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European Journal of Pharmacology 433 (2001) 115–121

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Effect of in vitro and in vivo aerosolized treatment with geniposide on tracheal permeability in ovalbumin-induced guinea pigs

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Received 10 May 2001; received in revised form 30 October 2001; accepted 2 November 2001

Abstract

The primary objective of this study was to investigate the effect of geniposide, a potent anti-inflammatory, on ovalbumin-antigen-induced tracheal permeability and transepithelial electrical resistance in guinea pigs. Two weeks after sensitization with ovalbumin (100 mg/ml), the permeability of guinea-pig tracheas was evaluated by flux measurements using the transcellular tracer, [^{14}C]estradiol, and the paracellular tracer, [^{14}C]mannitol. The effect of extracellular Ca^{2+} with geniposide was also studied, using deletion of Ca^{2+} in the donor chamber. The in vivo treatment effect of aerosolized geniposide on tracheal permeability in the ovalbumin-sensitized guinea pigs was also evaluated. The results indicate that tight junction permeability of ovalbumin-sensitized trachea was significantly dose dependent and decreased by geniposide (1–10 mM), as evidenced by substantial recovery of transepithelial electrical resistance and decreased transepithelial permeability of [^{14}C]mannitol at $(1.32 \pm 0.12) \times 10^{-5}$ cm/s. The effect of combination of the removal of extracellular Ca^{2+} with geniposide had no effect on tight junction permeability of ovalbumin-sensitized trachea and revealed that transepithelial electrical resistance and junction permeability did not recover. In addition, the cAMP levels and phosphodiesterase activity were not significantly influenced in ovalbumin-sensitized tracheal tissues after geniposide treatment. Inhaled geniposide (50 mM, 30 min after ovalbumin sensitization) significantly restored junction permeability induced by ovalbumin (100 mg/ml, 2 min). Junction permeability did not recover on pretreatment with geniposide (50 mM for 30 min over 16 days consecutive before ovalbumin sensitization) after exposure of conscious guinea pigs to aerosol ovalbumin. In conclusion, geniposide has inhibitory effects on ovalbumin-induced junction permeability and recovery of transepithelial electrical resistance in guinea pig trachea, showing its potential as anti-asthma therapy. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Permeability; Tight junction; Geniposide; Aerosol; Ovalbumin

1. Introduction

It is known that the increase in permeability of respiratory epithelium after exposure to antigens is due to damaged epithelial junctions (Hulbert et al., 1981). In addition, others (Boucher et al., 1980; Goto et al., 2000) have demonstrated that this altered permeability is associated with structural damage to the healing strands of tight junctions and adherent junctions. We also observed that tight junctions in sensitized guinea pigs were opened by ovalbumin (Liaw et al., 1999). The altered state of permeability associated with mucosal injury must eventually return to normal or these junctions of tracheal epithelium must be closed to prevent further exposure to the antigen (Mashito et al., 1999; Ohbayashi et al., 1999; Pons et al., 2000). Thus, a potential for closing tight junctions and repairing membrane permeability may be of interest for the treatment of asthma.

Geniposide is one of the major iridoid glucosides in the fruit of *Gardenia jasminoides* Ellis, which has been reported to possess anti-inflammatory activity (inhibition of 5-lipoxygenase) (Nishizawa et al., 1988), activity against tumor-promoting12-*O*-tetradecanoyl-phorbol-13-acetate (activation of protein kinase C) (Ueda and Iwahashi, 1991; Lee et al., 1995), and which have been used for treatment of a liver disorder (inhibition of P450-3A monooxygenase) (Kang et al., 1997). Some authors (Kaji et al., 1990; Huang et al., 1998; Li et al., 2000) also found that geniposide could promote collagen synthesis in false-aged rats, stimulate the proliferation of endothelial cells but, interestingly, also act as a cross-linking agent with low cytotoxicity and biocompatibility.

Additional studies on the in vivo pharmacological profile of geniposide demonstrated that it not only could counter

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PII: S0014-2999(01)01506-0

resistance to Ca^{2+}-induced mitochondrial membrane permeability but also could inhibit the rapid reduction of mitochondrial membrane potential (Yamamoto et al., 2000). Furthermore, Yamazaki et al. (1996) reported that geniposide could enhance responses of cells to carbachol and KCl-induced depolarization in terms of cytoplasmic free-Ca^{2+} concentration. They indicated that geniposide could induce cell differentiation through activation of components of the intracellular signal transduction pathway (cAMP and protein kinase). In addition, geniposide also inhibits the spontaneous pilocarpine-induced contraction of rat stomach and cathartic action (Endo and Taguchi, 1973). However, the effectiveness of geniposide on the recovery of junction permeability of the asthmatic trachea is still unknown. Therefore, the purpose of this project was to evaluate the repair of junction permeability of asthmatic trachea by geniposide, using radioactive markers in an in vitro guinea pig model of acute antigen ovalbumin-enhanced permeability. Secondly, the effects of geniposide on low extracellular Ca^{2+} concentration, cAMP levels, and phosphodiesterase activity in ovalbumin-induced tracheal permeability were also studied. Finally, the influence of aerosolized geniposide on the antigen-induced tracheal junction permeability was studied in the in vivo guinea pig.

2. Materials and methods

2.1. Animals and materials

Male Dunkin–Hartley guinea pigs that weighed 250–350 g from the Animal Center of National Taiwan University were used. Radioactive [^{14}C]mannitol and [^{14}C]estradiol were obtained from NEN Life Science Products (DuPont Chemical, Boston, MA, USA). Geniposide was obtained from the fruit of *G. jasminoides* Ellis with boiling water under reflux for 6 h. Geniposide was isolated following passage through charcoal and silica columns in methanol, with a 1:5 MeOH–$CHCl_3$ solvent system. The identity of geniposide was confirmed, based on measuring the melting point, and using FAB-Mass, infrared spectrometry, ^{1}H-nuclear magnetic resonance (^{1}H-NMR), and ^{13}C-NMR as well as commercial standards. The yield and purity of geniposide were 4.7% and 99%, respectively, as determined by high-pressure liquid chromatography (HPLC) and thin-layer chromatography (TLC). Ovalbumin was obtained from Sigma (St. Louis, MO, USA). All chemical reagents were of analytical grade and were used as received.

2.2. Immunization

Procedures for sensitization of guinea pigs followed the methods from our previous study (Liaw et al., 1999). Male Dunkin–Hartley guinea pigs were assigned at random to four groups. One group was first sensitized by the inhalation of aerosolized antigen (10 mg/ml of ovalbumin) for 30 min daily for 8 days, using a DeVilbiss nebulizer (25 × 15-cm chamber, Pulmo-Amide 5610D, Somerset, PA, USA) with a particle size range of 0.5–5 μm. The second challenge sensitization was performed on the 16th day after the first, using 100 mg/ml ovalbumin aerosol for 2 min. The second group, as a control group, was not sensitized with ovalbumin, and thus, served as the non-sensitized group. The other two groups were used for the in vivo aerosolized geniposide study in Section 2.5.

2.3. In vitro perfusion studies

The guinea pigs were killed with an overdose i.p. injection of 3% sodium pentobarbital. The tracheas were immediately removed, mounted on acrylic rings (0.24 cm^2), and placed in Ussing chambers as in our previous study (Liaw et al., 1999). Briefly, both surfaces of the trachea were bathed in glutathione bicarbonated Ringer (GBR) solution. All experiments were performed at 37 °C with a mixture of 95% O_2 and 5% CO_2 and at a pH adjusted to 7.4 with NaOH or HCl. The permeating [^{14}C]radioactive compound was sampled from the receiver compartment at fixed intervals and replaced with an equal volume of previously warmed

Table 1
Effect of geniposide on the in vitro ovalbumin-sensitized tracheal permeability of guinea pigs

Treatment	Apparent permeability coefficients ($\times 10^{-5}$ cm/s)	
	[^{14}C]estradiol[a]	[^{14}C]mannitol
Control	2.23 ± 0.27 ($n=5$)	1.49 ± 0.11 ($n=6$)
Control + 5 mM geniposide	nd[b]	1.46 ± 0.27 ($n=5$)
Control + 10 mM geniposide	2.46 ± 0.39 ($n=5$)	1.46 ± 0.12 ($n=7$)
Control − Ca^{2+}	nd	2.21 ± 0.22 ($n=7$)[c]
Control + 10 mM geniposide − Ca^{2+}	nd	1.91 ± 0.21 ($n=8$)[c]
Sensitized	1.96 ± 0.27 ($n=5$)	1.92 ± 0.12 ($n=9$)[c]
Sensitized + 1 mM geniposide	nd	2.26 ± 0.08 ($n=6$)
Sensitized + 5 mM geniposide	nd	1.53 ± 0.21 ($n=6$)
Sensitized + 10 mM geniposide	2.13 ± 0.49 ($n=6$)	1.32 ± 0.12 ($n=8$)[d]
Sensitized − Ca^{2+}	nd	1.31 ± 0.09 ($n=7$)[d]
Sensitized + 10 mM geniposide − Ca^{2+}	nd	1.63 ± 0.06 ($n=8$)[e]
Sensitized + 1 mM nifedipine	nd	1.20 ± 0.07 ($n=4$)[d]

[a] There was no statistically significant difference between the four estradiol treatment groups ($p > 0.1$).
[b] nd: not determined.
[c] Denotes a statistical significance at $p \leq 0.01$, as compared to the control mannitol group.
[d] Denotes a statistically significant decrease at $p \leq 0.0005$, as compared to the sensitized mannitol group.
[e] There was a statistically significant difference between treated and sensitized mannitol groups ($p < 0.01$).

GBR solution. The radioactive compounds were counted as the total number of disintegrations per minute (dpm) with a Beckman 6500 liquid scintillation counter.

2.4. Transepithelial electrical resistance measurement

Electrodes were prepared from silver wire, and resistance was measured using an Ag–AgCl four-electrode system (Liaw et al., 1999). Variable current pulses were given, and a digital multimeter measured the corresponding potential differences. Resistance was calculated from the slope of the applied current and potential difference plot.

2.5. In vivo aerosolized geniposide study

For the in vivo aerosolized geniposide study, a separate group of conscious male Dunkin–Hartley guinea pigs was treated with 50 mM of geniposide using a DeVilbiss nebulizer for 30 min after 2 min of second challenge sensitization. To evaluate the prevention ability of geniposide in ovalbumin-sensitized guinea pigs, the second group inhaled 50 mM geniposide for 30 min daily over 16 days, using a DeVilbiss nebulizer before the second challenge sensitization. These guinea pigs were then killed with an overdose injection of 3% sodium pentobarbital after 2 min of second challenge sensitization for further evaluation in an in vitro perfusion study.

2.6. Assay of adenosine 3,5-cyclic monophosphate (cAMP) and inhibition of c-AMP phosphodiesterase by geniposide

c-AMP was assayed with a c-AMP assay kit from NEN Life Science Products (Boston, MA, USA) which measured phosphodiesterase activity. The experimental procedures followed those of our previous studies (Liaw et al., 1999).

2.7. Apparent permeability coefficient calculation and data analysis

In vitro apparent permeability coefficients (P) were calculated from the equation

$$P = V/A \times 1/C \times \mathrm{d}C/\mathrm{d}t$$
$$= (\text{Fraction of dose transported})\ /\mathrm{d}t \times V/A,$$

where the fraction of the dose transported through the trachea can be calculated after correction for sampling and solution replacement at each time point. These values were then plotted versus time. Therefore, the apparent permeability coefficient, P (cm/s), could be calculated from the slope, using the receiver compartment volume, V (7 ml), and surface area of the tissue A (0.24 cm^2). Data from all experiments were pooled to determine the mean and standard error of the mean (S.E.M.). Analysis of variance (ANOVA), with Dunnett's multiple comparison tests at 95% confidence levels, was used to determine the significance of differences between each group of experiments.

3. Results

Paracellular permeability coefficients (mannitol) of the control and ovalbumin-sensitized guinea pig tracheas are shown in Table 1. The [^{14}C]mannitol permeability of the trachea in the sensitized group was significantly greater than that of the control group ([1.92 ± 0.12] vs. [1.49 ± 0.11] × 10^{-5} cm/s). Table 1 also shows the effects of three different concentrations (1, 5, and 10 mM) of geniposide on the permeability of mannitol in ovalbumin-sensitized tracheal tissues; the apparent permeability coefficients of mannitol

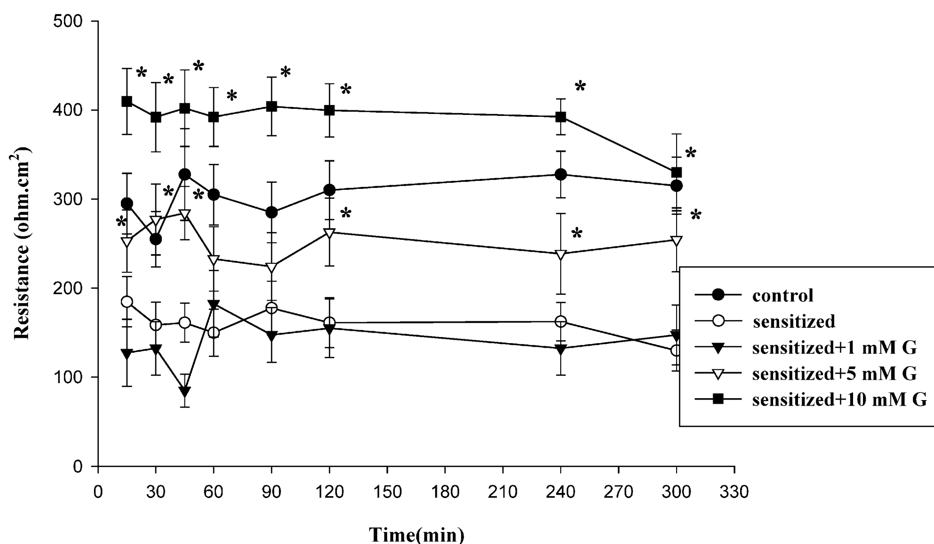

Fig. 1. Typical resistance–time profiles of ovalbumin-sensitized guinea-pig trachea, using three concentrations of geniposide (G). Error bars represent 1 S.E.M.; $n = 6–8$. * indicates a significant difference at $P < 0.01$ from the ovalbumin-sensitized group.

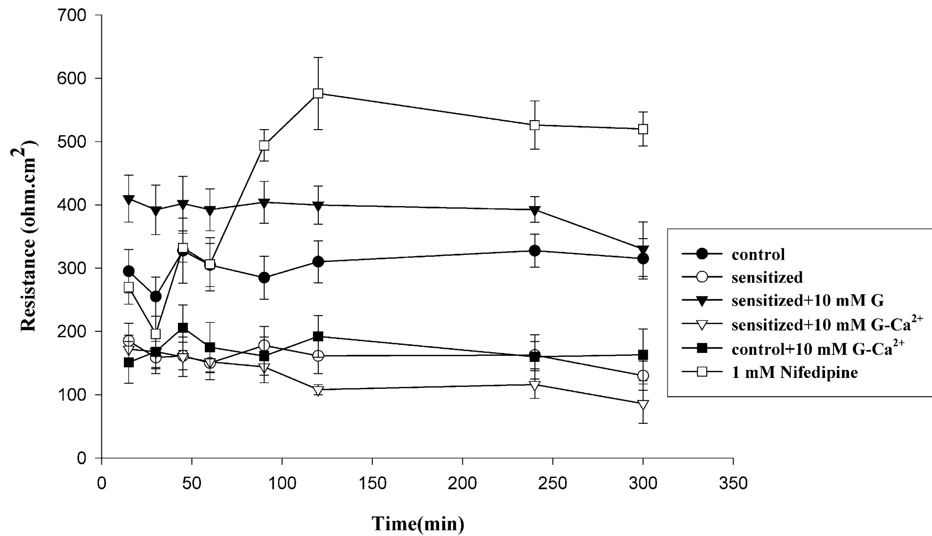

Fig. 2. Typical resistance–time profiles of ovalbumin-sensitized guinea-pig trachea with 10 mM geniposide (G) (▼), 10 mM geniposide without Ca^{2+} in the donor solution (▽), and the control group with 10 mM geniposide without Ca^{2+} in the donor solution (■), 1 mM nifedipine (□). Error bars represent 1 S.E.M.; $n=6–8$.

were (2.26 ± 0.08), (1.53 ± 0.21), and (1.32 ± 0.12) $\times 10^{-5}$ cm/s. Among these treatments, only 10 mM geniposide could decrease permeation of mannitol, and there was no significant difference from control tissues. In addition, in control tissues, there was no significant difference between effects for the two concentrations of geniposide. The apparent permeability coefficient of estradiol, an intracellular marker, also showed no significant differences between treatments ($p > 0.05$). Fig. 1 shows the electrical resistance profile of guinea-pig tracheas sensitized with aerosolized ovalbumin and treated with three concentrations of geniposide. The range of transepithelial electrical resistance of the control and 10 mM geniposide-treated tracheal tissues fell in the range of 300–425 Ω cm^2 over a 5-h period. On the other hand, in the 1 and 5 mM geniposide groups, resistance values decreased to around 98–280 Ω cm^2, indicating that these two concentrations could not repair tracheal junctional integrity after ovalbumin challenge.

To understand the relation between geniposide and the effect of extracellular Ca^{2+} on tracheal permeability, Ca^{2+} was removed from the medium in the donor chamber during a 5-h experiment. Control and ovalbumin-sensitized tracheal permeability were affected by the removal of Ca^{2+} from the donor chamber and mannitol junction permeability was (2.21 ± 0.22) and (1.31 ± 0.09) $\times 10^{-5}$ cm/s, respectively. After combination removal of Ca^{2+} from the extracellular bathing medium with the 10 mM geniposide-treated sensitized group, mannitol junction permeability increased from (1.31 ± 0.09) to (1.63 ± 0.06) $\times 10^{-5}$ cm/s, and a significant difference was found compared with that of the sensitized trachea group (1.92 ± 0.12 $\times 10^{-5}$ cm/s). However, there was no protection of junction permeability (1.91 ± 0.21 $\times 10^{-5}$ cm/s) in the control group with 10 mM geniposide in a low-Ca^{2+} extracellular medium. Furthermore, incubation with 1 mM nifedipine restored junction permeability of mannitol (1.20 ± 0.07 $\times 10^{-5}$ cm/s) to the same extent as 10 mM of geniposide did. A typical resistance–time profile of guinea pig trachea without Ca^{2+} is illustrated in Fig. 2. The resistance of samples without Ca^{2+} in the donor chamber decreased to around 90–200 Ω cm^2. For the 10 mM geniposide, 1 mM nifedipine, and control groups, the resistance values were all above 300 Ω cm^2. Using the trichloroacetic acid extraction method, the cAMP content in ovalbumin-sensitized tracheal tissues was found to be 127.5 ± 2.5 to 122.8 ± 2.4 pmol/ml ($p > 0.05$, $n = 6$) after 10 mM geniposide treatment. In addition, the inhibition of phosphodiesterase activity by 10 and 100 μg/ml geniposide was evaluated, and the effects on this enzyme activity were found to be only 19.62% and 10.02%, respectively. Taken together, these results suggest that the extracellular calcium concentration in the environment of the tissues influences the effect of geniposide on sensitized trachea more than the cAMP second messenger pathway.

Inhalation of 50 mM geniposide 30 min after ovalbumin challenge significantly decreased the apparent per-

Table 2
Effect of aerosolized geniposide on the in vivo ovalbumin-sensitized tracheal permeability of guinea pigs

Treatment	Apparent permeability coefficients [^{14}C]mannitol ($\times 10^{-5}$ cm/s)
Sensitized	1.92 ± 0.12 ($n=9$)
Aerosolized geniposide 50 mM + sensitized[a]	1.98 ± 0.06 ($n=5$)
Sensitized + aerosolized geniposide 50 mM[b]	0.75 ± 0.19 ($n=6$)[c]

[a] Pre-treatment with 50 mM geniposide for 30 min over 16 consecutive days before ovalbumin sensitization.
[b] Inhalation of 50 mM geniposide 30 min after ovalbumin sensitization.
[c] Denotes a statistically significant decrease at $p \leq 0.0005$, as compared to the sensitized mannitol group.

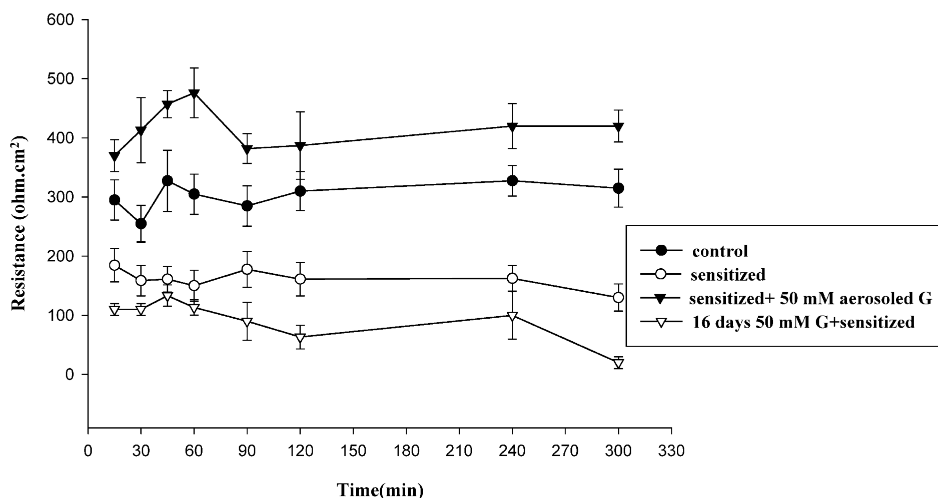

Fig. 3. Typical resistance–time profiles of ovalbumin-sensitized guinea-pig trachea with aerosolized 50 mM geniposide (G) (▼). Preventive ability of aerosolized 50 mM geniposide for 30 min, 16 days on ovalbumin-sensitized guinea pigs (▽). Error bars represent 1 S.E.M.; $n = 5-9$.

meability coefficients of mannitol from (1.92 ± 0.12) to $(0.75 \pm 0.19) \times 10^{-5}$ cm/s (Table 2). However, there was no influence of pretreatment with 50 mM geniposide for 30 min for 16 consecutive days before ovalbumin sensitization at $(1.98 \pm 0.06) \times 10^{-5}$ cm/s. Fig. 3 shows the electrical resistance property of guinea-pig tracheas with aerosolized 50 mM geniposide after ovalbumin sensitization. Transepithelial electrical resistance with aerosolized geniposide increased to around 389–460 Ω cm^2 which differs significantly from that of ovalbumin-sensitized trachea of guinea pigs.

4. Discussion

Ranga et al. (1983) have reported that after antigen (ovalbumin) challenge, there was a significant increase in the plasma levels of horseradish peroxidase, dextran, mannitol, and its correlates of enhanced transepithelial permeability of tight junctions. In our studies, after aerosol ovalbumin administration in guinea pig, tracheal permeability mainly consistently influenced a paracellular (tight junction) pathway up to $(1.96 \pm 0.27) \times 10^{-5}$ cm/s as well as resulting in decreased transepithelial electrical resistance.

Yamamoto et al. (2000) found that oral delivery of geniposide in mice could counter the Ca^{2+}-induced mitochondrial membrane permeability and inhibits the rapid reduction of membrane potential. In the present study, we have extended these observations to show that geniposide reduces tight junction permeability in guinea-pig trachea. These results are consistent with those of previous studies that showed a reduction in antigen-induced microvascular leakage in guinea-pig trachea by baicalin and recovery of junction permeability in vitro (Liaw et al., 1999). Bhat et al. (1993) reported that high extracellular Ca^{2+} levels and low intracellular Ca^{2+} levels are required for normal epithelium. In addition, decreasing extracellular Ca^{2+} may induce intracellular Ca^{2+} release from internal stores (mitochondria and endoplasmic reticulum) followed by opening of tight junctions of the trachea. This is consistent with our permeability and transepithelial electrical resistance results showing that both in control trachea with low extracellular Ca^{2+} medium and in ovalbumin sensitized.

Yamazaki et al. (1996) reported that geniposide could enhance the increase of cytoplasmic Ca^{2+} concentration induced by high K^{+} and the carbachol-induced depolarization with cytoplasmic Ca^{2+}-free condition and indicated that it could activate the intracellular signal pathway (protein kinases). Others (Anderson et al., 1993; Sandoval et al., 2001) suggested that the compound could affect tight junction permeability and may exert its action through mechanism involving Ca^{2+}, cytoskeleton, cadherin, and junction proteins (ZO-1,-2, cingulin, and Rab13, etc.). The inferred blocking properties of Ca^{2+} influx channels or inhibition of Ca^{2+} release from intracellular stores, or promotion of Ca^{2+} sequestration by intracellular stores we showed in our geniposide studies in guinea pig trachea, had also been demonstrated by Ivorra et al. (1992). Similar results were obtained with 1 mM nifedipine (a Ca^{2+} channel blocker) treatment in ovalbumin-sensitized trachea, namely decreased mannitol permeability of trachea. This was found with other calcium antagonists (diltiazem) that attenuate antigen-induced bronchoconstriction in various animal species (Fanta et al., 1982; Malo et al., 1983) and show some activity in asthmatic patients (Barnes, 1985). These characteristics make this iridoid attractive for exploration of its anti-inflammatory activity in vivo. Taken together, our results suggest that treatment of ovalbumin-sensitized tracheal tissues with 10 mM geniposide involves Ca^{2+} and plays a dominant role in repairing the junction structure.

When the concentration of geniposide was increased to over 10 mM, tracheal permeability increased to (1.93 ±

0.77) and $(2.19 \pm 0.69) \times 10^{-5}$ cm/s for 15 and 20 mM geniposide, respectively. Furthermore, geniposide was reported to be able to stimulate biliary excretion, to prevent hepatic damage induced by aflatoxin B1, and to have anti-inflammatory functions; however, hepatotoxicity was seen when it was administered at a high dose (Yamano et al., 1990). This is consistent with our finding that geniposide can be toxic to the structure of trachea tissues in high concentrations. However, mechanisms of damage to asthmatic trachea by higher doses of geniposide will need further investigation.

Inhalation of aerosolized geniposide (50 mM, 30 min after antigen exposure) by conscious guinea pigs prevented the junction permeability that appeared and restored the tissue integrity, affected in sensitized animals immediately after their exposure to the antigen aerosol. Lower doses of geniposide did not appear effective (data not shown). The dose of inhaled geniposide (50 mM) necessary to significantly restore the damage to the ovalbumin-sensitized trachea was higher than the corresponding inhaled or intratracheal doses of rolipram (10–500 μM) which markedly inhibited bronchoconstriction by an antigen in actively sensitized guinea pigs (Cortijo et al., 1993). Therefore, the preventive effect of 16 consecutive days-inhaled geniposide on antigen-induced acute asthma may be related to its low ability to inhibit phosphodiesterase IV activity.

However, the possible contribution of a variety of intracellular second messenger mechanisms to the inhibitory effect of geniposide should also be considered. Ullrich et al. (1993) indicated that inhibitors of dibutyl c-AMP hydrolytic and phosphodiesterase activities could increase the concentration of c-AMP in epithelial cells and preserve the structure of tight junctions. Although geniposide did not significantly increase the cAMP content in trachea, and the inhibition of phosphodiesterase activity only reached 19.62% with 10 μg/ml geniposide, it could augment the cAMP accumulation produced by the adenylyl cyclase activator. Pons et al. (2000) reported that glaucine did not increase cAMP, but could augment the cAMP accumulation produced by forskolin. On the other hand, cyclic nucleotides, such as cGMP, coupled to the nitric oxide system, are also of outstanding interest in this respect (Bredenbroker et al., 2001). Therefore, the contribution of these various actions to the inhibitory effect of geniposide cannot be ruled out by the present experiments.

In summary and conclusion, inhaled geniposide effectively reduced antigen-induced junction permeability, causing the recovery of transepithelial resistance in actively sensitized guinea pigs. The main mechanism of these inhibitory effects of geniposide is likely to be its known property of blocking calcium channels but the contribution of other pharmacological properties cannot be excluded. Geniposide has been used for years as an anti-inflammatory, but its potential use as an anti-asthmatic is uncertain since other more potent and selective agents appear advantageous for treating asthma.

Acknowledgements

The authors thank Dr. K.H. Hsieh for the technical help provided in the ovalbumin sensitization of the guinea pigs. Also the authors thank Drs. L.L. Yang and K.Y. Yen for their hospitality during my stay in the Graduate Institute of Pharmacognosy. This work was supported by a grant from the National Science Council, Republic of China (NSC84-2331-B038-030-M03).

References

Anderson, J.M., Balda, M.S., Fanning, A., 1993. The structure and regulation of tight junctions. Curr. Opin. Cell Biol. 5, 772–778.

Barnes, P.J., 1985. Clinical studies with calcium antagonists in asthma. Br. J. Clin. Pharmacol. 20, 289S–298S.

Bhat, M., Toledo-Velasquez, D., Wang, L.Y., Malanga, C.J., Ma, J.K.H., Rojanaskau, Y., 1993. Regulation of tight junction permeability by calcium mediators and cell cytoskeleton in rabbit tracheal epithelium. Pharm. Res. 10, 991–997.

Boucher, R.C., Johnson, J., Inoue, S., Hulbert, W., Hogg, J.C., 1980. The effect of cigarette smoke on the permeability of guinea pig airways. Lab. Invest. 43, 94–100.

Bredenbroker, D., Dyarmand, D., Meingast, U., Fehmann, H., Staats, P., Wichert, P.C., Wanger, U., 2001. Effects of the nitric oxide/cGMP system compared with the cAMP system on airway mucus secretion in the rat. Eur. J. Pharmacol. 411, 319–325.

Cortijo, J., Bou, J., Cardelus, I., Llenas, J., Morcillo, E., Gristwood, R.W., 1993. Investigation into the role of phophodiesterase IV in bronchorelaxation, including studies with human bronchus. Br. J. Pharmacol. 108, 562–568.

Endo, T., Taguchi, H., 1973. The constituents of *Gardenia jasminoides*, geniposide and gentibioside. Chem. Pharm. Bull. 12, 2624–2688.

Fanta, C.H., Venugopalan, C.S., Lacouture, P.G., Drazen, J.M., 1982. Inhibition of bronchoconstriction in the guinea pig by a calcium channel blocker, nifidipine. Am. Rev. Respir. Dis. 125, 61–66.

Goto, Y., Uchida, Y., Nomura, A., Sakamoto, T., Ishii, Y., Morishima, Y., Masuyama, K., Sekizawa, K., 2000. Dislocation of E-cadherin in the airway epithelium during an antigen-induced asthmatic response. Am. J. Respir. Cell Mol. Biol. 23, 712–718.

Huang, L.L., Sung, H.W., Tsai, C.C., Huang, D.M., 1998. Biocompatibility study of a biological tissue fixed with a naturally occurring cross-linking reagent. J. Biomed. Mater. Res. 42, 568–576.

Hulbert, W.C., Walker, D.C., Jackson, A., Hogg, J.C., 1981. Airway permeability to horseradish peroxidase in guinea pigs: the repair phase after injury by cigarette smoke. Am. Rev. Respir. Dis. 123, 320–326.

Ivorra, M.D., Lugnier, C., Schott, C., Catret, M., Noguera, M.A., D'ocon, P., 1992. Multiple actions of glaucine on cyclic nucleotide phosphodiesterases. Adrenoceptor and benzothiazepine binding site at the calcium channel. Br. J. Pharmacol. 106, 387–394.

Kaji, T., Miezi, N., Kaga, K., Ejiri, N., Sakuragawa, N., 1990. *Gardenia* fruit extract stimulates the proliferation of bovine aortic endothelial cells in culture. Planta Med. 56, 353–356.

Kang, J.J., Wang, H.W., Liu, T.Y., Chen, Y.C., Ueng, T.H., 1997. Modulation of cytochrome *P*-450-dependent monooxygenases, glutathione and glutathione *S*-transferase in rat liver by geniposide from *Gardenia jasminoides*. Food Chem. Toxicol. 35, 957–965.

Lee, M., Hsu, J., Wang, C., 1995. Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-caused tumor promotion in benqopyene-initated CD-1 mouse skin by geniposide. Anticancer Res. 15, 411–416.

Li, Y., Kamo, S., Metori, K., Koike, K., Che, Q., Takahashi, S., 2000. The promoting effect of eucommiol from *Eucommiae cortex* on collagen synthesis. Biol. Pharm. Bull. 23, 54–59.

Liaw, J., Gau, Y.Y., Chao, Y.C., 1999. Effect of baicalin on tracheal per-

meability in ovalbumin sensitized guinea pigs. Pharm. Res. 16, 1653–1657.

Malo, P.E., Wasserman, M.A., Griffin, R.L., 1983. The effects of nifedipine and verapamil on antigen-induced bronchoconstriction in dogs. Eur. J. Pharmacol. 92, 69–75.

Mashito, Y., Ichinose, M., Shirato, K., 1999. Bradykinin B2 antagonist HOE 140 inhibits late allergic microvascular leakage in guinea pig airways. Immunopharmacology 43, 249–253.

Nishizawa, M., Izuhara, R., Ksnrko, K., Koshihara, Y., Fujimoto, Y., 1988. 5-Lipoxygenase inhibitors isolated from *Gardeniae fructus*. Chem. Pharm. Bull. 36, 87–95.

Ohbayashi, H., Suito, H., Yoshida, N., Ilto, Y., Kume, H., Yamaki, K., 1999. Adrenomedullin inhibits ovalbumin-induced bronchoconstriction and airway microvascular leakage in guinea pigs. Eur. Respir. J. 14, 1076–1081.

Pons, R., Santamaria, P., Suchankova, J., Cortijo, J., Morcillo, E.J., 2000. Effects of inhaled glaucine on pulmonary responses to antigen in sensitized guinea pigs. Eur. J. Pharmacol. 397, 187–195.

Ranga, V., Powers, M.A., Padilla, M., Strope, G.L., Fowler, L., Kleinerman, J., 1983. Effect of allergic bronchoconstriction on airway epithelial permeability to large polar solutes in the guinea pig. Am. Rev. Respir. Dis. 128, 1065–1070.

Sandoval, R., Malik, A.B., Minshall, R.D., Kouklis, P., Ellis, C.A., Tiruppathi, C., 2001. Ca^{2+} signalling and PKCcalpha activate increased endothelial permeability by disassembly of VE-cadherin junction. J. Physiol. 533, 433–445.

Ueda, S., Iwahashi, Y., 1991. Production of anti-tumor-promoting iridoid glucosides in *Genipa americana* and its cell cultures. J. Nat. Prod. 54, 1677–1679.

Ullrich, O., Haase, W., Koch-Brandt, C., 1993. Elevated cAMP levels induce multilayering of MDCK cells without disrupting cell surface polarity. J. Cell Sci. 104, 719–726.

Yamamoto, M., Mirua, N., Ohtake, N., Amagaya, S., Ishige, A., Sasaki, H., Komatsu, Y., Fukuda, K., Terasawa, K., 2000. Genipin, a metabolite derived from the herbal medicine Inchin-ko-to, and suppression of Fas-induced lethal liver apoptosis in mice. Gastroenterology 118, 380–389.

Yamano, T., Tsujimoto, T., Ndoa, T., Shimizu, M., Ohmori, M., Morita, S., Yadmada, A., 1990. Hepatotoxicity of geniposide in rats. Food Chem. Toxicol. 28, 515–519.

Yamazaki, M., Chiba, K., Mohri, T., 1996. Neuritogenic effect of natural iridoid compounds on PC12h cells and its possible relation to signaling protein kinases. Biol. Pharm. Bull. 19, 791–795.