2009년 8월
박사학위논문

The effect of rocuronium through acetylcholine receptors on the contractility of non-pregnant isolated rat uterine myometrium

조선대학교 대학원

의학과

연화

The effect of rocuronium through acetylcholine receptors on the contractility of non-pregnant isolated rat uterine myometrium

백서 자궁평활근 절편에서 rocuronium이 acetylcholine receptor를 통해 자궁수축에 미치는 효과

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지도교수 송창훈

이 논문을 의학 박사학위신청 논문으로 제출함

2009년 4월

조선대학교 대학원

의 학 과

연 화

연 화의 박사학위논문을 인준함

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2009 年 6 月

조선대학교 대학원

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

The effect of rocuronium through acetylcholine receptors on the contractility of non-pregnant isolated rat uterine myometrium

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Background: Muscle relaxants are frequently used for relaxation of skeletal muscle in cesarean section. The present study aims to investigate the effects of rocuronium on uterus in rat model.

Methods: Tissue preparations were from non-pregnant rat uteri which were cut into 2 × 10 mm. Rocuronium (10^{-5} to 2 × 10^{-4} M) were added cumulatively to tissue baths filled with Kreb's solution which were pretreated with nothing, acetylcholine (10^{-5} M) or atropine (10^{-5} M).

Results: Although atropine (muscarinic receptor antagonist) had no effect on spontaneous uterine contraction it reacted to acetylcholine pretreated group. In other hand, rocuronium reduced not only spontaneous but also acetylcholine-induced uterine contraction in a

dose-dependant manner.

Conclusion: In conclusion, the relaxant effects of neuromuscular blockers on the uterine smooth muscle may be transmitted via nicotinic acetylcholine receptors in rat uterus.

Key Words: acetylcholine receptor, rat, uterine smooth muscle, rocuronium

Introduction

Neuromuscular-blocking agents block neuromuscular transmission at the neuromuscular junction, causing paralysis of the affected skeletal muscles. This is accomplished either by acting presynaptically via the inhibition of acetylcholine (ACh) synthesis or release, or by acting postsynaptically at the acetylcholine receptor. These drugs fall into two groups: depolarizing blocking agents and non-depolarizing blocking agents. Non-depolarizing blocking agents act by blocking the binding of ACh to its receptors, and in some cases, they also directly block the ionotropic activity of the ACh receptors (Bufler J,1996). Non-depolarizing neuromuscular blocking agent, rocuronium is an aminosteroidal muscle relaxant. And it is used for induction and maintenance of general anesthesia to facilitate endotracheal intubation and to provide for relaxation of skeletal muscle during surgery or mechanical ventilation. Introduced in 1994, rocuronium has rapid onset, and intermediate duration of action (Hunter JM,1995).

The relaxant mechanism of non-depolarizing neuromusclar blocking agents on the skeletal mus치e are well delineated however, besides their main actions there involvement of muscarinic receptors caused cardiac, respiratory, central nervous and gastrointestinal tract side effects (E. Sylvester Viti, 2006). Reviews discuss (cf. Bowman,

1990; Hunter, 1987, 1995) that several muscle relaxants (MRs), except vecuronium and pipecuronium, are able to produce tachycardia. And it has been shown (Torocsik et al., 1989) that MRs inhibit the M_3 type of muscarinic receptors of the longitudinal muscle of the guinea-pig ileum. Some nondepolarizing MRs possess antimuscarinic activity, and inhibit M_2 receptors located on the axon terminals of cholinergic innervation of airways. Thus, with M_2 receptor block, the release of ACh is enhanced (as a result of suspension of negative feedback modulation), subsequently producing a bronchospasmus, an effect mediated via M_2 receptors. And it has been generally accepted that in uterine smooth muscle from many species shows heterogeneous populations of muscarinic receptors, with both M_2 and M_3 receptors involved in the contractile function (Abdalla FM et al., 2000; Boxall DK et al., 1998; Choppin A et al., 1999).

The current study attempts to investigate the effects of rocuronium on rat uterine smooth muscle, moreover to study the possible underlying mechanisms because there is no relevant studies on the effects of nondepolarizing neuromuscular blocking agent on the uterine tissue.

MATERIALS AND METHODS

The protocol for this study was approved by the animal care and use committee at Chosun University School of Medicine.

1. Tissue preparation

Sprague-Dawley rats weighing 200-250 g were used as experimental animals. It was anesthetized via inhalation of carbon dioxide and the uterus was excised. The mywastrial tissue specimens were dissected into 2 mm wide and 10 mm long strips in a Pstri dish filled with Krebs solution with the following composition: 118.3 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.2 mM $MgCl_2$, and 11.1 mM glucose.

2. Measurements of myometrium contraction

The strips were positioned perpendicularly in organ bath, and the baths were filled with Krebs solution bubbled with 95% oxygen and 5% carbon dioxide at 37 ℃. One end of each strip was connected to the bottom of the organ bath and the other end was connected to a strain gauge transducer (FTO3 Grass Instruments Co, Mass USA). The data from the transducer were recorded on a computer via an interface (Powerlab data recoding system; AD Instruments Pty Ltd., Castle Hill, Australia). As an index of myometrium contraction, the amplitude was calculated as the average for the myometrium

contractions in a 15 minute period, while the frequency was determined by the number of myometrium contractions in a 15 minute period. The strips were allowed to equilibrate at resting tension (2 g) for 60 minute. Rocuronium from 10^{-5} to 2×10^{-4} M were added at cumulative manner in 15 intervals. ACh (10^{-5} M) and atropine (10^{-5} M) were added and equilibrated for 30 minutes. During the last 15 min of equilibration, the baseline contraction data were measured and recorded. The parameters of the contraction before application of each drug were used as controls. The inhibitory effects were compared with the control, and were described as % inhibition. EC_5 (effective concentration of 5% reduction), EC_{25}, EC_{50}, EC_{75}, and EC_{95} on active tension and frequency of contraction were calculated using a probit model.

3. Statistical analysis

The results are expressed as the mean ± the standard deviation (SD). The values of EC_{50} between pretreatments were compared using two-tailed student's t-test. The probability values were adjusted with Bonferroni correction. P-value < 0.05 were considered statistically significant.

RESULTS

1. Inhibitory effects of rocuronium on the contraction of raw myometrium, myometrium pretreated with acetylcholine and myometrium pretreated with atropine

Rocuronium (10^{-5} to 2×10^{-4} M) decreased both active tension and frequency of spontaneous uterine contraction in a concentration-dependent manner ($P < 0.05$, Figure. 1).

Moreover, rocuronium inhibited ACh (10^{-5} M) and atropine (10^{-5} M) pretreated muscle at concentration of 10^{-5} to 2×10^{-4} M and 10^{-6} to 2×10^{-5} M, respectively .

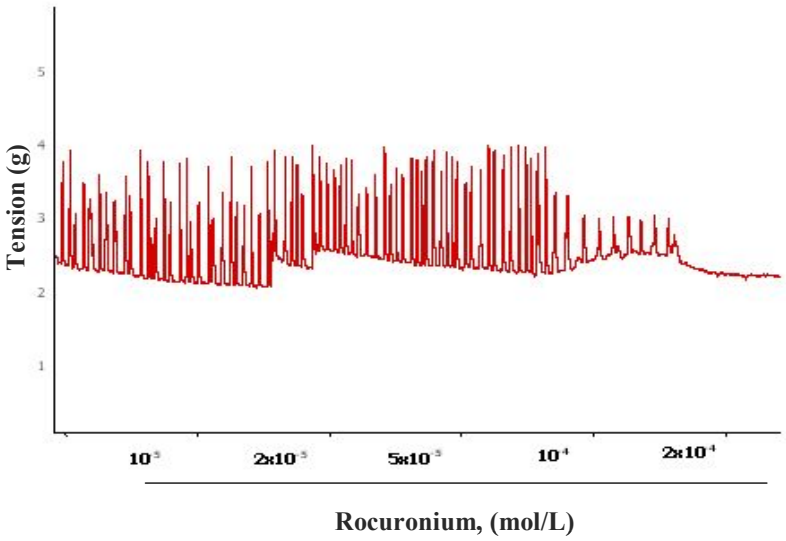

Fig. 1. Respresentative trace shows the effects of rocuronium on spontaneous isolated non-pregnant rat myometrium. The concentrations of rocuronium are cumulative concentrations (10^{-5} to $2x10^{-4}$ M) by 15-minute intervals.

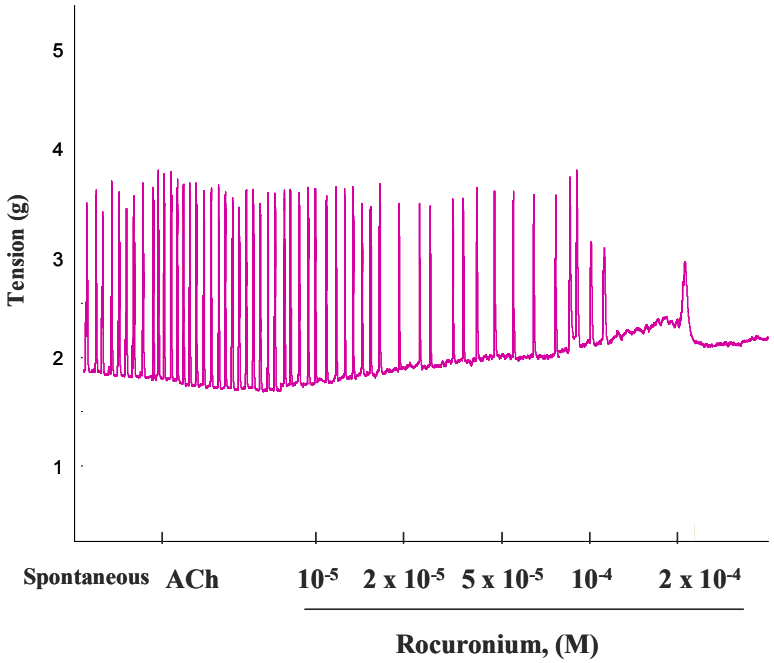

Fig. 2 Respresentative trace shows the effects of rocuronium on isolated non-pregnant rat myometrium pretreated with acetylcholine. The concentrations of rocuronium are cumulative concentrations (10^{-5} to $2x10^{-4}$ M) by 15-minute intervals.

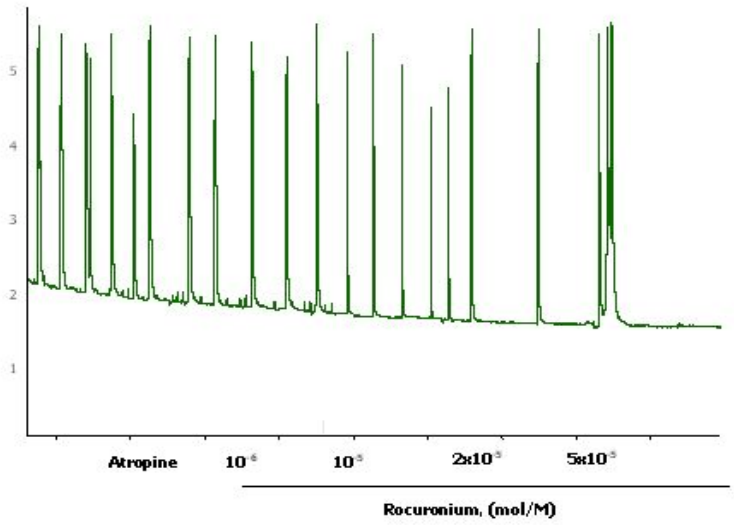

Fig. 3 Respresentative trace shows the effects of rocuronium on isolated non-pregnant rat myometrium pretreated with atropine. The concentrations of rocuronium are cumulative concentrations (10^{-5} to $2x10^{-4}$ M) by 15-minute intervals.

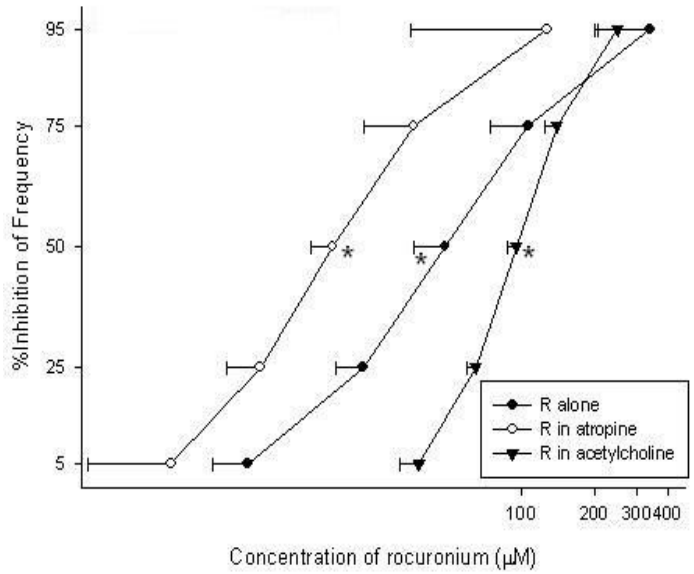

Fig. 4. Rocuronium has different inhibitory effects on the frequencies of contractions of raw uterine muscle (EC_{50}=4.80 X 10^{-5}), those of uterine muscle pretreated with atropine(10^{-5} M)(EC_{50}=1.65 X 10^{-5}), and those of uterine muscle pretreated with acetylcholine(10^{-5} M) (EC_{50}=9.44 X 10^{-5}). The frequencies of contractions of uterine muscles pretreated with atropine are most susceptible to rocuronium. The values are mean and SD of rocuronium concentrations. EC_{50} represents 50% effective concentration of maximal effect. *: $p < 0.001$, compared with each other

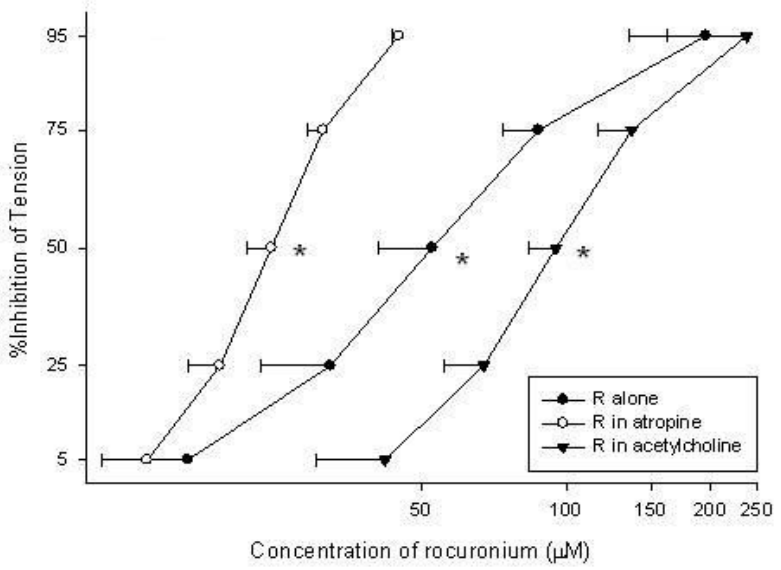

Fig. 5. Rocuronium has different relaxant effects on active tensions of raw uterine muscle (EC_{50}=5.26 X 10^{-5}), those of uterine muscle pretreated with atropine(10^{-5} M) (EC_{50}=2.43 X 10^{-5}), and those of uterine muscle pretreated with acetylcholine(10^{-5} M) (EC_{50}=9.51 X 10^{-5}). The tensions of uterine muscles pretreated with atropine are most susceptible to rocuronium. The values are mean and SD of rocuronium concentrations. EC_{50} represents 50% effective concentration of maximal effect. *: $p < 0.001$, compared with each other

2. Effective concentration (M) of rocuronium on uterine smooth muscle

The EC_{50} of rocuronium, ACh pretreated and atropine pretreated group on active tension in the uterine smooth muscle were 5.26 (1.19) × 10^{-5} M, 9.51 (1.13) × 10^{-5} M, 2.43 (0.26) × 10^{-5} M, respectively (Table 1). And the EC_{50} of rocuronium, ACh pretreated and atropine pretreat group on frequency of contraction were 4.80 (1.21) × 10^{-5} M, 9.44 (0.74) × 10^{-5} M, 1.65 (0.32) × 10^{-5} M, respectively. (Table 2).

Table 1. Effective Concentrations (M) of Rocuronium on Active Tension (g) in the uterine smooth muscle

Drug	EC_5	EC_{25}	EC_{50}	EC_{75}	EC_{95}
Rocuronium	1.63(0.55) × 10^{-5}	3.23(0.90) ×10^{-5}	5.26 1.19) × 10^{-5}	8.75 (1.38) × 10^{-5}	1.95 (0.59) × 10^{-4}
Rocuronium+ ACh	4.19 (1.18) × 10^{-5}	6.73 (1.15) × 10^{-5}	9.51 (1.13) × 10^{-5}	1.37 (2.05) × 10^{-5}	2.37 (0.75) × 10^{-4}
Rocuronium+ Atropine	1.34 (0.27) × 10^{-5}	1.90 (0.27) × 10^{-5}	2.43 (0.26) × 10^{-5}	3.12(0.22) × 10^{-5}	4.48 (0.15) × 10^{-5}

Data are expressed mean ± SD. from 15 independent experiments. EC: effective concentration

Table 2. Effective Concentrations (M) of Rocuronium on Frequency (f for 15 min) of contraction in the uterine smooth muscle

Drug	EC_5	EC_{25}	EC_{50}	EC_{75}	EC_{95}
Rocuronium	7.35 (0.21) × 10^{-6}	2.20 (0.51) × 10^{-5}	4.80 (1.21) × 10^{-5}	1.06 (3.24) × 10^{-4}	3.37 (1.32) × 10^{-4}
Rocuronium+ ACh	3.74 (0.64) × 10^{-5}	6.43 (0.53) × 10^{-5}	9.44 (0.74) × 10^{-5}	1.40 (1.57) × 10^{-5}	2.47 (0.48) × 10^{-4}
Rocuronium+ Atropine	3.5 (0.19) × 10^{-6}	8.3 (0.28)×10^{-6}	1.65 (0.32) × 10^{-5}	3.57 (0.13) × 10^{-5}	1.26 (0.91) × 10^{-4}

Data are expressed mean ± SD from 15 independent experiments. EC: effective concentration

Discussion

Neuromuscular blocking agents are frequently used as muscle relaxant for obstetric anesthesia, but there are few studies evaluating the effects of them on isolated uterine muscle.

Rocuronium has aminosteroidal structures that contain acetylcholine-like moieties. Thus, this compounds are structrually related to ACh. Rocuronium can block neural transmission by competitive inhibition of ACh, binding at nicotinic and muscarinic receptors (Miller, 1994). As it has been well established that spontaneous rhythmic contractions of rat uterus are primarily dependent on the influx of extracelluar calcium through L-type channals (Wray et al., 2001). They are abolished when the extracellular medium is made of Ca^{2+}-free or L-type calcium channel blocking agent. Although, pacemaker cells in the uterus are not characterized as yet, spontaneous contractions originate from the depolarization of uterine cell membrane and a consequential opening of voltage-dependent L-type calcium channels (Wray,1993; Shmigol et al., 1998).

In the present study I investigated if the parasympathetic nervous system is responsible for relaxant effects of rocuronium by inhibiting the effect of ACh on rat uterus. Rocuronium from concentration 10^{-5} to 2×10^{-4} M reduced the activity of spontaneous

uterine contraction by a dose dependant manner, to the point of completely arresting uterine contraction (Fig. 1). But ACh antagonist, atropine (10^{-5} to 10^{-2} M) alone has minor effects on frequency and amplitude of spontaneous uterine contraction (data not shown).

On the other hand, when the muscles are pretreated with atropine (10^{-5} M) rocuronium inhibited active tension and frequency of uterine contraction at concentration 10^{-6} to 2×10^{-5} M which is 10 times les 10 tn when rocuronium treated alone in spontaneous contraction (Fig. 3). In other experiments where muscles are pretreated with ACh (10^{-5} M), rocuronium (10^{-6} to 2×10^{-5} M) also has similar inhibitory effects with active tensions and frequencies (Fig. 2).

In a word, those effects are best explained as being due to nicotinic receptor blockade and to some extent antimuscarinic actions on rat uterine smooth muscle. But relaxant effects of rocuronium are only showed minor differences in spontaneous and ACh pretreated muscle groups so in future study it need to check in low concentrations.

When refer to the EC_{50} of rocuronium with tension of uterine contraction are 5.26×10^{-5}, 9.51×10^{-5}, $2.43 \times 10\text{-}^{5}$ M and with frequency of contraction are 4.80×10^{-5}, 9.44×10^{-5}, 1.65×10^{-5}M in spontaneous, those of uterine muscle pretreated with atropine(10^{-5} M), and those of uterine muscle pretreated with acetylcholine(10^{-5}

M), respectively. They were greater than the effective free fractions observed in patients undergoing neuromuscular blockade. After intravenous adminstration, plasma concentration of rocuronium vary between 0.6 and 2.6 × 10^{-6} M (Servin et al., 1993). Protein binding fraction for rocuronium has been reported to be 25% (Weirda and Proost, 1995). Therefore, active serum concentration in patients under neuromuscular blockade shoulde be expected to range from 0.45 to 1.95 × 10^{-6} M for rocuronium, and be considerably less than those that produce effects in my study. At the same time, it is well established that in rat, compared to human, higher doses for neuromuscular blocking action are required (Marshall et al., 1994).

In conclusion, the present demonstrates rocuronium inhibit frequency and active tension of spontaneous, ACh pretreated and atropine pretreated uterine contraction. These data suggest that the inhibitory effects of rocuronium may be mediated by binding with ACh receptors to make uterine relaxation in rat uterine model. But it still remains to find the specific ACh receptors in rat uterus and investigates the blocking mechanisim in molecular level. And because expression of ACh receptors are heterogeneous we can confirm relaxant effects of rocuronium and the ACh receptor types in different species of uteri. We must pay attention to muscarinic effects of rocuronium in different tissues besides its main actions on skeletal muscles.

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국문초록

백서 자궁 평활 근 절편에서 자궁수축에 대한 rocuronium의 acetylcholine receptor를 통한 효과

근이완제는 산부인과 수술마취에서 자주 사용된다. 이러한 근이완제는 작용기전이 각각 다른 탈분극성 근이완제와 비탈분극성 근이완제로 분류한다. 최근에 산과마취에 많이 사용되는 비탈분극성 근이완제가 자궁 평활근에 대한 작용은 정확히 밝혀지지 않았다.

본 연구에서는 비탈분극성 근이완제인 rocuronium이 자궁수축에 미치는 효과를 관찰하고 rocuronium이 자궁수축에 대한 작용기전을 연구하고자 적출된 백서 자궁 평활근절편의 수축실험 모델을 이용하여 실험을 수행하였다. 결과 10^{-5} M에서 2×10^{-4} M까지 농도의 rocuronium은 자발성 자궁수축, 아세틸콜린에 의한 자궁수축 및 아트로핀을 선투여한 자궁수축을 억제시켰다.

본 연구는 rocuronium의 자궁수축에 대한 억제농도와 무스카린 리셉터의 존재 가능성에 대한 연구로써 비탈분극성 근이완제가 골격근뿐만 아니라 평활근에도 작용함을 밝힘으로써 산부인과 영역에서 산과 환자관리에 근이완제를 향후 더 많이 응용할 수 있으리라 생각한다.

감사의 글

시간이 물 흐르듯 지나가고 3년간의 박사과정도 이제 마무리를 하게 됩니다. 3년 동안 참 많이 고민하고 갈등하고 우왕좌왕했습니다. 기다면 길고 짧다면 짧은 3년 동안 배운 것도 많겠지만 아쉬움이 많이 남고 후회도 많이 됩니다.

박사과정 동안 저에게 아낌없는 격려와 도움을 주셨던 송창훈 교수님께 감사를 드리고 많이 부족한 저의 논문을 끝까지 지도해 주시고 조언을 해주신 안태훈 교수님 정말 감사하고 미안합니다. 저의 논문을 심사해주시고 꼼꼼히 수정해주신 이수일 교수님, 이국현 교수님, 소금영 교수님께도 감사를 드립니다.

저에게 도움과 격려를 아끼지 않았던 선후배, 친구들, 그리고 사랑하는 나의 가족 모두 감사합니다.

마지막으로 JB 후원에 감사드립니다.

저작물 이용 허락서

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논문제목	한글: 백서 자궁평활근 절편에서 rocuronium이 acetylcholine receptor를 통해 자궁수축에 미치는 효과 영어 :The effect of rocuronium through acetylcholine receptors on the contractility of non-pregnant isolated rat uterine myometrium				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(o) 반대( )

2009년 6 월 18 일

저작자: 연화 (서명 또는 인)

조선대학교 총장 귀하