown in Figure 6.

For the results shown in Figure 6A, a one way ANOVA test for all the groups suggested that the difference amongst those groups was significant ($p < 0.001$), and the Tukey's post test (comparing every two groups) indicated that 65 µM $CoCl_2$ caused a significant reduction in the relative cell number from the control ($p < 0.001$), but 10 – 100µM cromakalim in combination with 65 µM $CoCl_2$ did not have any significant effect on the relative cell number from the same dose of $CoCl_2$ alone.

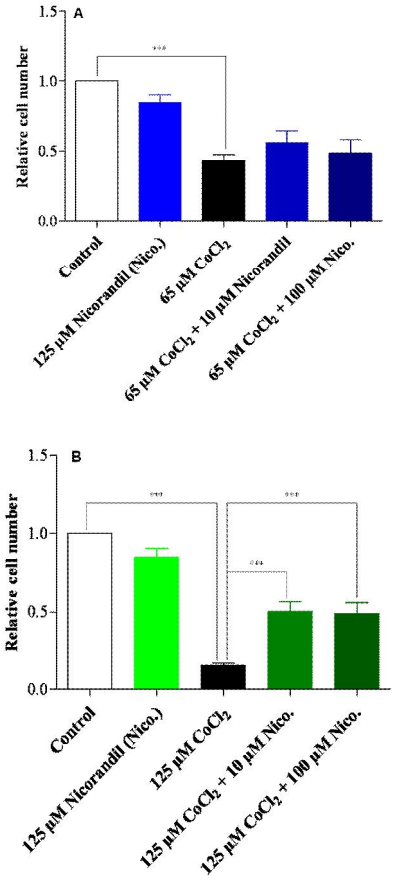

Figure 5 The effect of nicorandil (Nico.) in combination with $CoCl_2$ insult in MOG-G-UVW cells. A: 65 µM $CoCl_2$, the results are the means of 30 for 65 µM $CoCl_2$ alone and the control, and 9 for 125 µM nicorandil alone, 65 µM $CoCl_2$ + 10 µM nicorandil and 65 µM $CoCl_2$ + 100 µM nicorandil. And B: 125 µM $CoCl_2$, the results are the means of 12 for $CoCl_2$ alone and the control, and 9 for 125 µM nicorandil alone, 125 µM $CoCl_2$ + 10 µM nicorandil and 125 µM $CoCl_2$ + 100 µM nicorandil.property is similar to the effect of diazoxide on $CoCl_2$ in MOG-G-UVW cells.

From the results shown in Figure 6B, it can be seen that cromakalim did not protect the cells from a high dose of $CoCl_2$ (125 µM) either. A one way ANOVA test for all the groups suggested that the

difference amongst those groups was significant ($p < 0.001$), the Tukey's post test (comparing every two groups) indicated that 125 µM $CoCl_2$ alone caused a significant reduction in the relative cell number from the control ($p < 0.001$), and the Dunnett's post test (comparing each group with the $CoCl_2$ alone) indicated that 100 µM cromakalim in combination with 125 µM $CoCl_2$ also had a significant effect on the relative cell number from the same dose of $CoCl_2$ alone ($p < 0.05$).

To summarise the cell proliferation MTS assays results on K_{ATP} activators, it can be reported that none of them can protect MOG-G-UVW cells against a low dose of $CoCl_2$ (65 µM). However, against a high dose of $CoCl_2$ (125 µM), diazoxide and nicorandil protect MOG-G-UVW cells, and cromakalim has the opposite effect by further reducing the relative cell number from 125 µM $CoCl_2$ alone.

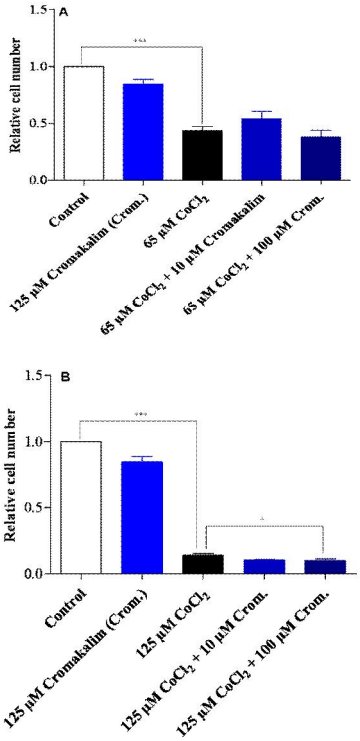

Figure 6 The effect of cromakalim (Crom.) in combination with $CoCl_2$ in MOG-G-UVW cells. A: 65 µM $CoCl_2$, the results are the means of 30 for 65 µM $CoCl_2$ alone and the control, and 6 for 125 µM cromakalim alone, 65 µM $CoCl_2$ + 10 µM cromakalim and 65 µM $CoCl_2$ + 100 µM cromakalim. And B: 125 µM $CoCl_2$, the results are the means of 21 for 125 µM $CoCl_2$ alone and the control, and 6 for 125 µM cromakalim alone, 125 µM $CoCl_2$ + 10 µM cromakalim and 125 µM $CoCl_2$ + 100 µM cromakalim. combination with $CoCl_2$ insult are shown in Figure 7.

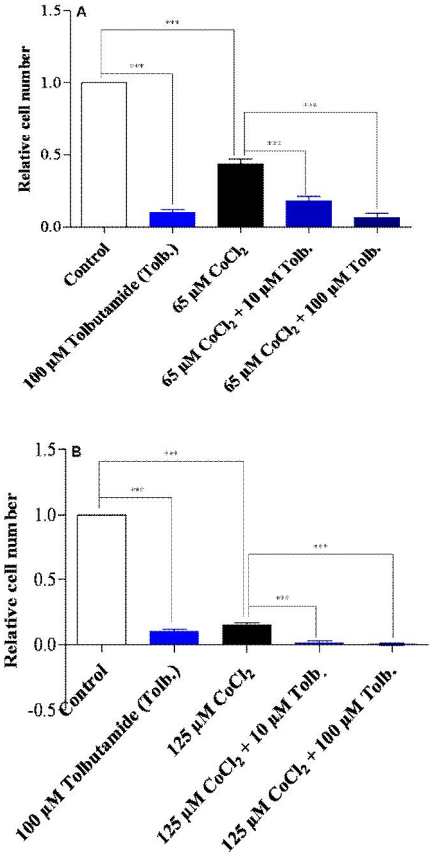

Figure 7 The effect of tolbutamide (Tolb.) in combination with $CoCl_2$ in MOG-G-UVW cells. A: 65 µM $CoCl_2$, the results are the means of 30 for 65 µM $CoCl_2$ alone and the control, and 6 for 100 µM tolbutamide alone, 65 µM $CoCl_2$ + 10 µM tolbutamide and 65 µM $CoCl_2$ + 100 µM tolbutamide. And B: 125 µM $CoCl_2$, the results are the means of 12 for 125 µM $CoCl_2$ alone and the control, and 6 for 100 µM tolbutamide, 125 µM $CoCl_2$ + 10 µM tolbutamide and 125 µM $CoCl_2$ + 100 µM tolbutamide.

3.4 K_{ATP} blocker effect on $CoCl_2$ insult

In addition to K_{ATP} activators, the blockers, tolbutamide and glibenclamide, were also tested in MOG-G-UVW cells. The results of tolbutamide in Figure 6.

For the results shown in Figure 7A, a one way ANOVA test for all the groups suggested that the difference amongst those groups was significant ($p < 0.001$), and the Tukey's post test (comparing every two groups) indicated that both 65 µM $CoCl_2$ alone and 100 µM tolbutamide alone caused significant reductions in the relative cell numbers from the control ($p < 0.001$ for both), and 10 µM or 100 µM tolbutamide in combination with 65 µM $CoCl_2$ also

had a significant effect on the relative cell number in comparison to the same dose of $CoCl_2$ alone ($p < 0.001$ for both).

Similar effects can be seen with a higher dose of $CoCl_2$ (125 μM). The Tukey's post test (comparing every two groups) for all the groups in Figure 7B indicated that 10 μM or 100 μM tolbutamide in combination with 125 μM $CoCl_2$caused a significant reduction in the relative cell number from the same dose of $CoCl_2$ alone ($p < 0.001$). Therefore, it can be concluded that 100 μM tolbutamide alone reduces the relative cell number, and 10 μM – 100 μM tolbutamide cannot protect MOG-G-UVW cells from either a low dose (65 μM) or a high dose (125 μM) of $CoCl_2$, and may in fact further reduce the relative cell number from the same dose of $CoCl_2$ alone.

In addition to tolbutamide, another K_{ATP} blocker, glibenclamide, has also been tested, but only negative results were achieved. The cell density was 5,000 per well in 24 well plates here. The EC_{50} of $CoCl_2$ in these experiments was estimated at about 500 μM. The glibenclamide results are shown in Table 2.

Table 2 The effect of glibenclamide in combination with an EC_{50} of $CoCl_2$ insult in MOG-G-UVW cells (n = 2)

Glibenclamide concentration (in combination with 500 μM $CoCl_2$)	Relative cell number
10 μM	0.621
50 μM	0.586
100 μM	0.428
500 μM	0.348

The Bonferronni's post test (comparing every two groups) did not suggest any significant difference in the relative cell number. Hence, it can be concluded that glibenclamide cannot protect MOG-G-UVW cells from a $CoCl_2$ insult.

Therefore, to summarise the results of the K_{ATP} channel blockers against the $CoCl_2$ insult, it can be concluded that neither tolbutamide nor glibenclamide was protective. In addition, tolbutamide in combination with $CoCl_2$ insult may even further reduce the relative cell number compared to the same dose of $CoCl_2$.

4. Discussion

From the PCR results shown above, it can be seen that the messages of K_{ATP} subunits exist in the MOG-G-UVW cell line, and the subunit composition of K_{ATP} channel in MOG-G-UVW cell line might (tentatively) be $K_{ir}6.2$ with SUR2A.

For the possible role, if any, of the K_{ATP} channel in cell death, previous studies have reached different conclusions, as discussed in the introduction. Some studies believe that the activation of K_{ATP} channel can induce cell death when there was no insult applied to the cells, for example both diazoxide and cromakalim can cause a reduction in cell numbers (Burg et al., 2006, Wang et al., 2007), and the blockade of K_{ATP} channel, with for example glibenclamide, may increase cell p roliferation(Wang et al., 2007). However, in combination with insults, such as hypoxia and Aβ, a K_{ATP} channel activator like diazoxide might then be neuroprotective(Goodman & Mattson, 1996).

Interestingly, the results in this study did not exactly concur with those studies above. K_{ATP} channel activators alone, such as diazoxide, nicorandil and cromakalim, tended to cause a reduction in the relative cell number from the control, but not significantly. Only 125 μM diazoxide alone caused a significant reduction in the relative cell number compared to the control, and K_{ATP} channel activators, like diazoxide and nicorandil, protected the cells from a high dose of $CoCl_2$ (125 μM), although the results were negative in the case of a low dose of insult (65 μM). In addition, K_{ATP} channel blockers, for example tolbutamide and glibenclamide, cannot protect the cells from $CoCl_2$ insult, and tolbutamide in combination with $CoCl_2$ insult may even cause a further reduction in the relative cell number from the same dose of $CoCl_2$ alone.

From the results shown before, a K_{ATP} channel blocker alone, such as tolbutamide, cannot increase cell proliferation in MOG-G-UVW, but causes a reduction in the relative cell number from the control. Additionally, not all K_{ATP} channel activators may protect the cells from $CoCl_2$ insult. Cromakalim may somehow aggravate the insult of a high dose of $CoCl_2$ (125 μM).

These results might be explained by considering the balance of $[Ca^{2+}]_i$ or K^+ release from the cell. K_{ATP} channel activator would reduce the open probability of Ca^{2+} channels on both ER membrane and cell membrane(Brini, 2003). In normal conditions without any insult, such an effect might lower the $[Ca^{2+}]_i$ to below a 'normal' physiological level. On the other hand, as introduced before, a K_{ATP} channel blocker might reduce cellular K^+ release and might cause the mitochondrial membrane hyperpolarisation.

In the hypoxia environment, such as exists with a $CoCl_2$ insult, two opposite results might follow the activation of the K_{ATP} channel. It can reduce the open probability of Ca^{2+} channels, but can also cause a K^+ release or even efflux. Hence, whether the K_{ATP} channel activator is neuroprotective or not may depend on the balance of those two opposing effects.

In addition, the difference in the effects of cromakalim and the other two K_{ATP} channel activators

might be explained by the selectivity to different K_{ATP} subunits. From the RT-PCR results shown before, it can be seen that, in MOG-G-UVW cell line, the expression of $K_{ir}6.2$ is higher than that of $K_{ir}6.1$, and the expression of SUR2A is higher than that of the other two SUR subunits. If there is any possible selectivity with respect to the different K_{ATP} channel subunits in the activators, it might explain the negative results shown by cromakalim.

5. Conclusions and future work

To summarise all the results and discussion, it may be suggested that:

The messages of K_{ATP} channel subunits exist in the mRNA of MOG-G-UVW cell line, and the main subunit composition of the K_{ATP} channel in this cell line might be $K_{ir}6.2$ with SUR2A.

K_{ATP} activators, such as diazoxide, nicorandil and cromakalim, cannot increase the cell proliferation of MOG-G-UVW cells and cannot protect the cells from a low dose of $CoCl_2$ (65 μM). However, with a high dose of $CoCl_2$ (125 μM), diazoxide and nicorandil protect the MOG-G-UVW cells, but cromakalim causes a further reduction in the relative cell number from $CoCl_2$ alone.

K_{ATP} channel blockers, such as tolbutamide and glibenclamide, cannot increase cell proliferation in MOG-G-UVW cells, and tolbutamide alone may even cause a reduction in the relative cell number. Neither of the two blockers can protect MOG-G-UVW cells from $CoCl_2$ insult, and tolbutamide may even cause a further reduction in the relative cell number from the same dose of $CoCl_2$ alone.

For the future work on K_{ATP} channel, the possible selectivity, if any, for different K_{ATP} subunits in the activators should be investigated to find out whether this is the reason for cromakalim to be different from the other two activators. If this is the case, the expression of different K_{ATP} subunits in neurones can be studied with Q-PCR to find out what the main subunit composition of K_{ATP} channel in neurones is. Furthermore, the K_{ATP} channel activators which can target those subunits, for example $K_{ir}6.2$ and/or SUR2A might point the way to a K^+ channel strategy for neuroprotection.

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Authors Contributions

Dr. ZHANG Jin wrote the paper and did the RT-PCR work. Dr. Zhang, Miss. Marwa KHUDHEYER and Miss Jasmin NOWAK did the cell proliferation MTS assays. Dr. Zhang and Dr. Dwaine BURLEY analysed the data. Dr. Burley and Prof. Kenneth WANN designed the research. And, Prof. Wann supervised this study as the group leader.

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