

Dexmedetomidine impairs P-glycoprotein-mediated efflux function in L02 cells via the adenosine 5'-monophosphate-activated protein kinase/nuclear factor- κ B pathway

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Abstract. Dexmedetomidine (DEX) a type of the anaesthetic that has been widely used in anaesthesia and intensive care. However, whether DEX affects the pharmacokinetics of drugs remains elusive. As hepatic P-glycoprotein (P-gp) serves a critical role in the disposition of drugs, the present study aimed to address whether P-gp function could be affected by DEX *in vitro*. In the present study, L02 cells (a normal human liver cell line) were exposed to DEX for 24 h and P-gp function was evaluated by the intracellular accumulation of Rhodamine 123. The results indicated that P-gp function was significantly impaired by DEX treatment and that the mRNA levels and protein levels of P-gp were downregulated in a dose- and time-dependent manner. Importantly, DEX-induced downregulation of P-gp was associated with adenosine 5'-monophosphate-activated protein kinase (AMPK) activation, as it was significantly attenuated by AMPK inhibition using dorsomorphin. Furthermore, the results revealed that changes in the subcellular localisation of nuclear factor (NF)- κ B following AMPK activation were involved in the P-gp regulation in response to DEX treatment. Collectively, these results suggested that DEX impairs P-glycoprotein-mediated efflux function in L02 cells via the AMPK/NF- κ B pathway, which provided direct evidence that the hepatic disposition of drugs may be affected by DEX through the downregulation of P-gp.

Introduction

Accruing evidence indicates that anaesthetics not only exert anaesthetic action but also affect a variety of physiological changes, including haemo-dynamics (1), immune regulation (2), inflammatory responses (3) and ischaemia/reperfusion (I/R) injury during severe surgeries (4). Dexmedetomidine (DEX) is one of the anaesthetics that have been widely used for anaesthesia and intensive care. However, in addition to its sedative, anxiolytic, analgesic and anaesthetic effects, DEX has recently been extensively studied for its non-anaesthetic effect. For example, it has been shown to exert a protective effect on specific organs, including the brain, heart, lung and kidney, likely via decreasing cell apoptosis (5,6) and inhibiting pro-inflammatory cytokine release (7,8). To date, few studies have examined the effects of DEX on the pharmacokinetics of drugs. Given that hepatocytes play a critical role in the pharmacokinetics of drugs and that DEX is rapidly distributed and extensively metabolised in the liver, we hypothesised that the hepatic expression of genes responsible for the modulation of pharmacokinetics may be influenced by DEX.

The liver is one of the most important organs responsible for the metabolism and biliary excretion of drugs, and hepatic drug transporters play important roles in the hepatic disposition of drugs by modulating the uptake of drugs from the blood into the hepatocytes or the efflux of drugs into bile. Basolateral transport systems (e.g., organic cation transporters such as OCT1 and organic anion-transporting polypeptides such as OATP1B1) are responsible for the translocation of molecules across the sinusoidal membrane into hepatocytes, whereas active canalicular transport systems [e.g., P-glycoprotein (P-gp) and multidrug resistance proteins (MRPs)] are responsible for the biliary excretion of drugs, phase I or phase II drug metabolites, and endogenous compounds into bile (9,10). P-gp, a 170 kDa product encoded by ABCB1 (also termed as MDR1) was the first ATP-binding cassette (ABC) transporter identified in the canalicular membranes of normal hepatocytes. As one of the most studied drug transporters, it plays a significant role in the active efflux of drugs, and most therapeutic agents that cross the cell plasma membrane via passive diffusion (lipophilic drugs) are P-gp substrates, including anticancer drugs, antibiotics, antiemetics, antiepileptics and steroids (11,12). In

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Abbreviations: DEX, dexmedetomidine; P-gp, P-glycoprotein; AMPK, adenosine 5'-monophosphate-activated protein kinase; NF- κ B, nuclear factor- κ B

Key words: dexmedetomidine, P-glycoprotein, L02, AMPK pathway, NF- κ B

addition to P-gp, MRP2 also mediates the biliary elimination of different organic anions, including glutathione-S-conjugates of leukotriene, of bilirubin and of oestrogens; MRP3 and MRP4, which are present at the sinusoidal pole of hepatocytes, contribute to the transport of xenobiotics, nucleoside analogues and antiviral drugs for secondary renal elimination (13,14).

Currently, though evidence of the relationship between DEX and hepatic drug metabolism is insufficient and the mechanism remains elusive, a number of *in vitro* and *in vivo* studies have revealed that DEX can elicit various non-anaesthetic effects via various signalling pathways, including ERK, Akt and adenosine 5'-monophosphate-activated protein kinase (AMPK) (15-17). In contrast, the expression of canalicular drug transporters has been recently reported to be partially mediated by various second messengers, as well as by protein kinases, in response to different endogenous or exogenous stimuli (18,19). In this study, we examined the effect of DEX on the expression of hepatic drug transporters by using L02 cells (a normal human liver cell line) as an *in vitro* model. In particular, we investigated the effect of DEX on the expression and function of P-gp *in vitro* and explored the possible signal transduction pathways by using specific inhibitors. Our findings may provide a new perspective on the non-anaesthetic effect of DEX regarding P-gp function and hepatic drug disposition.

Materials and methods

Chemicals. Cell culture reagents were purchased from Thermo Fisher Scientific (Waltham, MA, USA). DEX was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). PD98059 (ERK inhibitor), LY294002 (PI3K/Akt inhibitor), SB600125 (JNK inhibitor), SB203580 (p38 inhibitor) and dorsomorphin (AMPK inhibitor) were purchased from ApexBio (Houston, TX, USA). Nuclear factor (NF)- κ B, GAPDH, Histone 3 and P-gp were purchased from Abcam (Cambridge, UK). ATP1A1 was purchased from Proteintech (Wenzhou, China). Phosphorylated AMPK (p-AMPK) at Thr172 and total AMPK were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA).

Cell culture and treatment. The normal human liver cell line L02 (obtained from the Chinese Academy of Science Committee Type Culture Collection Cell Bank) was cultured in high-glucose Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum. The cells were maintained in a humidified incubator at 37°C in an atmosphere containing 5% CO₂. The medium was changed every two days. The L02 cells were exposed to 0.1, 0.2, 0.5, 1, 2, 5 and 10 μ M DEX for 12-24 h to test its effect on P-gp. In some experiments, the cells were pretreated with the following inhibitors for 1 h before exposure to DEX: PD98059 (10 μ M), LY294002 (20 μ M), SB600125 (10 μ M), SB203580 (10 μ M) and dorsomorphin (10 μ M). As the stock solution of inhibitors was dissolved in DMSO, an equal volume of DMSO (final concentration <0.1%) was added to the control cells.

Cell viability assay. Cell viability was evaluated using the Cell Counting kit-8 (CCK-8) assay kit (Dojindo Laboratories, Kumamoto, Japan). After DEX treatment, the cells were

incubated with 100 μ l of 10% CCK-8 reagent for 30 min at 37°C. The absorbance of each well was measured using a Multiskan Spectrum (Thermo Fisher Scientific) at 450 nm.

Intracellular Rhodamine 123 accumulation assay. The efflux function of P-gp was evaluated by measuring the accumulation of Rhodamine 123 (a specific substrate for P-gp) as described previously (20). Briefly, the cells were incubated with 5 μ M of Rhodamine 123 in culture medium at 37°C for 1 h. After incubation, the unabsorbed Rhodamine 123 was removed, and the cells were washed three times with ice-cold PBS. The cells were harvested and then lysed with 0.3% Triton X-100. The amount of Rhodamine 123 was determined using a Multiskan Spectrum (Thermo Fisher Scientific) and normalised to the protein content. Verapamil (100 μ M) was used as a positive control for the specific inhibition of P-gp function.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from L02 cells using TRIzol (Life Technologies, Grand Island, NY, USA), and cDNA was synthesised with an RNA isolation plus kit (Applied Biosystems, Foster City, CA, USA). Q-PCR was performed using a 7900HT Fast Real-Time PCR system (Applied Biosystems), and the relative mRNA levels were calculated as a fold change using the comparative quantitative cycle (Cq) method ($\Delta\Delta$ Cq) with GAPDH used as the internal control. The primers are listed in Table I.

Western blot analysis. P-gp was extracted from the membrane proteins as described previously (21). Briefly, the membranous fractions were isolated by homogenising the cells in a buffer containing 12 mM of Tris-HCl, 5 mM of EGTA, 300 mM of mannitol and a cocktail of protease inhibitors. Unbroken cells and nuclei were precipitated by centrifugation. The resulting supernatant was centrifuged at 18,000 g for 1 h to give a total plasma membrane protein, and the resulting pellet was resuspended in 1% sodium dodecyl sulfate containing a cocktail of protease inhibitors (Thermo Fisher Scientific). Samples (20 μ g of protein) were subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and the content of P-gp was determined using a mouse monoclonal antibody against P-gp (Abcam; 1:500). ATP1A1 (Proteintech; 1:1,000) served as loading control. For total protein extraction, the cells were harvested and lysed on ice for 30 min in 100 μ l of RIPA buffer containing a cocktail of protease and phosphatase inhibitor. Samples containing 20 μ g of extracted protein were loaded, and the expression of p-AMPK, AMPK and NF- κ B (p65) was determined using a rabbit monoclonal antibody against p-AMPK (1:1,000), a rabbit monoclonal antibody against AMPK (both Cell Signaling Technology; 1:1,000) and a rabbit monoclonal antibody against NF- κ B (Abcam; 1:2,000), respectively. Cytoplasmic and nuclear fractions were extracted using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific). Approximately 20 μ g of cytoplasmic proteins and 8 μ g of nuclear proteins were loaded. Histone 3 and GAPDH served as loading controls for nuclear fractions and cytoplasmic fractions, respectively. Immunodetection was performed

Table I. Primers for reverse transcription-quantitative polymerase chain reaction.

Gene	Full name	Sequences, forward/reverse 5'-3'
<i>CYP7A1</i>	Cytochrome P450 7A1	GCTCTTTACCCACAGTTAATGC TTGTCTTCCCGTTTTTCATCA
<i>CYP27A1</i>	Cytochrome P450 27	CAAGGACTTTGCCACATGC TGGCAGAACACAAACTGGGT
<i>P-gp</i>	P-glycoprotein	GTTCTTTTCAGTCAATGGCAAC TCTAATTGCTGCCAAGACCTC
<i>MRP2</i>	Multidrug resistance-associated protein 2	GGACAGTGACAAGGTAATGGTCC TGCCGCACTCTATAATCTTCCC
<i>MRP3</i>	Multidrug resistance-associated protein 3	TCTGTCTGGCTGGAGTCG TCCGTTGAGTGGGAATCAGCA
<i>MRP4</i>	Multidrug resistance-associated protein 4	CCCTTTAAGGAGCACACGGA TACCTCTGTAAAGGCATTCCACAGT
<i>GAPDH</i>	Glyceraldehyde-3-phosphate dehydrogenase	TATTGTTGCCATCAATGACCC ACTCCACGACGTACTCAGC

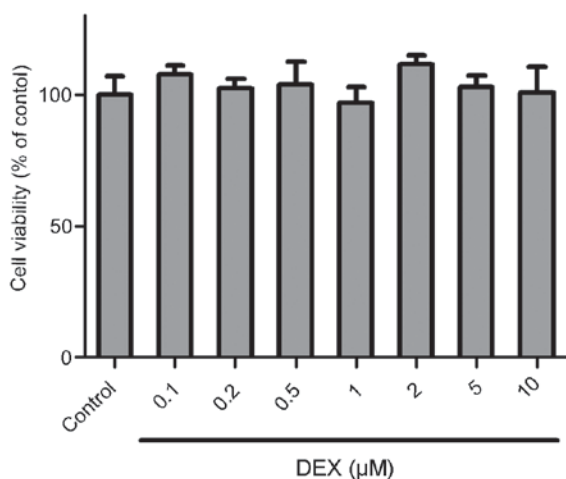


Figure 1. Effect of DEX on the viability of L02 cells. L02 cells were treated with 0.1-10 μM of DEX for 24 h. Cell viability was assessed by CCK-8 assay. The data are presented as the mean \pm SD (n=6-8/group). DEX, dexmedetomidine; CCK-8, Cell Counting kit-8; SD, standard deviation.

with an enhanced chemiluminescence detection system (Thermo Fisher Scientific).

Immunofluorescence staining. Cells were fixed in 3.7% formaldehyde at room temperature for 15 min and then permeabilised with 0.3% Triton-X100 for 15 min. After incubation with primary antibodies against P-gp at 4°C overnight, the cells were incubated with Alexa Fluor 488-conjugated secondary antibodies for 1 h. The nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). All images were visualised and processed using a Leica application suite (LAS AF Lite; Leica, Mannheim, Germany).

Statistical analysis. Statistical analyses were performed using GraphPad Prism 5 (La Jolla, CA, USA). The data are expressed as the means \pm standard deviation (SD). For the comparisons of multiple groups, data were evaluated by one-way analysis

of variance, followed by the Newman-Keuls post hoc test. Comparisons between two groups were done with a Student's t-test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

The effect of DEX on the cell viability, and the expression of genes encoding drug metabolising enzymes and transporters in L02 cells. As indicated by CCK-8, no significant alterations were observed in the cells treated with various concentrations of DEX (0.1-10 μM), suggesting that the viability of L02 cells was not affected by DEX (Fig. 1). Q-PCR analysis revealed that only P-gp expression was significantly downregulated by DEX treatment (~45%; $P < 0.05$), with no significant effects observed on the expression of CYP7A1, CYP27A1, MRP2, MRP3 and MRP4 (Fig. 2).

The efflux function of P-gp was suppressed by DEX treatment in L02 cells. The intracellular accumulation of Rhodamine 123 was dose-dependently increased by DEX treatment at 0.1-1 μM . As shown, though the intracellular accumulation of Rhodamine 123 was not significantly increased upon DEX at lower doses (0.1 and 0.2 μM), it was dose-dependently increased by DEX treatment at 0.5-10 μM (Fig. 3A). As treatment with DEX at 1 μM elicited the most significant impact on P-gp expression, with no more significant effects observed at the higher concentrations, this concentration was subsequently used in the time course study. As shown in Fig. 3B, though the intracellular accumulation of Rhodamine 123 was unchanged at 6 h post-DEX treatment, it was significantly increased at 12 h and 24 h post-DEX treatment. Consistently, representative images showing the visualised accumulation of Rhodamine 123 (analysed by grey value) mirrored the significantly increased intracellular accumulation of Rhodamine 123 by DEX treatment (Fig. 3C). Taken together, these results indicated that the efflux function of P-gp was suppressed by DEX treatment in L02 cells.

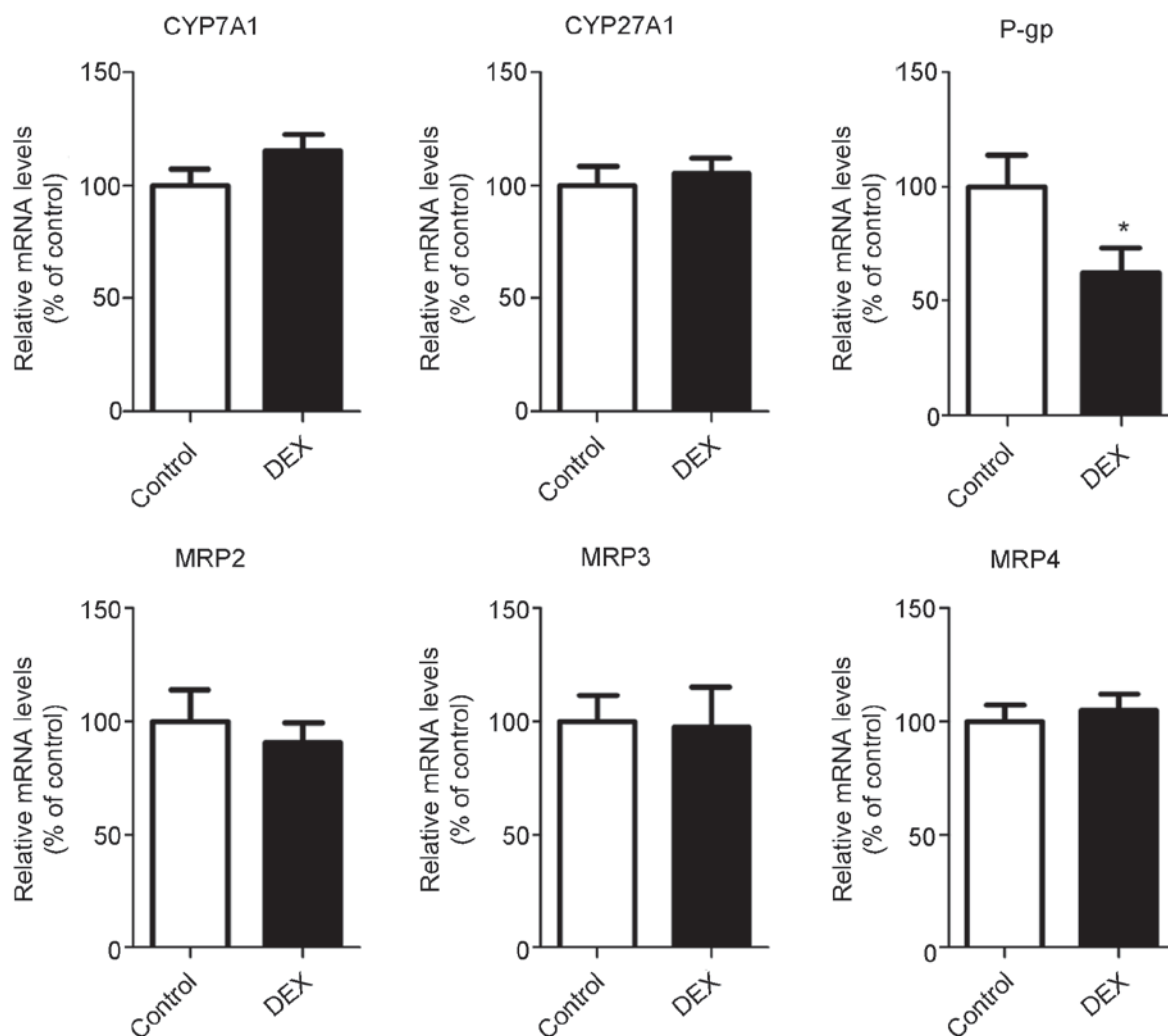


Figure 2. Effect of DEX on the expression of genes encoding drug metabolising enzymes and transporters in L02 cells. Cells were treated with 1 μ M of DEX for 24 h, and the mRNA levels of target genes were determined by q-PCR. The data are presented as the mean \pm SD (n=3). *P<0.05, compared with control. DEX, dexmedetomidine; MRP, multidrug resistance protein; q-PCR, quantitative polymerase chain reaction; SD, standard deviation.

DEX downregulated the expression of P-gp in a dose- and time-dependent manner. As shown, the mRNA levels of P-gp were decreased on DEX treatment in a dose- and time-dependent manner. After a 24 h incubation with DEX, the levels were significantly decreased to 76.9% (P<0.05) and 57.7% (P<0.05) at 0.5 and 1 μ M, respectively. In contrast, the downregulation of P-gp was evident at both 12 and 24 h post-DEX treatment (Fig. 4A). Consistently, Western blot analysis substantially mirrored the results of the Q-PCR (Fig. 4B). Moreover, immunofluorescence staining demonstrated that downregulation of P-gp expression in L02 cells was evident after DEX treatment (Fig. 4C). Taken together, these results suggested that DEX downregulated the expression of P-gp in a dose- and time-dependent manner.

DEX downregulated P-gp expression via AMPK activation. Possible signalling pathways responding to DEX treatment were examined by using specific inhibitors, including ERK, PI3K/Akt, p38, JNK and AMPK. As shown, DEX-induced elevation of Rhodamine 123 accumulation was significantly attenuated by pretreatment with an AMPK inhibitor (dorsomorphin), with no significant impact observed in the other

groups (Fig. 5A). Activation of the AMPK pathway in response to DEX was confirmed by Western blot analysis. As shown, though no changes in the total amount of AMPK were observed within the period of time (0, 30, 60, 120, and 180 min), phosphorylated levels of AMPK (p-AMPK) were already evident at 60 min after DEX stimulation and decreased until 180 min (Fig. 5B). Consistent with the functional assay, pretreatment with dorsomorphin partially restored the expression of P-gp at both the mRNA levels and protein levels (Fig. 5C and D). Collectively, these results suggested that the AMPK pathway may play a pivotal role in mediating the regulation of P-gp upon DEX treatment *in vitro*.

DEX activated the AMPK pathway and induced the nuclear exclusion of NF- κ B. As one of the down-stream proteins of the AMPK pathway, NF- κ B was examined, as early studies indicated that activated NF- κ B may accumulate in the nucleolus and serve as an enhancer of P-gp transcription (22,23). Thus, we hypothesised that DEX may either reduce the total amount of NF- κ B or alter its subcellular distribution, in turn leading to the repression of P-gp transcription. First, changes in the subcellular distribution of NF- κ B (p65) were examined. As shown

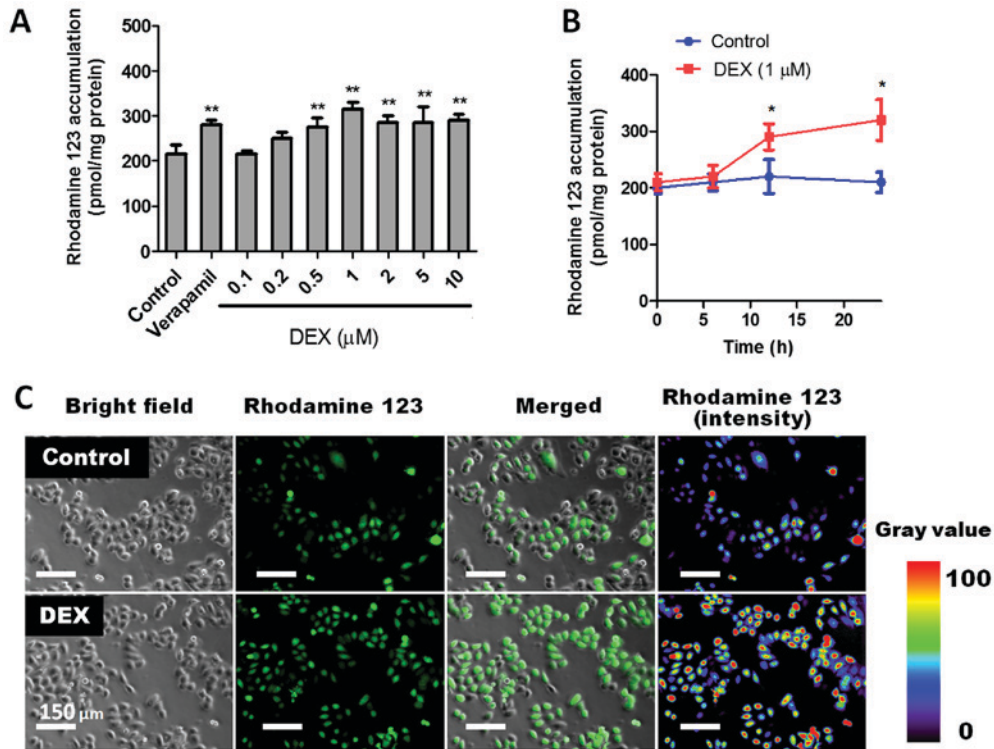


Figure 3. Effect of DEX on P-gp function in L02 cells. (A and B) Intracellular accumulation of Rhodamine 123 in L02 cells. The cells in (A) were treated with 0.1-10 μM of DEX for 24 h, and the cells in (B) were treated with 1 μM of DEX for 6, 12 and 24 h. The data are presented as the mean ± SD (n=3-4/group). *P<0.05, **P<0.01 compared with control. (C) Representative images of Rhodamine 123 accumulation in L02 cells. Cells were treated with 1 μM of DEX for 24 h. The intensity was shown as a heat spectrum, analysed using LAS AF Lite software by grey value. Scale bar, 150 μm. Three experiments were performed that showed similar results. DEX, dexmedetomidine; P-gp, p-glycoprotein; SD, standard deviation.

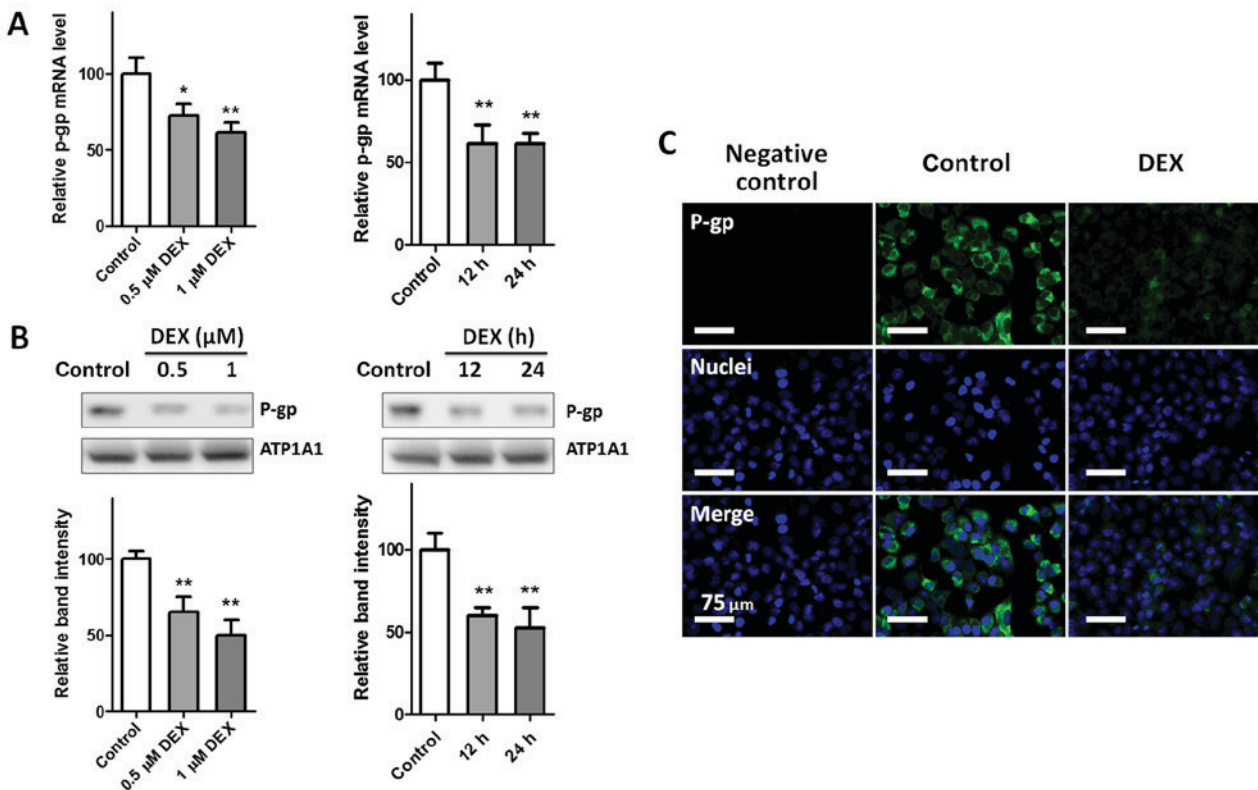


Figure 4. Effect of DEX on P-gp expression in L02 cells. (A) P-gp mRNA levels in L02 cells. Cells were treated with 0.5-1 μM of DEX for 12-24 h. Total RNA was extracted, and the mRNA levels of P-gp were determined by Q-PCR. The data are presented as the mean ± SD (n=3). *P<0.05, **P<0.01 compared with control. (B) Representative blots of P-gp in L02 cells. Cells were treated as described in (A). (C) Representative images of P-gp staining in L02 cells. Cells were treated with 1 μM of DEX for 24 h. Notably, a significant reduction in P-gp (green) expression was observed upon DEX treatment. Scale bar, 75 μm. Three experiments were performed that showed similar results. DEX, dexmedetomidine; P-gp, p-glycoprotein; SD, standard deviation.

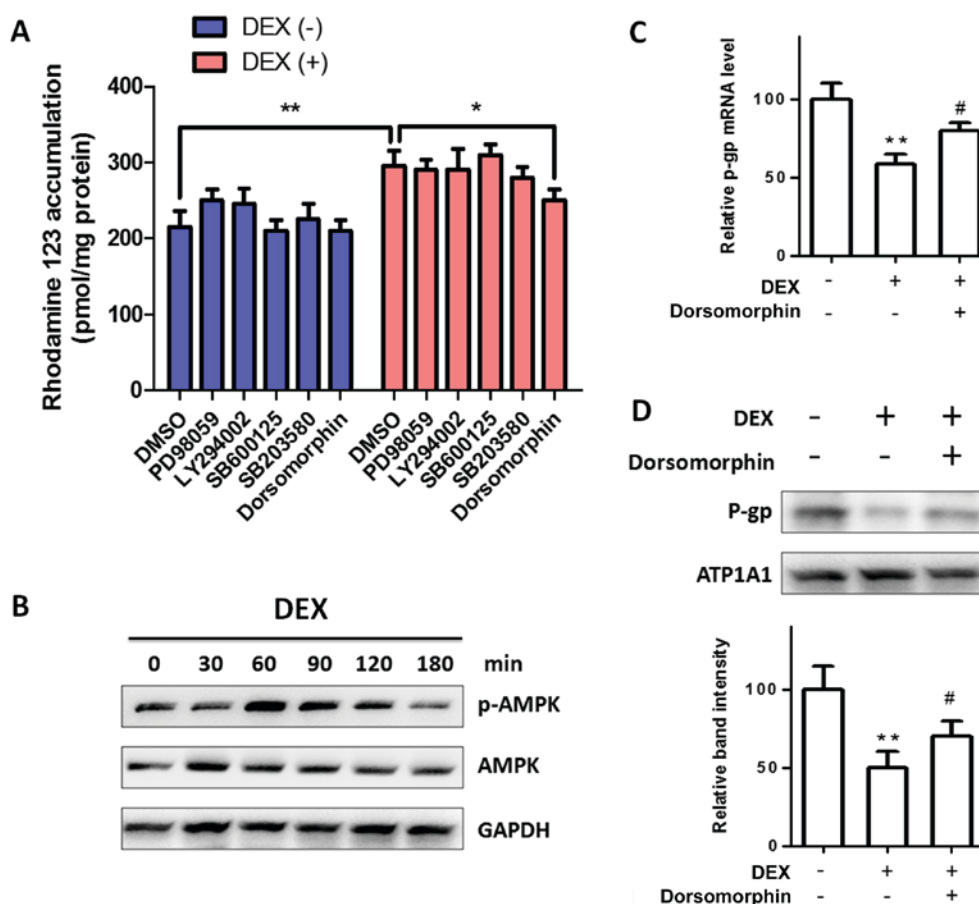


Figure 5. DEX suppressed the function and expression of P-gp via the AMPK pathway. (A) Intracellular accumulation of Rhodamine 123 in L02 cells. Cells were pre-treated with specific inhibitors as described in the 'materials and methods'. Note that pretreatment with AMPK inhibitor (dorsomorphin) significantly reduced Rhodamine 123 accumulation in DEX-treated cells. The data are presented as the mean \pm SD (n=3). *P<0.05, **P<0.01 as indicated. (B) Activation of the AMPK pathway responding to DEX treatment. The phosphorylation level and total amount of AMPK were examined for the indicated periods. (C and D) Changes in the mRNA levels and protein levels of P-gp by inhibition of the AMPK pathway. Blocking the AMPK pathway with dorsomorphin significantly attenuated the downregulation of P-gp expression. The data are presented as the mean \pm SD (n=3). **P<0.01 compared to control. #P<0.05 compared to DEX. DEX, dexmedetomidine; P-gp, p-glycoprotein; AMPK, adenosine 5'-monophosphate-activated protein kinase; SD, standard deviation.

in Fig. 6A, though the total amount of NF- κ B (p65) was not changed in the whole cell lysate, it was reduced in the nucleus but increased in the cytoplasm with DEX treatment over the period of time, suggesting that NF- κ B (p65) translocated from the nucleus to the cytoplasm in response to DEX treatment. Importantly, pretreatment with dorsomorphin inhibited AMPK activation and abolished these changes in the NF- κ B distribution (Fig. 6B). By measuring band intensity, the results revealed that the relative distribution of NF- κ B in the nucleus shifted from 68 to 30% upon DEX treatment and returned to baseline upon AMPK inhibition (Fig. 6C). Taken together, these results suggested that DEX induced downregulation of P-gp transcription through the AMPK-mediated nuclear exclusion of NF- κ B. A schematic representation is shown in Fig. 6D.

Discussion

General anaesthesia is often repeated within a short period of time with the same anaesthetic agent. Though DEX has been routinely used for surgical operations, its non-anaesthetic effects need to be further studied. To date, an area that has not been fully studied is the effect that DEX may have on hepatic drug transporters and metabolic enzymes. In this study, we examined

the effect of DEX on the expression of drug transporters, including P-gp and MRP2/3/4, in L02 cells and demonstrated that DEX may suppress the expression of P-gp and impair P-gp-mediated efflux function *in vitro*. As P-gp plays a pivotal role in the hepatic disposition of drugs, this may have potential clinical significance, as the repeated administration of DEX may affect drug disposition, drug bioavailability and even drug-drug interactions *in vivo*.

In this study, the dosages of DEX ranging from 0.1-10 μ M were referenced to a study conducted by Kharasch *et al.* (24), in which approximately 2 μ M was used to examine its influence on the metabolism of alfentanil in human liver microsomes *in vitro*. As shown, our results revealed no detectable cytotoxicity under the dosages ranging from 0.1-10 μ M (Fig. 1), suggesting that changes in the expression of drug transporters are independent on the cell viability. Importantly however, DEX elicited dose- and time-dependent inhibitory effects on P-gp function, consistent with its downregulated expression at both the mRNA levels and protein levels (Figs. 3 and 4). Interestingly, these results obtained *in vitro* are partially in line with a rodent study conducted previously by Nakazato *et al.* (25), in which they found that the mRNA expression of P-gp was significantly decreased in the liver 6 h after DEX administration but returned

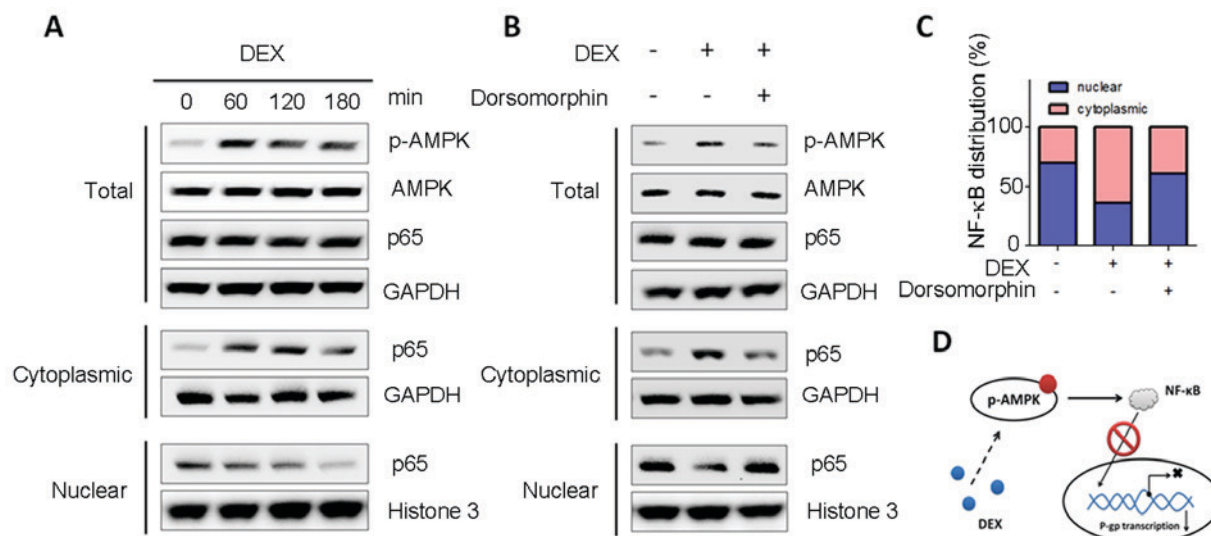


Figure 6. DEX induced the nuclear exclusion of NF- κ B via the AMPK pathway. (A) Alterations in the subcellular distribution of NF- κ B. The cells were treated with DEX (1 μ M) for the indicated periods. Note that the expression levels of NF- κ B (p65) were time-dependently reduced in the nucleus but increased in the cytoplasm upon DEX treatment. (B) DEX-induced nuclear exclusion of NF- κ B was reversed by inhibition of the AMPK pathway. The cells were pre-treated with dorsomorphin for 1 h prior to DEX treatment, and changes in the subcellular distribution of NF- κ B were examined at 60 min after DEX treatment. The data shown are representative of one of three experiments. (C) The relative distribution of NF- κ B (nuclear/cytoplasmic) analysed by the band intensity in (B). (D) Schematic representation showing that DEX suppresses p-gp expression through the nuclear exclusion of NF- κ B via the AMPK pathway. DEX, dexamethasone; NF, nuclear factor; AMPK, adenosine 5'-monophosphate-activated protein kinase; p-AMPK, phosphorylated AMPK; SD, standard deviation.

to baseline at 24 h after awakening from the anaesthesia. Combined with results of Keiko *et al*, it suggested that the effect of DEX on P-gp expression was inducible both *in vivo* and *in vitro* and that this effect may be reversible upon DEX withdrawal.

To our knowledge, studies that have explored the effect of DEX on the hepatic expression of P-gp *in vitro* are limited. However, one aspect requires comment here. In this study, L02 cells instead of HepG2 cells were used as a model *in vitro*, as L02 is a normal human liver cell line that has been approved as suitable for studying liver function and various non-cancerous liver diseases *in vitro* (26-29). For example, Liu *et al* examined the effect of synthetic muscone on the expression of the CYP1A2 and CYP3A4 enzymes in L02 cells, as well as in the liver tissue of Kunming mice, and demonstrated that muscone can induce the hepatic expression of CYP1A2 and CYP3A4 both *in vitro* and *in vivo* (29). In this study, we did not use hepatoma-derived HepG2 cells due to the possibility that the high abundance of P-gp in this cell line may not reflect the effect of DEX in normal liver tissue. However, though the expression of P-gp was nearly undetectable in the total protein extract of L02 cells due to its relatively low abundance compared with HepG2 cells, it could be clearly detected in the membrane fractions.

In an attempt to further understand the underlying mechanism, we examined possible signalling pathways that may mediate the regulation of P-gp upon DEX treatment, including ERK, PI3K/Akt, JNK, p38 and AMPK. These pathways were examined because: i) Previous studies demonstrated that DEX may elicit various non-anesthetic effects via these pathways (17,30) and ii) these pathways have been reported to be involved in the regulation of P-gp expression in response to a variety of extracellular stimuli (31,32). In the present study, though it lacks statistical significance, it seems that the intracellular accumulation of Rhodamine 123 in L02 cells was slightly

increased by inhibition of the ERK and PI3K/Akt pathways, suggesting that these pathways may contribute to the activation of P-gp expression under normal conditions. In contrast, elevated Rhodamine 123 accumulation by DEX treatment was significantly attenuated by AMPK inhibition. Consistently, the downregulated expression of P-gp was partially restored at both the mRNA levels and protein levels. These results suggested that AMPK activation plays a pivotal role in the regulation of P-gp upon DEX treatment (Fig. 5). Recently, an increasing number of studies has revealed that NF- κ B is an important down-stream effector responding to DEX treatment, contributing to a variety of DEX-associated physiological or pharmacological actions in hepatocytes (8,33). In contrast, it has also been shown to play a critical role in P-gp expression as a transcriptional enhancer (34,35). In this study, though the total amount of NF- κ B remained stable, its subcellular distribution was significantly altered upon DEX treatment. Importantly, this alteration seems to be associated with AMPK activation, as it was significantly abolished by blocking the AMPK pathway (Fig. 6). Taken together, we propose that DEX activates the AMPK pathway, which in turn induces nuclear exclusion of NF- κ B. As a result, NF- κ B loses its transcriptional activity on P-gp, leading to the downregulation of P-gp and impaired P-gp efflux function.

In conclusion, this study revealed an important non-anaesthetic effect of DEX on the expression of hepatic P-gp, which demonstrated that DEX may impair P-gp-mediated efflux function in L02 cells via the AMPK/NF- κ B pathway. Because DEX is currently widely used as a preoperative anaesthetic, this study provided direct evidence that the expression and function of drug transporters may be affected by DEX treatment; therefore, the changes in drug deposition, drug bioavailability and drug-drug interaction should be taken into consideration for the patients receiving DEX administration.

Acknowledgements

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References

- Kumar A, Sinha C, Kumar A and Kumari P: The effect of intravenous dexmedetomidine compared to propofol on patients hemodynamics as a sedative in brachial plexus block: A comparative study. *Anesth Essays Res* 11: 201-205, 2017.
- Zhang X, Wang J, Qian W, Zhao J, Sun L, Qian Y and Xiao H: Dexmedetomidine inhibits tumor necrosis factor- α and interleukin 6 in lipopolysaccharide-stimulated astrocytes by suppression of c-Jun N-terminal kinases. *Inflammation* 37: 942-949, 2014.
- Liu Z, Wang Y, Wang Y, Ning Q, Zhang Y, Gong C, Zhao W, Jing G and Wang Q: Dexmedetomidine attenuates inflammatory reaction in the lung tissues of septic mice by activating cholinergic anti-inflammatory pathway. *Int Immunopharmacol* 35: 210-216, 2016.
- Chen Z, Qiu PY and Ma CG: Dexmedetomidine preconditioning protects against retinal ischemia/reperfusion injury and inhibits inflammation response via toll-like receptor 4 (TLR4) pathway. *Biomed Pharmacother* 93: 1018-1024, 2017.
- Choi IY, Hwang L, Jin JJ, Ko IG, Kim SE, Shin MS, Shin KM, Kim CJ, Park SW, Han JH and Yi JW: Dexmedetomidine alleviates cerebral ischemia-induced short-term memory impairment by inhibiting the expression of apoptosis-related molecules in the hippocampus of gerbils. *Exp Ther Med* 13: 107-116, 2017.
- Fu C, Dai X, Yang Y, Lin M, Cai Y and Cai S: Dexmedetomidine attenuates lipopolysaccharide-induced acute lung injury by inhibiting oxidative stress, mitochondrial dysfunction and apoptosis in rats. *Mol Med Rep* 15: 131-138, 2017.
- Yan X, Cheng X, Zhou L, He X, Zheng W and Chen H: Dexmedetomidine alleviates lipopolysaccharide-induced lung injury in Wistar rats. *Oncotarget* 8: 44410-44417, 2017.
- Wang Y, Wu S, Yu X, Zhou S, Ge M, Chi X and Cai J: Dexmedetomidine protects rat liver against ischemia-reperfusion injury partly by the α 2A-adrenoceptor subtype and the mechanism is associated with the TLR4/NF- κ B pathway. *Int J Mol Sci* 17: pii: E995, 2016.
- Mottino AD and Catania VA: Hepatic drug transporters and nuclear receptors: Regulation by therapeutic agents. *World J Gastroenterol* 14: 7068-7074, 2008.
- Konig J, Muller F and Fromm MF: Transporters and drug-drug interactions: Important determinants of drug disposition and effects. *Pharmacol Rev* 65: 944-966, 2013.
- Chen Z, Shi T, Zhang L, Zhu P, Deng M, Huang C, Hu T, Jiang L and Li J: Mammalian drug efflux transporters of the ATP binding cassette (ABC) family in multidrug resistance: A review of the past decade. *Cancer Lett* 370: 153-164, 2016.
- Nakanishi T and Tamai I: Interaction of drug or food with drug transporters in intestine and liver. *Curr Drug Metab* 16: 753-764, 2015.
- Wen J, Luo J, Huang W, Tang J, Zhou H and Zhang W: The pharmacological and physiological role of multidrug-resistant protein 4. *J Pharmacol Exp Ther* 354: 358-375, 2015.
- Keppler D: Multidrug resistance proteins (MRPs, ABCs): Importance for pathophysiology and drug therapy. *Handb Exp Pharmacol* 2011: 299-323, 2011.
- Xia M, Ji NN, Duan ML, Tong JH, Xu JG, Zhang YM and Wang SH: Dexmedetomidine regulate the malignancy of breast cancer cells by activating α 2-adrenoceptor/ERK signaling pathway. *Eur Rev Med Pharmacol Sci* 20: 3500-3506, 2016.
- Wang Y, Wu C, Han B, Xu F, Mao M, Guo X and Wang J: Dexmedetomidine attenuates repeated propofol exposure-induced hippocampal apoptosis, PI3K/Akt/Gsk-3 β signaling disruption, and juvenile cognitive deficits in neonatal rats. *Mol Med Rep* 14: 769-775, 2016.
- Sun Y, Jiang C, Jiang J and Qiu L: Dexmedetomidine protects mice against myocardium ischaemic/reperfusion injury by activating an AMPK/PI3K/Akt/eNOS pathway. *Clin Exp Pharmacol Physiol* 44: 946-953, 2017.
- Mayati A, Moreau A, Le Vée M, Stieger B, Denizot C, Parmentier Y and Fardel O: Protein kinases C-mediated regulations of drug transporter activity, localization and expression. *Int J Mol Sci* 18: pii: E764, 2017.
- Shan E, Zhu Z, He S, Chu D, Ge D, Zhan Y, Liu W, Yang J and Xiong J: Involvement of pregnane X receptor in the suppression of carboxylesterases by metformin in vivo and in vitro, mediated by the activation of AMPK and JNK signaling pathway. *Eur J Pharm Sci* 102: 14-23, 2017.
- Yan JK, Gong ZZ, Zhang T and Cai W: Sodium butyrate attenuates soybean oil-based lipid emulsion-induced increase in intestinal permeability of lipopolysaccharide by modulation of P-glycoprotein in Caco-2 cells. *Biochem Biophys Res Commun* 482: 791-795, 2017.
- Yan J, Jin G, Du L and Yang Q: Modulation of intestinal folate absorption by erythropoietin in vitro. *Mol Pharm* 11: 358-366, 2014.
- Wang Z, Zhang L, Ni Z, Sun J, Gao H, Cheng Z, Xu J and Yin P: Resveratrol induces AMPK-dependent MDR1 inhibition in colorectal cancer HCT116/L-OHP cells by preventing activation of NF- κ B signaling and suppressing cAMP-responsive element transcriptional activity. *Tumour Biol* 36: 9499-9510, 2015.
- Kim HG, Hien TT, Han EH, Hwang YP, Choi JH, Kang KW, Kwon KI, Kim BH, Kim SK, Song GY, *et al*: Metformin inhibits P-glycoprotein expression via the NF- κ B pathway and CRE transcriptional activity through AMPK activation. *Br J Pharmacol* 162: 1096-1108, 2011.
- Kharasch ED, Hill HF and Eddy AC: Influence of dexmedetomidine and clonidine on human liver microsomal alfentanil metabolism. *Anesthesiology* 75: 520-524, 1991.
- Nakazato K, Yoshida Y, Takemori K, Kobayashi K and Sakamoto A: Expressions of genes encoding drug-metabolizing enzymes are altered after sevoflurane, isoflurane, propofol or dexmedetomidine anesthesia. *Biomed Res* 30: 17-24, 2009.
- Lu Y, Shen T, Yang H and Gu W: Ruthenium complexes induce HepG2 human hepatocellular carcinoma cell apoptosis and inhibit cell migration and invasion through regulation of the Nrf2 pathway. *Int J Mol Sci* 17: pii: E775, 2016.
- Xiong J, Wang K, He J, Zhang G, Zhang D and Chen F: TFE3 alleviates hepatic steatosis through autophagy-induced lipophagy and PGC1 α -mediated fatty acid β -Oxidation. *Int J Mol Sci* 17: 387, 2016.
- Zhao B, Xie GJ, Li RF, Chen Q and Zhang XQ: Dexamethasone protects normal human liver cells from apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand by upregulating the expression of P-glycoproteins. *Mol Med Rep* 12: 8093-8100, 2015.
- Liu S, Cheng Y, Rao M, Tang M and Dong Z: Muscone induces CYP1A2 and CYP3A4 enzyme expression in L02 human liver cells and CYP1A2 and CYP3A11 enzyme expression in kunming mice. *Pharmacology* 99: 205-215, 2017.
- Liao Z, Cao D, Han X, Liu C, Peng J, Zuo Z, Wang F and Li Y: Both JNK and P38 MAPK pathways participate in the protection by dexmedetomidine against isoflurane-induced neuroapoptosis in the hippocampus of neonatal rats. *Brain Res Bull* 107: 69-78, 2014.
- Wang H, Jia XH, Chen JR, Yi YJ, Wang JY, Li YJ and Xie SY: HOXB4 knockdown reverses multidrug resistance of human myelogenous leukemia K562/ADM cells by downregulating P-gp, MRP1 and BCRP expression via PI3K/Akt signaling pathway. *Int J Oncol* 49: 2529-2537, 2016.
- Yan JK, Zhu J, Gu BL, Yan WH, Xiao YT, Zhou KJ, Wen J, Wang Y and Cai W: Soybean oil-based lipid emulsion increases intestinal permeability of lipopolysaccharide in Caco-2 cells by downregulation of P-Glycoprotein via ERK-FOXO 3a pathway. *Cell Physiol Biochem* 39: 1581-1594, 2016.
- Chi X, Wei X, Gao W, Guan J, Yu X, Wang Y, Li X and Cai J: Dexmedetomidine ameliorates acute lung injury following orthotopic autologous liver transplantation in rats probably by inhibiting Toll-like receptor 4-nuclear factor kappa B signaling. *J Transl Med* 13: 190, 2015.
- Requenez-Contreras JL, López-Castillejos ES, Hernández-Flores R, Moreno-Eutimio MA, Granados-Riveron JT, Martínez-Ruiz GU and Aquino-Jarquín G: MiR-138 indirectly regulates the MDR1 promoter by NF- κ B/p65 silencing. *Biochem Biophys Res Commun* 484: 648-655, 2017.
- Chen Q, Bian Y and Zeng S: Involvement of AP-1 and NF- κ B in the up-regulation of P-gp in vinblastine resistant Caco-2 cells. *Drug Metab Pharmacokinet* 29: 223-226, 2014.



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