Inhibitory Effects of Magnolol on Distal Colon of Guinea Pig *in Vitro*

Zhao Xiang BIAN,^{*,a} Gen Shui ZHANG,^{a,b} Ka Leung WONG,^a Xu Guang HU,^{a,c} Liang LIU,^a Zhijun YANG,^a and Min LI^a

^a School of Chinese Medicine, Hong Kong Baptist University; Kowloon Tong, Hong Kong SAR; ^b Guangzhou College of Medicine; Guangzhou, 510182 P. R. China; and ^c Guangzhou College of Pharmacy; Guangzhou, 510006 P. R. China.

Received September 21, 2005; accepted December 27, 2005

The influence of plant product magnolol (0–100 μM) on the contractile activity of isolated colonic muscle strips in guinea pig and related mechanism were investigated. Magnolol did not affect the base tone of colon muscle strips, but it dose-dependently inhibited 40 mM KCl-, 1 μM carbachol (CCh)- and 10 μM serotonin (5-HT)-induced contractions at concentrations higher than 10 μM . And also, magnolol inhibited the 5-HT- or CCh-induced muscle contraction in calcium-free buffer. Furthermore, magnolol inhibited the KCl-induced contraction under the condition of procaine. In addition, inhibition rate of nifedipine plus magnolol on muscle strips was lower than that of nifedipine alone. Moreover, magnolol dose-dependently decreased the velocity of pellet propulsion in the concentration range of 0.1–10 μM , and totally inhibited pellet propulsion at the concentration higher than 30 μM . Thus, it can be concluded that magnolol may 1) block receptor-operated cation channels and the voltage dependent Ca^{2+} channel, and 2) inhibit calcium release from the sarcolemmal membrane (SR) through blocking InsP_3 -sensitive and ryanodine-sensitive pathways. This explains, at least partially, that *Cortex magnoliae officinalis* exerts therapeutic effects on gastrointestinal disease through relaxation of GI tract smooth muscles.

Key words magnolol; smooth muscle; Ca^{2+} channel

Magnolol (MAG), 5,5-Di-2-propenyl-[1,1-biphenyl]-2,2-diol (Fig. 1), is the major phenolic compound purified from the medical plant *Cortex magnoliae officinalis*,¹⁾ which has been used for lower gastrointestinal disorders (e.g., diarrhea, constipation) in Traditional Chinese Medicine for long time.²⁾ Major findings about the pharmacological activity of MAG involved: 1) inhibition of the respiratory burst in formylmethionyl-leucyl-phenylalanine activated neutrophils³⁾; 2) suppression of the overproduction of nitric oxide and tumor necrosis factor (TNF)- α in lipopolysaccharide activated macrophages⁴⁾; 3) protection of transplanted organs from lipid peroxidation⁵⁾; 4) prevention of chemical hypoxia in cortical neurons of rats⁶⁾; and 5) antioxidative effect on neutrophil adhesion *in vitro* and *in vivo* in rats.^{7–9)} Furthermore, MAG has previously been reported to inhibit smooth muscle contraction in porcine trachea, with possible mechanism of blockade of calcium influx through voltage dependent Ca^{2+} channels.¹⁰⁾ Recently, one study reported that MAG can inhibit contractility of the upper gastrointestinal tract smooth muscles of isolated gastric fundus strips of rats and isolated ileum of guinea pigs with a calcium-antagonistic effect,¹¹⁾ but no research have been done about MAG on the lower gastrointestinal tract. The characteristics of different parts of the gastrointestinal tract are not the same, especially in terms of motility,¹²⁾ permeability,¹³⁾ and response to calcium manipulation.¹⁴⁾ Thus, it is necessary to study the effect of MAG on colon, in order to understand the pharmacological action of MAG in lower gastrointestinal disorder.

Fig. 1. Molecular Structure of Magnolol

In this study, pharmacological effects of MAG on muscle strips isolated from distal colon of guinea pig were investigated with the presence of activators and inhibitors of muscle contraction, and isolated colon segments of guinea pig were used to test the effect of MAG on the velocity of artificial pellet propulsion.

MATERIALS AND METHODS

Tissue Preparation Male guinea pigs (200–250 g) were fasted for 24 h, and then sacrificed by stunning and cervical dislocation, and 2-cm pieces of the colon were dissected from the colon segment 1.5 to 2 cm from anus. Luminal contents were washed out with Krebs-bicarbonate buffer. The segments were then opened along the mesenteric border, cut into 10 mm $\times$ 4 mm strips approximately along the circular and longitudinal axis individually and transferred into cold Krebs-bicarbonate buffer. The mucosa was carefully dissected away to expose the muscularis externa. Tissues were mounted for tension recording and allowed to equilibrate for 1–2 h in 10-ml chambers containing Krebs-bicarbonate buffer (118 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4 , 1.2 mM MgSO_4 , 2.5 mM CaCl_2 , 25 mM NaHCO_3 and 11 mM Glucose, pH 7.4, at 37°C), and saturated with 95% O_2 and 5% CO_2 . The colonic longitudinal muscle strips (LMS) and circular muscle strips (CMS) were initially maintained at 0.5 g tension and equilibrated for 60 min. The bath solution was replaced every 20 min to keep the solution fresh during equilibration.

Measurement of Contractile Activity The mechanical responses in preparations were recorded with an isometric transducer (AD instruments, Australia) and a polygraph. For the MAG induced inhibition of preparations, responses were calculated as percentages of the contraction induced by different stimulators, such as serotonin (5-HT), KCl or carbachol (CCh), which was applied at the beginning of the experiment for calibration.

* To whom correspondence should be addressed. e-mail: bzxiang@hkbu.edu.hk

Measurement of Velocity of Pellet Propulsion (VPP)

Male guinea pigs (200–250 g) were fasted for 24 h, sacrificed by stunning and cervical dislocation, and then the whole colon was obtained. The proximal 3 to 4 cm and distal 1 to 2 cm were discarded. The remaining pellet-containing portion was incubated in Krebs-bicarbonate medium at 37 °C for 30 min, to allow spontaneous evacuation of the pellets. Each distal colon was cut into two equal segments and secured with pins placed at intervals through the attached mesentery. After 30 min equilibration, an artificial fecal pellet similar in shape and size to colonic fecal pellets (10-mm long × 2-mm wide) was inserted into the oral end of the segment to calculate VPP. It was calculated on the basis of the time taken by a pellet to transverse a 3-cm segment of isolated colon. The basal VPP was first determined using three successive pellets inserted at 5 min intervals. The segments were allowed to equilibrate again in fresh buffer for 30 min. And then MAG was added into buffer directly to test the effect on VPP. The results of MAG on VPP were expressed as percentage of basal velocity in millimeters per second.¹⁵⁾

Protocol of Experiments. Test One: Effects of MAG on Muscle Strip Contractions Induced by 5-HT, CCh and KCl Effects of MAG (0 μ M, 1 μ M, 10 μ M, 30 μ M, 100 μ M) on muscle strip contractions induced by three different stimulants (10 μ M serotonin, 1 μ M CCh and 40 mM KCl) were tested separately. The procedures were as follows: muscle strips were treated with 40 mM KCl, 10 μ M 5-HT and 1 μ M CCh separately at first, the contraction results of each stimulant were recorded as the baseline. After washout for 30 min, the strips were pre-exposed to MAG with different concentration separately for 5 min, and activated by 10 μ M 5-HT, 1 μ M CCh or 40 mM KCl respectively again. The muscle strip contraction were recorded and compared with respective calibration.

Test Two: Effects of MAG on Muscle Strip Contractions Induced by 5-HT and CCh in Calcium Free Krebs-bicarbonate Buffer Effects of MAG on muscle strip contractions induced by 5-HT or CCh in the calcium-free media were tested respectively. The procedure was same as that of test one, but no calcium existed in the buffer. This experiment aimed to study the effect of 100 μ M MAG on 1 μ M CCh-induced or 10 μ M 5-HT-induced LMS contractions in calcium-free Krebs-bicarbonate buffer. LMS were treated with CCh or 5-HT for 1 min first, then treated with MAG for 5 min respectively, and finally activated by CCh or 5-HT again.

Test Three: Effects of MAG Plus Nifedipine on The Contraction of Muscle Strips Longitudinal strips were pretreated with 0.5 μ M nifedipine or 0.5 μ M nifedipine plus 100 μ M MAG respectively. Then muscle strips were contracted by 40 mM KCl. The outcomes of contraction were compared with that of pretreatment with 100 μ M MAG.

Test Four: Effect of MAG on KCl-Induced Contraction with 1 mM Procaine Muscle strips were exposed to 10 mM procaine for 1 min and then contracted by 40 mM KCl for calibration, and the results were recorded as baseline for control. After 15 min washout until the muscle strips returned to steady state, MAG (0, 30, 100 μ M) and 10 mM procaine were added into organ bath chamber for 1 min, followed by 40 mM KCl to induce contraction. The contraction outcomes were compared with respective baselines and calculated as per-

centages of the contractile response obtained in calibration.

Test Five: Effect of MAG on Velocity of Pellet Propulsion MAG was directly added to the organ bath solution, with different final concentrations (0 μ M, 0.1 μ M, 1 μ M, 10 μ M) in the organ bath solution respectively, and the velocity of pellet propulsion was tested separately.

Drugs and Solutions MAG (99% purity), purchased from the National Institute for the Control of Pharmaceutical & Biological Products of P. R. China, was dissolved in dimethyl sulfoxide (DMSO). CCh, 5-HT, nifedipine, thapsigargin and procaine were purchased from Sigma Chemical Co. (Sigma-Aldrich China Inc). The concentrations of drugs described in this paper refer to the final bath concentrations.

Statistical Analysis Results were expressed as mean $\pm$ S.D. with the number of experiments given in parentheses. For the comparison of data, paired or unpaired Student's *t* tests or analysis of variance (ANOVA) were used where applicable. Differences were considered to be significant when $p \leq 0.05$.

RESULTS

Test One: Inhibitory Effects of MAG on 5-HT-, CCh- or KCl-Induced Contraction ($n=8$) in the Guinea Pig Isolated Colon Muscle Strips MAG did not influence the baseline of preparations (data were not shown in this paper). As shown in Figs. 2a, b and c, pre-exposure of the preparations to 1 μ M MAG for 1 min cannot significantly inhibit KCl-, 5-HT- or CCh-induced LMS and CMS contraction, while pre-exposure to MAG at concentrations higher than 10 μ M dose-dependently inhibited 5-HT-, KCl- or CCh-induced LMS and CMS contraction. The inhibition rates of MAG at concentrations of 10 μ M, 30 μ M or 100 μ M on 5-HT-induced contraction of LMS were 12%, 28% and 45% respectively, while on CMS they were 22%, 29% and 40%, respectively. As for CCh-induced contraction of LMS, the inhibitory rates of MAG at concentrations of 10 μ M, 30 μ M or 100 μ M were 12%, 21% and 27% respectively, while for CMS they were 23%, 30% and 36% respectively. The inhibition rate of MAG at concentrations of 10 μ M, 30 μ M or 100 μ M on KCl-induced contractions of LMS were 15%, 30% and 41% respectively, while for CMS they were 22%, 34% and 39% respectively.

Test Two: Inhibitory Effects of MAG on Contractions of the Guinea Pig Isolated Colon Muscle Strips Induced by 5-HT-, CCh-Induced Contraction in Calcium-Free Buffer ($n=8$) As shown in Fig. 3a, MAG dose-dependently inhibited the 5-HT-induced contraction in LMS in calcium-free buffer. The inhibitory rate of MAG at the concentration of 1 μ M, 10 μ M, 30 μ M or 100 μ M were 38%, 63%, 76% and 92% respectively. Fig. 3b showed that 100 μ M MAG inhibited CCh-induced LMS contraction in calcium-free buffer, with the inhibitory rate 36%.

Test Three: Inhibitory Effect of MAG vs. MAG Plus Nifedipine on KCl-Induced Contraction in the Guinea Pig Isolated Colon Muscle Strips ($n=8$) Nifedipine, a classic calcium channel blocker, can decrease the intracellular calcium concentration ($[Ca^{2+}]_i$). As shown in Fig. 4, when LMS was pre-exposed to 1 μ M nifedipine alone or 1 μ M nifedipine plus 100 μ M MAG separately, their inhibition rates on 40 mM KCl-induced contraction were 92% and 71%.

Fig. 2. Mag-Induced Inhibition of 5-HT, CCh, and KCl-Induced Muscle Strips Contraction

Graph showing the concentration-related inhibitory effects of pretreatment with MAG (1–100 μ M) on the submaximal contractions induced by KCl (40 μ M, $n=8$, Fig. 2a), 5-HT (10 μ M, $n=8$, Fig. 2b) and CCh (1 μ M, $n=8$, Fig. 2c) in the guinea pig. Data are shown as means $\pm$ S.E. * Significance at $p < 0.05$ vs. 0 μ M magnolol (blank), $n=8$.

There were significant difference ($p < 0.05$) between two groups. As showed in Fig. 2a and Fig. 4, the inhibition rate of 100 μ M MAG on KCl-induced contractions of LMS were 41%, with significant difference in contrast with that of nifedipine or MAG plus nifedipine ($p < 0.05$).

Test Four: Inhibitory Effect of MAG on KCl-Induced Contraction with 1 mM Procaine Condition ($n=8$) The effect of MAG on KCl-induced contraction under pretreatment with 1 mM procaine, a ryanodine receptor inhibitor, was investigated. As shown in Fig. 5, MAG significantly inhibited the contraction induced by 40 mM KCl between 1–100 μ M concentrations.

Test Five: Inhibitory Effect of MAG on Velocity of Pellet Propulsion ($n=6$) As shown in Fig 6, MAG dose-dependently decreased the VPP at the concentration range of 0.1–10 μ M. At the concentration higher than 30 μ M, MAG totally inhibited pellet propulsion in colon segment.

DISCUSSION

The present study found that 1) MAG did not affect the base tone of colon muscle strips, but dose-dependently inhibited high K^+ , CCh- and 5-HT-induced contractions at concentrations higher than 10 μ M; and 2) MAG inhibited CCh-

Fig. 3. Mag-Induced Inhibition of 5-HT, CCh-Induced Muscle Strips Contraction in Calcium-Free Krebs-Bicarbonate Buffer

Graph showing the concentration-related inhibitory effects of pretreatment with MAG (1–100 μ M) on the submaximal contractions induced by 5-HT (10 μ M, $n=8$, Fig. 3a) in the guinea pig and inhibitory effects of pretreatment with MAG (100 μ M) on the submaximal contractions induced by CCh (1 μ M, $n=8$, Fig. 3b) in the guinea pig. Data are shown as means $\pm$ S.E. * Significance at $p < 0.05$ vs. 0 μ M magnolol (blank).

Fig. 4. Inhibitory Effect of Magnolol on KCl (40 μ M)-Induced Contraction of LMS in Presence or Absence of Nifedipine

Inhibition rate of 1 μ M nifedipine alone, 1 μ M nifedipine plus 100 μ M MAG and 100 μ M MAG alone on 40 μ M KCl-induced contraction were 92%, 71% and 41% respectively. Data are shown as means $\pm$ S.D. * Significance at $p < 0.05$ vs. magnolol alone, $n=8$.

and 5-HT-induced contractions in calcium-free Krebs-bicarbonate buffer; 3) the inhibition rate of nifedipine plus MAG on LMS contractions was higher than that of MAG, but lower than that of nifedipine alone on LMS; 4) MAG dose-dependently inhibited KCl-induced contraction under 1 mM procaine condition; and 5) MAG dose-dependently decreased the VPP at the concentration range of 0.1–10 μ M, and totally inhibited pellet propulsion in colon segment at the concentration higher than 30 μ M. The data indicate that MAG not only blocked voltage dependent Ca^{2+} channel and non-selective cation channel, but also inhibiting calcium release from the sarcolemmal membrane (SR) via inositol trisphosphate ($InsP_3$)-sensitive and ryanodine-sensitive receptors.

Fig. 5. Inhibitory Effect of Magnolol on KCl-Induced Contraction with the Condition of 1 μ M Procaine

Magnolol dose-dependently inhibited 40 μ M KCl-induced longitudinal muscle contraction. Data are shown as means $\pm$ S.E. * Significance at $p < 0.05$ vs. 0 μ M magnolol (blank), $n = 8$.

Fig. 6. Inhibitory Effect of Magnolol on Velocity of Pellet Propulsion in Guinea Pig Colon Segments

At the concentration of 0.1–10 μ M, Magnolol diminished the velocity of pellet propulsion in guinea pig colon segments. Data are shown as means $\pm$ S.D. * Significance at $p < 0.05$ vs. 0 μ M magnolol (blank), $n = 6$.

Therefore the mechanism of inhibition on lower gastrointestinal tract smooth muscle is different from that on porcine trachea, which is achieved by blockade of calcium influx through voltage dependent Ca^{2+} channels only.

It is well known that the coordinated contractions and relaxations of the smooth muscle cells are the basis of gastrointestinal motility. Previous studies showed that the inhibitory effect of MAG on muscle contractions induced by stimulants does not rely on β -adrenergic-¹⁶⁾ and protein Kinase A activity,¹⁷⁾ thus, intracellular $[\text{Ca}^{2+}]_i$ and sensitivity of contractile apparatus to calcium ion may be targets of MAG. Factors that increase $[\text{Ca}^{2+}]_i$ in visceral smooth muscle cells involve¹⁸⁾ 1) influx of Ca^{2+} through the voltage-operated calcium channel (VOCC), receptor-operated calcium channel (ROCC), anti-dromic $\text{Na}^+/\text{Ca}^{2+}$ exchangers, and so on; 2) release of Ca^{2+} from the Ca^{2+} store, mainly from sarcolemmal membrane (SR), and this release takes the form of calcium induced calcium release (CICR) and InsP_3 -induced Ca^{2+} release (IICR) (induced by activation of ryanodine receptor and InsP_3 receptors); 3) reuptake of Ca^{2+} into the SR; and 4) extrusion of Ca^{2+} via Ca^{2+} -ATPase and orthodromic $\text{Na}^+/\text{Ca}^{2+}$ exchange. Therefore, these factors affecting the intracellular $[\text{Ca}^{2+}]_i$ may be the targets related with the mechanism by which MAG influences colon muscle tissue contraction.

It is well known that high K^+ -induced smooth muscle contractions are mediated by activation of the VOCC. Our results showed that MAG dose-dependently inhibited KCl-induced muscle strips contraction, suggesting MAG blocked

the calcium influx through VOCC. It has been reported that MAG can inhibit the porcine trachea smooth muscle contraction, with the possible mechanism being blockade of calcium influx through VOCC.¹⁰⁾ Thus, these findings suggest that VOCC may be one target for the inhibitory effect of MAG on smooth muscle.

Whether MAG has any effects on ROCC? In order to discover this puzzle, we tested the effect of MAG on the CCh-induced muscle strips contraction. Data showed that 1) 100 μ M MAG significantly inhibited CCh-induced muscle strips contractions in calcium-containing Krebs-bicarbonate buffer with the inhibition rate 27%; and 2) MAG significantly inhibited CCh-induced contractions in Ca^{2+} -free buffer with inhibition rate 36%. CCh can induce graded depolarization and/or reduction in action potential of membranes through integrating with muscarinic receptors, thus initiating excitation and contraction of GI tract smooth.¹⁹⁾ Our results suggested that MAG can inhibit receptor-operated channel. For two major ionic currents in visceral smooth muscle that permeate several cations exist, one is a receptor-operated nonselective channel, and the other is a hyperpolarization-activated channel. In the process of receptor-operated channels, the GTP binding protein-mediated signal transduction pathway plays a key role. These data indicate that MAG can inhibit the ROCC. Therefore, MAG can inhibit both VOCC and ROCC, thus blocking the influx of calcium through sarcolemmal membrane.

On the other hand, the inhibition of MAG on muscle contraction may not only target on the VOCC and ROCC, inhibition of calcium release from SR may contribute to this inhibitory effect too. This is because that KCl-induced influx of calcium through the sarcolemmal membrane causes the increase of $[\text{Ca}^{2+}]_i$, and such increase of $[\text{Ca}^{2+}]_i$ subsequently induces the calcium release from SR. Based on this rationale, the inhibition of MAG on muscle contraction may not only on the VOCC, but another possibility exist, that is inhibition on SR. Whether MAG can inhibit calcium release from SR? In order to discover this, calcium-free buffer was selected to test the effect of MAG on 5-HT- and CCh-induced muscle strips contraction, thus to avoid the extracellular calcium affecting the concentration of calcium in cytosol. The results showed that MAG still dose-dependently inhibited the contractions induced by two activators. These data indicate that MAG can inhibit the calcium release from SR. It is thought that activation of a given receptor, such as CCh on muscarinic receptor and serotonin on 5-HT receptor,²⁰⁾ modifies an ionic channel through activation of the receptor's coupled G protein. This may involve¹⁸⁾ 1) direct regulation of ion channels without the synthesis of second messengers (e.g., ROCC), 2) regulation of ion channels through an increase in the Ca^{2+} concentration in the cytosol via the actions of InsP_3 and initiated by activation of Gq/11 (e.g., K^+ channels, Cl^- channels), or 3) regulation of ion channels through an increase or decrease in the amount of cAMP induced via the actions of Gs or Gi/o (e.g., K^+ channels, Cl^- channels). Our results showed that in calcium-free media, MAG still inhibited the muscle strip contraction induced by CCh and 5-HT, indicating that MAG may inhibit the action of InsP_3 . Previous studies showed 5-HT can cause intracellular calcium release from SR.^{21,22)} Present study documented that MAG decreased 5-HT (10 μ M)-induced contraction in a dose-de-

pendent manner at concentrations ranging from 1–100 μM , and also MAG significantly decreased the VPP at the concentration of 0.1–10 μM , and totally inhibited the pellet propulsion when higher than 30 μM concentration. These data mean that blockage of calcium release from SR is another mechanism underlining the inhibitory effect of MAG on colon muscle contractions. Interestingly, 30–100 μM MAG totally inhibited the pellet propulsion in colon segment. Any other mechanisms exist about MAG on pellet propulsion? It is well known that pellet propulsion is controlled by enteric nervous system, smooth muscle contraction, and many intrinsic neurons and neurotransmitters. A propagated peristaltic reflex initiated by muscle stretch or mucosa stimulation mediates the propulsion of colonic contents. The reflex involves sequential activation of sensory neurons coupled *via* modulatory interneurons to ascending excitatory and descending inhibitory motor neurons.²³⁾ Main neurons in the intrinsic nerve circuits that are activated by appropriate stimuli are the intrinsic primary afferent neurons (IPANs). It has been reported that fecal pellets trigger the release of 5-HT, which acts *via* both 5-HT₃ and 5-HT₄ receptors to regulate propulsive activity in guinea pig colon.¹⁵⁾ But further study is necessary to solve the questions whether MAG inhibits the 5-HT release from EC cells, and whether and how MAG affects the binding of MAG on 5-HT receptors.

Our data also showed that MAG significantly inhibited the contraction induced by 40 mM KCl between 1–100 μM concentrations under the presence of procaine. Procaine is an inhibitor of ryanodine receptors. When ryanodine receptor is blocked, the change of intracellular calcium concentration is mainly due to the influx of extracellular calcium through VOCC and ROCC, and activation of InsP₃-mediated calcium release from SR. The inhibitory effects of MAG under the condition of procaine reconfirmed the function of MAG on the VOCC and calcium release mediated through the InsP₃-receptor. As for inhibitory rate of MAG on voltage-dependent calcium channel, our data showed that the inhibitory effect was mild. It is well known that nifedipine is a classic antagonist of calcium channels.^{24,25)} Comparing inhibitory rate of nifedipine alone (92%) with that of MAG alone (41%) on KCl-induced contraction of LMS, we found that MAG inhibited the muscle strips contraction about 41% at the concentration of 100 μM , but adding nifedipine with MAG significantly enhanced the inhibitory rate about 30%, up to 71%. This result suggested that the inhibitory effect of 100 μM MAG on muscle strips is not mainly through nifedipine-specific receptors. Combining MAG and nifedipine together led higher inhibitory effect than that of MAG alone, but the inhibitory rate lower than that of nifedipine alone, suggested that there may have competition between two agents on blockage of calcium channel.

Limitation Recent investigations^{26,27)} found that smooth muscle tone appears to depend on not only incoming extracellular signals $[\text{Ca}^{2+}]_e$, but also a network of activating and inhibiting intracellular signals. During the regulation of smooth muscle contraction one key event is the phosphorylation/dephosphorylation of the regulatory light chains of myosin II (rMLC) catalyzed, respectively, by the Ca^{2+} - and calmodulin-activated myosin light-chain kinase (MLCK) and a type 1 myosin phosphatase. When $[\text{Ca}^{2+}]_i$ increase, Ca^{2+}

binds to calmodulin and activates MLCK, leading to phosphorylation of rMLC predominantly at Ser-19. Phosphorylation of rMLC allows the myosin ATPase to be activated by actin and then muscle contracts. The activity of MLCP is inhibited by a group of kinases involving Rho-associated kinase, and protein kinase C, leading to an increase in Ca^{2+} sensitivity.²⁸⁾ ROCKs (which are also known as Rho kinases) are the first group effectors of Rho to be discovered, and were initially characterized for their roles in mediating the formation of RhoA-induced stress fibres and focal adhesions through their effects on the phosphorylation of myosin light chain (MLC).²⁹⁾ In our study, we have not investigated whether MAG affected the network of activating and inhibiting intracellular signals, especially about the phosphorylation/dephosphorylation of rMLC and subsequent events. Further studies will help to clarify the detailed mechanism of MAG on muscle contraction.

CONCLUSION

Taken together, our results show that MAG is able to inhibit colon muscle contractions. The mechanism of inhibitory effect of MAG involves, at least, blockage the calcium influx through VOCC and ROCC, and inhibition of calcium release from SR. This result explains the therapeutic effects of *Cortex magnoliae officinalis* on lower gastrointestinal disease.

Acknowledgements This work is supported by the Faculty Research Grant, Hong Kong Baptist University. And authors want to thank Dr. Martha Dahlen for her assistance in editing of English writing of this paper.

REFERENCES

- 1) Zhang G. D., *Zhongguo Zhong Yao Za Zhi*, **14**, 565–568, 576 (1989).
- 2) Chen Q. S., "Pharmacology and Applications of Chinese Material Media," Vol. 2, ed. by Chang H. M., But P. P. H., World Scientific Singapore, 1986, pp. 878–880.
- 3) Wang J. P., Hsu M. F., Raung S. L., Chang L. C., Tsao L. T., Lin P. L., Chen C. C., *J. Pharm. Pharmacol.*, **51**, 285–294 (1999).
- 4) Son H. J., Lee H. J., Yun-Choi S. H., Ryu J. H., *Planta Med.*, **66**, 469–471 (2000).
- 5) Chiu J. H., Wang J. C., Lui W. Y., Wu C. W., Hong C. Y., *J. Surg. Res.*, **82**, 11–16 (1999).
- 6) Lee M. M., Hsieh M. T., Kuo J. S., Yeh F. T., Huang H. M., *Neuroreport*, **9**, 3451–3456 (1998).
- 7) Haraguchi H., Ishikawa H., Shirataki N., Fukuda A., *J. Pharm. Pharmacol.*, **49**, 209–212 (1997).
- 8) Shen Y. C., Sung Y. J., Chen C. F., *Eur. J. Pharmacol.*, **343**, 79–86 (1998).
- 9) Kong C. W., Tsai K., Chin J. H., Chan W. L., Hong C. Y., *Shock*, **13**, 24–28 (2000).
- 10) Ko C. H., Chen H. H., Lin Y. R., Chan M. H., *Planta Medica*, **69**, 532–536 (2003).
- 11) Zhang W. W., Li Y., Wang X. Q., Tian F., Cao H., Wang M. W., Sun Q. S., *World J. Gastroenterol.*, **11**, 4414–4418 (2005).
- 12) Sarna S. K., *Gastroenterology*, **89**, 894–913 (1985).
- 13) Davis G. R., Santa Ana C. A., Morawski S. G., Fordtran J. S., *Gastroenterology*, **83**, 844–850 (1982).
- 14) Forrest A., Molleman A., Parsons M., *Euro. J. Pharmacol.*, **509**, 77–783 (2005).
- 15) Jin J. G., Foxx-Orenstein A. E., Grider J. R., *J. Pharmacol. Exp. Ther.*, **288**, 93–97 (1999).
- 16) Tsai T. H., Lee T. F., Chen C. F., Wang L. C., *Neurosci. Lett.*, **186**, 49–52 (1995).

- 17) Wang J. P., Hu M. F., Raung S. L., Chang L. C., Tsao L. T., Lin P. L., Chen C. C., *J. Pharm. Pharmacol.*, **51**, 285—294 (1999).
- 18) Kuriyama H., Kitamura K., Itoh T., Inoue R., *Physiol. Rev.*, **78**, 811—920 (1998).
- 19) So I., Kim K. W., *J. Smooth Muscle Res.*, **39**, 231—247 (2003).
- 20) Berridge M. J., Bootman M. D., Roderick H. L., *Nat. Rev. Mol. Cell. Biol.*, **4**, 517—529 (2003).
- 21) Inoue R., Isenberg G., *Am. J. Physiol.*, **258**, C1173—C1178 (1990).
- 22) Nilius B., Droogmans G., *Physiol. Rev.*, **81**, 1415—1459 (2001).
- 23) Foxx-Orenstein A. E., Grider J. R., *Am. J. Physiol.*, **271** (3 Pt 1), G433—G437 (1996).
- 24) Hering S., Beech D. J., Bolton T. B., Lim S. P., *Pflugers Archiv.*, **411**, 590—592 (1988).
- 25) Advenier C., Cerrina J., Duroux P., Floch A., Renier A., *Br. J. Pharmacol.*, **82**, 727—733 (1984).
- 26) Leung T., Chen X. Q., Manser E., Lim L., *Mol. Cell. Biol.*, **16**, 5313—5327 (1996).
- 27) Somlyo A. P., Somlyo A. V., *J. Physiol.*, **522**, 177—185 (2000).
- 28) Pfitzer G., *J. Appl. Physiol.*, **91**, 497—503 (2001).
- 29) Riento K., Ridley A. J., *Nat. Rev. Mol. Cell. Biol.*, **4**, 446—456 (2003).