

Supernatants from *Staphylococcus Aureus* Grown in the Presence of β -Lactam Antibiotics Induce Release of Nitric Oxide from Mouse Macrophages

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Abstract

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Introduction: Septic shock is now recognized to be associated with multiple critical organ failure, usually attributable to the uncontrolled release of multiple pro inflammatory cytokines such as $TNF\alpha$, $INF\gamma$, IL-6, IL-1, and NO (nitric oxide). NO is one of the most important pro inflammatory mediators in sepsis and septic shock. It is a potent vasodilator, and appears to be responsible for hypotension in sepsis patients. On the other hands, antibiotic action on the microbes in the host can result in release of bacterial components that will trigger a host pro inflammatory response. In the present study we examined the effects of a number of β -lactam antibiotics (cloxacillin, ampicillin or ceftazidim) on the release of bacterial products from *Staphylococcus aureus* and their effect on NO production by mouse macrophages.

Material and Methods: MICs and MBCs of antibiotics for *S. aureus* were determined by standard macrodilution method. *S. aureus* was incubated in absence (control) or presence of MBC concentrations of three β -lactam antibiotics. Supernatants of the cultures obtained by filtration were added to plastic adherent peritoneal murine macrophages. After 24 hrs incubation, macrophage supernatants were tested for the presence of nitric oxide by Griess reagents.

Results: Supernatants from bacteria incubated with β -lactam antibiotics induced significantly higher nitric oxide levels than those obtained from bacteria incubated with culture medium only (no antibiotics). The effects of the three β -lactam antibiotics to generate nitric oxide release from *S. aureus* were similar.

Conclusions: The results indicate that release of bacterial components during β -lactam antibiotic treatment of *S. aureus*, can play an important role in proinflammatory response.

Keywords: *S. aureus*, β -lactam antibiotics, nitric oxide, septic shock, macrophage



Effect of Rolipram, a Type 4-Specific Phosphodiesterase Inhibitor, on Unit Activity of Paragigantocellularis Neurons and Withdrawal Signs in Morphine Dependent Rats

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Abstract

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Introduction: Effect of rolipram, as a type 4-specific phosphodiesterase inhibitor on spontaneous activity of paragigantocellularis nucleus (PGi) and precipitation of withdrawal signs in morphine dependent rats was studied.

Material and Methods: Extracellular single unit recording was used to record the spontaneous activity of PGi neurons in urethane-anesthetized NMRI male rats (250-350g). Rolipram (0.1, 1 and 10 μ M) was microinjected into PGi. To assess the behavioral signs, frequency analysis was used.

Results: The results showed that rolipram microinjection (0.1 and 1 μ M) in control rats had no significant effect on the spontaneous activity of the PGi neurons. Rolipram microinjection (10 μ M) in control rats increased and in morphine dependent ones decreased neuronal activity significantly. Intranuclear microinjection of rolipram (10 μ M) produced chewing and teeth chattering in control rats while induced ejaculation and writhing in dependent ones. Restlessness, forepaw tremor, jumping and ptosis as withdrawal signs didn't appear and signs such as teeth chattering and writhing decreased by applying 10 μ M of rolipram into the PGi nucleus before subcutaneous injection of naloxone.

Conclusion: It is concluded that adaptive changes in activity of cAMP pathway in PGi neurons following chronic morphine exposure may play an important role in the development of dependence on morphine.

Keywords: Paragigantocellularis nucleus, cAMP, Rolipram, Single unit recording, Morphine



Effect of Aspirin on Morphology of CA1 Hippocampal Neurons Following Ischemia Induction in Male Rat

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Introduction: The present study evaluated morphological changes of CA1 hippocampal neurons following transient bilateral common carotid and permanent left middle cerebral arteries occlusion, also the neuroprotective effects of aspirin i.p injection was inducing assessed after ischemia in male rat.

Material and Methods: In this experiment, 4 groups of animals were used including; control, sham, ischemia and aspirin. In ischemia group, the skull was drilled and left middle cerebral artery was cauterized. Then, common carotid arteries were isolated from vagus nerve and jugular vein, and were clogged for 90 min. In aspirin group, rats received 30mg/kg aspirin (i.p) 30min after ischemia induction. 48 hr after surgery, the brain was removed, and hippocampus was separated and fixed. All samples were stained with hematoxylin and eosin, and morphology of CA1 was assessed in all groups.

Results: Following ischemia, pyramidal neurons of CA1 hippocampus developed necrosis and became degenerated. These neurons had dense cytoplasm and pyknotic nuclei, and cytoplasmic eosinophilia had increased. Aspirin injection, 30 min after ischemia induction, improved the state of neurons, which means decreased the pyknotic nuclei.

Conclusion: These result suggested that, this model of ischemia has a severe effect on pyramidal neurons of CA1, and single dose of Aspirin injection decreases the effect of ischemia and neuronal injuries.

Keywords: Cerebral Ischemia, Common carotid artery, Middle cerebral artery, Aspirin, CA1 hippocampus



The Effect of Granulocyte-Macrophage Colony Stimulating Factor on Development and Quality of Murine Two-Cell Embryos

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Introduction: Murine preimplantation embryos expressed Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) receptor and it is known as a growth factor. In this study capacity of two cell mouse embryos were studied in the presence and absence of GM-CSF.

Material and Methods: Female NMRI mice were superovulated using routine technique. 299 2-cell embryos were cultured in one group as the control (T6 medium supplemented with 5mg/ml bovine serum albumin) and in the other group as experimental (T6 medium supplemented with 5mg/ml bovine serum albumin and 2 ng/ml GM-CSF). Developmental rate of embryos was assessed daily. The blastocysts were stained and their total cell number were counted. The diameter of blastocysts were measured using calibrated eye piece.

Results: The cleavage rate of embryos in the presence of GM-CSF was increased during 48 and 72 hours after culturing. The blastocyst formation rate in the control and GM-CSF groups were 65.78 % and 74.82% and the diameter of blastocysts in these groups were 125.40 and 128.02 micrometer, respectively. Also total cell number of blastocysts in previous groups were 74.20 and 80. There was statistically significant differences between these groups in regard to blastocyst diameter and total cell number.

Conclusion: It seems the GM-CSF is a suitable embryotropic factor.

Keywords: GM-CSF, Two cell embryos, Blastocyst



Evaluating FK506 (Tacrolimus) Effects on Cord Blood Hematopoietic Progenitor Cells

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Introduction: Umbilical cord blood is a rich source of hematopoietic cells and can be used in the clinical setting for hematopoietic cell transplantation in graft versus host disease (GVHD). A number of different drugs have been used as prophylaxis against GVHD including corticosteroids, Cyclosporine A (Cy A) and FK506. In this study, the in vitro effects of FK506 on cord blood mononuclear cells were assessed.

Material and Methods: After cord blood collection and separation of mononuclear cells, the viability of them was assessed. Then, following 7-day liquid culture in the DMEM medium and in the presence of early acting growth factors such as SCF, FL, TPO and IL-6 and different doses of FK506, cells were counted and myeloid/erythroid ratios were determined. Furthermore, the percentage of CD34+ cells was assessed by flow cytometry. In this study, clonogenic assay was carried out in two stages (before and after 7- day liquid culture).

Results: Under the mentioned culture conditions, cell counts and M/E ratios did not change in comparison with control ($P=0.126$ and $P=0.819$, respectively). The percentage of CD34+ cells rose considerably ($P=0.018$). Compared with control, colony numbers decreased in the first stage ($P=0.000$), whereas the number of colonies did not change in the second ($P=0.109$).

Conclusion: Our results demonstrate that FK506 is not only an immunosuppressive agent but also has stimulating effects on expansion and clonogenicity of cord blood mononuclear cells. But it has no effect on differentiation of these cells.

Keywords: FK506, Umbilical Cord Blood, Clonogenic Assay, Expansion



Effect of Polarized Human Uterine Epithelial Cells on Mouse Embryo Development and Blastocyst Cellularity

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Introduction: The aim of this study was to investigate the growth and development of mouse two cell embryo on human uterine polarized monolayers.

Material and Methods: Human endometrial tissue was obtained from patients who had undergone total hysterectomy. Then, epithelial cells were isolated by collagenase I 0.25% and cultured on either ECM Gel coated Millipore filter Insert (polarized) or plastic surface(non-polarized). Epithelial Nature of cells cultured on plastic confirmed using immunohistochemistry and the polarized state of cells cultured on ECM Gel evaluated byTEM transmission electron microscopy. Two cell embryo of superovulated NMRI mouse were then flushed and cultured either on polarized or non polarized cells and medium alone. The rate of development in all groups were daily determined and statistically compared. At the end of cultivation period, trophoectoderm and ICM part of expanded blastocyst from each group were differentially stained by propidium iodide and hochst and the mean number of blastocyst were counted and statistically compared.

Results: Our results showed that the cells cultured on ECM Gel had highly polarized columnar shape compared to flattened shape of cells cultured on plastic surface. The two cell embryo cultured on polarized monolayer had higher developmental rate than those from non-polarized cells, although there was no statistic difference, but the blastocyst from polarized monolayer had significantly more mean cell number compared to non-polarized group.

Conclusion: Taken Together, we concluded that polarized cells could improve the embryo development from two cell stage in the term of quality (increasing blastocyst cellularity)rather than developmental rate.

Keywords: Polarized culture, uterine epithelium, mouse embryo development



Differentiation of P19 Embryonic Carcinoma Cells to Erythroid and Non Erythroid Cells in the Presence of Interleukin 3, 6 and Erythropoietin

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Introduction: Setting up a system to study the in vitro hematopoiesis is helpful to understand the mechanism of hematopoiesis. The effects of several cytokines and hormones such as erythropoietin (EPO) in differentiation of hematopoietic stem cells have been investigated in vivo. There are no records considering the potential differentiation of P19 cell line (embryonic carcinoma cells) to hematopoietic cells. The aim of this study was to use this cell line as a model to evaluate the effects of interleukin 3, 6 and erythropoietin on the differentiation of stem cells.

Material and Methods: P19 cells were cultured directly on semisolid medium supplemented with 10% fetal calf serum (FCS). The embryoid bodies were formed 9 days after culturing. The cells were trypsinized and dissociated to single cells. These cells were cultured on the semisolid medium containing 10% FCS, 10 ng/ml IL-3 and IL-6 and in the presence and absence of 20 u/ml erythropoietin. The colonies were formed after 14 days. Then the colonies were studied by benzidine and giemsa staining.

Results: Our results showed that in the presence of IL-3, IL-6 and EPO the positive and negative benzidine colonies were 54% and 46%, respectively. In the absence of EPO the mentioned colonies were 14% and 86%, respectively. There was a statistically significant difference between the positive and negative colonies in both groups ($p < 0.001$).

Conclusion: Under the effects of IL-3, IL-6 and EPO, the P19 cells could differentiate to erythroid and non-erythroid lineage and in the presences of EPO the umber of erythroid colonies increased.

Keywords: Embryonic carcinoma cells, Interleukin, Erythropoietin



Effect of Cysteamine on In Vitro Maturation, Resumption of Meiosis and Embryo Development of Immature Mouse Oocytes

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Introduction: In this study *in vitro* maturation, resumption of meiosis and development of immature mouse oocytes in the presence of cysteamine was evaluated.

Material and Methods: Immature oocytes recovered from NMRI mouse strain (4-6 weeks) were categorized in three experimental and one control group. The control included 345 immature oocytes which were matured in MEM α medium contain 5% FCS. In group one 292 immature oocytes were matured MEM α medium contain 5% FCS and 100 μ m cysteamine. In the second group 237 immature oocytes were matured in MEM α medium contain 5% FCS, 7.5IUhcG, 100mIUrFSH and in the third group 264 immature oocytes were matured in MEM α medium contain 5% FCS, 7.5IUhCG, 100mIUrFSH, 100 μ m cysteamine. Fertilization and embryo development were performed in T6 medium.

Results: The percentage of resumption of meiosis and IVM in control and the three experimental groups were 74.2, 99.2, 74.24, 85.6 and 59.7, 81.2, 52.74, 85.6 respectively.

Statistically significant difference was seen regarding in vitro maturation and resumption of meiosis between control and experimental group 1 and group 3 (P=0.0001).

The embryo formation in group 1 and group 3 was higher compared to the other groups (without cysteamines). The embryo formation after 24 hours in group 1 (P=0.0001) and group 3 (0.0001) increased significantly compared to the second group.

Conclusion: The result of this study showed that cysteamine (100 μ m) enhances in-vitro maturation, resumption of meiosis and has no significant effect on embryo development.

Keyword: Immature oocytes, Cysteamine, Glutathione, Mouse

