

EFFECT OF SEVOFLURANE EXPOSURE ON ADRIAMYCIN-INDUCED INJURIES TO MYOCYTESLi-hua Fan¹, Xin Han¹, Qing-quan Lian², Jun Li², Hou-xing Lei¹, Nling Zhang¹, Xiang-dong Xu¹, Xiang-hong Lu¹

The study is to investigate the potential protective effect of sevoflurane on adriamycin induced myocyte injury. We prepared primary myocyte culture from neonatal rats and subjected the cells to adriamycin treatment. We found that adriamycin treatment induced cell apoptosis, decreased cell vitality, which could be reversed with sevoflurane exposure after the drug treatment, potentially through the regulation of Bcl-2 anti-apoptotic pathway. The study suggested the potential roles of sevoflurane in clinical management of patients after adriamycin chemotherapy.

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ЭФФЕКТ ВОЗДЕЙСТВИЯ СЕВОФЛЮРАНА НА АДРИАМИЦИН-ИНДУЦИРОВАННЫЕ ПОВРЕЖДЕНИЯ МИОЦИТОВLi-hua Fan¹, Xin Han¹, Qing-quan Lian², Jun Li², Hou-xing Lei¹, Nling Zhang¹, Xiang-dong Xu¹, Xiang-hong Lu¹

Целью исследования является изучение потенциального защитного эффекта севофлюрана на адриамицин- индуцированные повреждения миоцитов. Мы подготовили первичную культуру миоцитов новорожденных крыс и подвергли клетки воздействию адриамицина. Мы обнаружили, что воздействие адриамицина индуцирует апоптоз клеток, снижает жизнеспособность клеток, что может быть обратимо воздействием севофлюрана после медикаментозного лечения, возможно, через регулирование Bcl-2 антиапоптозных путей.

В исследовании предлагается потенциальная роль севофлюрана в области клинического ведения пациентов после химиотерапии адриамицином.

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Ключевые слова: анестетики, севофлюран, миоцит, химиотерапия, адриамицин.

Introduction

The previous exposure to chemotherapy drugs can increase the cell death after anesthesia and surgical procedures [1]. For instance, the adriamycin (Doxorubicin) that is widely used to leukemia and lymph tumors can induce injury to the heart even after the end of drug administration [2–4]. Therefore the searching of safety anesthesia agent is important in clinical management of patients following adriamycin chemotherapy.

Sevoflurane is one of the commonly used anesthetics, and has been found to inhibit the cellular injury caused by ischemia-reperfusion in the rat heart [5, 6]. The potential mechanism involves the upregulation of Bcl-2, for example. In present study we investigated if sevoflurane can also be protective for adriamycin induced cellular injury with cultured myocytes from neonatal rats. This would provide guidelines for clinical safety in management of patients underwent chemotherapy with adriamycin.

Materials and methods

Animal and cell culture. SPF level SD rats with pregnancy 16–18 days were purchased from Lasike animal company (SCXK-Hu-2007–0005), and maintained in the SPF animal facility of key lab of anesthesia, Wenzhou Medical College, the second affiliated hospital until delivery.

For primary myocyte culture [7], 10 SD rats at P0 were sacrificed and the heart was harvested. The tissue was dissected in 4 degree DMEM (Gibco, US) and

rinsed in 4 degree D-hank solution (Suolaibao, Beijing, China). Then the cells were dissociated with 6 mL 0.125% trypsin (Gibco, US) for 3 minutes at 37 degree before the supernatant was collected. The digestion was stopped with 10% fetal bovine serum (FBS) -DMEM medium. The processes were repeated for 3–4 times and finally all supernatant was filtered to remove all remained tissues. The dissociated myocytes were seeded at 1X10⁵ / mL in 25 mL bottle in 5% CO₂ incubator (Thermo, US) for 48 hours. Then the cells were treated with 0.1% trypsin and reseeded at 5 X 10⁴ /mL in 96 well plate (200 µL/well) or 6 well plate (2 mL/well with 24 mm X 24 mm cover slip inside) with 10% FBS-DMEM medium for 24 hours before experiment.

Treatments. The cells were randomly assigned into four groups (n=15 each): Control (C) group: no treatment; Adriamycin (D) group: 1 µM/L adriamycin (Meilun, Dalian, China) in presence for 24 hours; Sevoflurane (S) group: no treatment before Sevoflurane exposure; Adriamycin+ Sevoflurane group (DS) group: 1 µM/L adriamycin in presence for 24 hours before Sevoflurane exposure.

For Sevoflurane exposure, 95% air-5% CO₂ mixture was passed through Vapor 2000 sevoflurane flowmeter (Draeger, Germany) at 2 L/min, and the concentration of sevoflurane was monitored by Vamos monitor for anesthetics (Draeger, Germany) to be stable at 2.4%. The cells were treated for 2 hours before additional 2 hours incubation.

Cell vitality. For Trypan Blue staining, 2 μ L of 4% Trypan Blue solution was mixed with 18 μ L cell suspension for 1 minute. Then the cells were counted within 3 minutes on the counting slide.

For MTT method, the cultured cells in 96 well plate were treated with 20 μ L 5 mg/mL MTT solution and 200 μ L DMEM for 4 hours. Then 150 μ L DMSO was added for 10 minutes with shaking. Finally the plate was read with Multiskan MK3 at 490 nm for optic density (repeated for 3 times each).

Immunocytochemistry. The cells were fixed with 4% PFA at room temperature for 30 minutes before wash with PBS and 0.5% triton treatments. Then the primary antibody (mouse- α -SA, 1:200; mouse-Bax, 1:100; mouse-Bcl-2, 1:100) was incubated together with the cell overnight at 4 degree before PBS wash. Finally the secondary antibody was added for 2 hours at room temperature before visualization and image acquisition.

ELISA for cTnI and NT-proBNP measurement. The concentrations of cTnI and NT-proBNP were measured as described in the kit. Briefly, the sample was mixed with antibody, HRP-Streptavidin for incubation for 1 hour before visualized with solution A and B for 10 minutes in the dark. Finally the reaction was stopped with stop solution. The optical density was read at 450 nm and was calculated for sample concentration based on the standard curve from the standard samples.

Western blot for NF- κ B. The total proteins were extracted and 30 μ g protein sample was put on SDS-PAGE electrophoresis before PVDF-membrane transfer. Then the membrane was blocked with 5% milk powder solution for 2 hours then incubated with primary antibody at 1:1000 for 2 hours at 37 degree before secondary antibody incubation for 1 h at 37 degree. Finally the ECL kit was used for visualization and the band was scanned with TYTOOPH system for analyses. The β -actin band was used as internal control.

Flow cytometry for cell apoptosis examination (AnnexinV/PI double staining). The cells after treatment were treated with AnnexinV antibody-FITC and propidium iodide (PI) to analyze the pure apoptotic cell population.

Statistics. The data were analyzed with SPSS 17.0 software (Chicago, US). The data in normality distribution was represented with mean \pm SD and intergroup comparisons were performed with One-way ANOVA. $P < 0.05$ was considered as statistically significant.

Results

Cell vitality; the cTnI and NT-proBNP concentration

We found that the average survival rate of primary myocyte culture was 95%. With MTT method, the MTT values in D and DS group decreased in compared to the C group, suggesting for the toxicity of adriamycin treatment. The sevoflurane did not decrease the cell vitality since the S group showed no changes.

In addition, in compared to C group, we found that the cTnI and NT-proBNP concentration increased in D group but not the S and DS group. Interestingly, DS group showed lower cTnI and NT-proBNP concentration in compared to D group.

The cell apoptosis rate and protein expression levels

We found that in C group the cell apoptosis rate was low, which increased significantly in D group. The adriamycin treatment induced cell apoptosis was reduced by sevoflurane exposure (DS group).

Interestingly, these changes in cell apoptosis accompanied series changes of protein expression. The increase in Bax/Bcl-2 ratio in D group was eliminated by sevoflurane exposure (DS group). In addition, this accompanied with the increase in NF- κ B protein expression.

Discussion

The present study set up a model of primary myocyte culture from neonatal rat with improvement from previous studies (95% survival rate and 83% purity). We found that as previously described [7], low concentration adriamycin treatment could trigger cell apoptosis after 24 hours incubation. In addition, we showed that the sevoflurane exposure, which is a common anesthetic in clinical management, could reduce the toxicity of adriamycin treatment. This validated the use of sevoflurane during anesthesia of patients underwent adriamycin chemotherapy.

Table 1

The cell vitality; the cTnI and NT-proBNP concentration in the medium (n=5)

Group	MTT value	cTnI (ng/ml)	NT-proBNP (ng/L)
C	0.62 \pm 0.28	7.9 \pm 2.7	164 \pm 20
D	0.39 \pm 0.14 ^a	11.4 \pm 1.6 ^a	228 \pm 12 ^a
S	0.62 \pm 0.34	7.2 \pm 1.3	168 \pm 33
DS	0.41 \pm 0.16 ^{ab}	7.8 \pm 0.5 ^b	180 \pm 34 ^b

^a $P < 0.05$ in compared to C group; ^b $P < 0.05$ in compared to D group.

Table 2

The cell apoptosis rate and protein expression levels (n=5)

Group	Cell apoptosis (%)	NF- κ B	Bax	Bcl-2	Bax/Bcl-2
C	1.9 \pm 0.8	0.75 \pm 0.04	2.3 \pm 0.7	1.6 \pm 0.4	1.3 \pm 0.4
D	37.8 \pm 4.5 ^a	1.21 \pm 0.13 ^a	2.8 \pm 1.2 ^a	1.3 \pm 0.3 ^a	2.2 \pm 0.4 ^a
S	1.6 \pm 0.4 ^b	0.74 \pm 0.05 ^b	2.4 \pm 0.8 ^b	1.6 \pm 0.6	1.5 \pm 0.3
DS	10.4 \pm 1.1 ^{ab}	0.91 \pm 0.06 ^{ab}	2.5 \pm 0.7 ^b	1.7 \pm 0.7 ^b	1.7 \pm 0.3 ^{ab}

^a $P < 0.05$ in compared to C group; ^b $P < 0.05$ in compared to D group.

cTnl is a sensitive and specific biomarker for heart injury in the early phase [8, 9]. NT-proBNP is an important biomarker for heart failure [10] and is ready to be detected in the culture medium. The present study utilized ELISA method to detect the changes of the two molecules and showed that sevoflurane exposure could actually prevent the further damage after adriamycin treatment.

We further explored the potential molecular signaling pathways involved in the protective effects of sevo-

flurane exposure. As expected, the Bcl-2 pathway is upregulated following adriamycin treatment, which was prevented by sevoflurane exposure, suggesting the potential target of sevoflurane [11]. In addition, the NF- κ B protein expression is associated with the changes described.

In conclusion, we believe that sevoflurane might be a safe anesthetic for patients underwent adriamycin chemotherapy.

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