Slow Release of Tetracycline from a Mucoadhesive Complex with Sucralfate for Eradication of *Helicobacter pylori*

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Treatment composed of a gastric mucoadhesive antibiotic with slow release drug delivery is expected to be effective for the eradication of *Helicobacter pylori* (*H. pylori*). In this study, we evaluated the slow release property of the tetracycline–sucralfate acidic complex. Tetracycline was the antibiotic selected because of its complexation capacity with sucralfate. Sustained release was tested using two different dissolution test methods: paddle and flow-through cell. The adhesive paste formed from the acidic complex displayed a longer sustained release profile of tetracycline using flow-through cell method. The milder conditions of the flow-through cell method better mimicked the fasted state of the stomach, suggesting that the oral administration with fasting is appropriate for the acidic complex. Furthermore, the paste formation protected the tetracycline from decomposition under an acidic condition, which apparently contributes to long-term release. Change in the zeta potential of the acidic complex particles was helpful in clarifying the release mechanisms of the tetracycline. The data indicated that the immediate release of tetracycline in the early stage of the test was indispensable to the subsequent paste formation that enables slow release. If administrated orally with fasting, the acidic complex rapidly adheres to the gastric mucosa and sustains long-term release of the tetracycline to the gastric lumen or mucus layer. This antibiotic delivery mechanism, which requires only a minimum dosage, may be effective for efficient eradication of *H. pylori*.

Key words tetracycline; sucralfate; *Helicobacter pylori*; sustained release; zeta potential

Since the discovery of *Helicobacter pylori* (*H. pylori*) in 1983,[1,2] *H. pylori* infection has been found to be one of the most common bacterial infections worldwide. In addition, the chronic inflammation of gastric mucosa from *H. pylori* infection has been associated with stomach cancer.

The administration of a proton pump inhibitor with antibiotics[3—8] is generally and most frequently used for the eradication of *H. pylori*; however, this treatment is unsatisfactory because of attendant problems such as resistance and side effects from the high dosages required.[9—15] Especially, the high resistance rate of *H. pylori* to clarithromycin is a critical cause of the decrease in eradication in recent years.

Retention of an antibiotic on gastric mucosa infected with *H. pylori* provides the time needed for the antibiotic to attack the *H. pylori* and thus, a mucoadhesive formulation would not only continuously deliver the antibiotic but also avoid repeated administrations that require a greater amount of antibiotics, which leads to resistance, side effects, and poor compliance.

Our concept for eradication of *H. pylori* is illustrated in Fig. 1 and follows three formulation criteria. (1) Mucoadhesion to retain the antibiotics on the surface of the gastric mucosa, (2) sustained release of the antibiotics, and (3) sufficient activity of the released antibiotics. Mucoadhesion and sustained release of an antibiotic in the stomach are essential.[16] For example, formulation using amoxicillin-loaded chitosan microspheres has been reported to be very effective.[17] We have reported the acidic complexation of tetracycline with sucralfate and the excellent mucoadhesive property of the acidic complex (CO).[18,19] The CO is remarkably simple to prepare and formulation does not require special technology. The aim of this study is to clarify the sustained release of the tetracycline from the CO, the secondary function of the CO in achieving eradication of *H. pylori*.

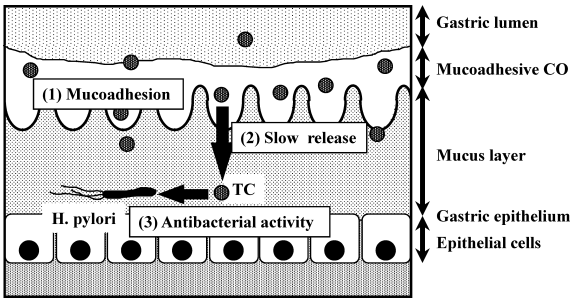

Fig. 1. Strategy for *H. pylori* Eradication Using a Mucoadhesive Complex Containing TC

Experimental

Materials The mucoadhesive material sucralfate (SF), with a loss-on-drying (LOD) moisture content (105 °C, 3 h) of 9.1%, was supplied by Chugai Pharmaceutical Co. (Tokyo, Japan). Amoxicillin (AMPC), metronidazole (MNZ), tetracycline (TC), and doxycycline (DC) were purchased from Sigma Co. (St. Louis, MO, U.S.A.). All other reagents used were of the highest purity reagent grade.

Interaction of Antibiotics with SF Antibiotics often used for eradication of *H. pylori* (AMPC, MNZ, TC, and DC) were tested. To prepare the aqueous dispersion system consisting of an antibiotic and SF, 100 mg of the antibiotic was added to 50-ml test tubes with 500 mg of SF (anhydride from LOD at 20 ml) reacted with water.

To test the influence of the addition of an acid, each antibiotic was dissolved with 1 M HCl (100 mg at 1 ml) and added to a test tube containing an aqueous dispersion of SF (500 mg at 19 ml) according to the practical preparation of a CO.

After shaking (rotary type, MK200D, Yamato Scientific, Tokyo, Japan) at 200 rpm for 30 min, the supernatants were separated by centrifugation at 1630×***g*** for 5 min (himac CF7D2 Hitachi, Tokyo, Japan) and removed. The precipitates in each tube were re-dispersed with 20 ml of fresh water with shaking and centrifugation repeated under the same conditions until no free antibiotic was detected in the washing water. The supernatants obtained

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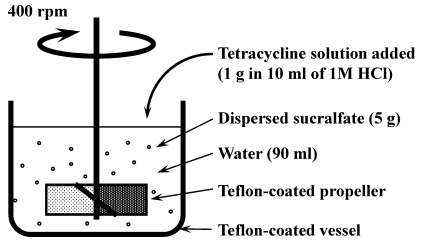

Fig. 2. Schematic Illustration of the Complexation Procedure

were diluted to an appropriate volume and the antibiotic content determined by spectrophotometry at the absorption maximum of each antibiotic (AMPC, 228 nm; TC, 355 nm; MNZ, 320 nm; and DC, 345 nm). The preliminary examination showed that decomposition of the antibiotics during the test was almost nonexistent, and so the amount of antibiotic interacted with SF was determined by subtracting the total amount of free antibiotic from the initial amount of antibiotic added.

Preparation of Mucoadhesive Formulation of TC TC was used because of its high interactive ratio with SF. Five grams of SF, corrected for water content, were dispersed into 90 ml of water in a vessel. A solution of TC with 1 M HCl (1 g in 10 ml) was added to the dispersion system and stirred at 400 rpm using a propeller type agitator with four blades, shown in Fig. 2. The entire dispersing system was filtered and the obtained product on the filter was re-dispersed in an appropriate amount of fresh water to remove the free TC and then re-filtered. The process was repeated and the resultant product freeze-dried (Neocool, Yamato Scientific) for 3 d or longer after prefreezing at −100 °C. The obtained powdered product was compared with the physical mixture (PM) of TC and SF.

Determination of the TC Content in the CO The amount of TC in the CO was determined by measuring the absorbance of the TC solution obtained by extraction from the CO with 2 M of acetic acid–ammonium acetate buffer as reported in our previous paper.[18,19] The homogeneous aqueous dispersion of CO (50 mg at 50 ml) was prepared and 3 ml of the dispersion removed and diluted to 50 ml with the buffer. After gentle shaking, the SF was removed by centrifugation and the adsorption of the resultant TC solution was measured at 355 nm. The moisture content of the CO was determined by LOD (105 °C, 3 h).

Release Tests of TC The profile for TC release from the CO was evaluated using the paddle and the flow-through cell methods. Water or No. 1 medium was thermally controlled at 37 °C for use as the test medium. With each evaluation method, the CO or PM (including *ca.* 7 mg of TC) was introduced into the test medium as the dispersion with a small amount of water to ensure homogeneous dispersion in the system.

With the paddle method, the test samples were introduced to 900 ml of test medium rotating at 100 rpm. To check the stability of TC in the acidic test medium, the change in the concentration of TC was monitored after introducing the original TC powder. With the flow-through cell method, dispersion of the CO or PM was introduced to the cell just prior to the test, which was carried out at a flow rate of 8 ml/min. The concentration of TC dissolved in the medium was measured spectrophotometrically at a wavelength of 355 nm (UV-160A, Shimadzu Corporation, Kyoto, Japan) over time.

Zeta Potential Measurement of the CO Particles CO particles (2 mg) were suspended in distilled water (100 ml) and in the TC saturated solution (100 ml). The zeta potential of the particles was measured at room temperature. Measurements (10) were taken at various intervals from 5 min to 8 h after the addition of 500 μl of 0.1 M HCl (see Fig. 5). The change in the pH of each suspension was also measured (Zetamaster, Malvern, U.K.).

Results and Discussion

Interaction of the Antibiotics with SF Several types of antibiotics were tested for complexation with SF and two of the antibiotics belonging to the tetracycline family interacted with SF despite repeated washing with water. The interaction rate of TC and DC were 23.0% and 15.2%, respectively, as shown in Table 1. MNZ and AMPC did not interact with SF.

When the interactive test was carried out in an acidic condition, the interaction rate of TC and DC increased significantly: 71.9% and 79.1%, respectively. MNZ and AMPC did not show an increased interaction rate even under acidic conditions and were completely removed from the system by repeated washing with water.

Table 1. Percentage (%) of Antibiotics Interacted with SF from Shaking under Non-acidic and Acidic Conditions

	Interaction under non-acidic condition	Interaction under acidic condition
TC	23.0±1.66	71.9±0.94
DC	15.2±0.59	79.1±0.58
AMPC	0.5±1.68	1.0±0.64
MNZ	−1.0±3.94	0.5±0.61

The results are expressed as the mean±S.D. (n=3).

To confirm the influence of the addition of the antibiotic to the acid, the antibiotic was added to the SF dispersion in advance separately from acid. The interaction rate was the same regardless of the method by which the antibiotic was added.

The pH of all tested systems was kept constant by the neutralization property of SF. The soluble status of each antibiotic was observed and estimated according to its solubility.

There are two possible mechanisms of interaction between an antibiotic and SF. One is the chemical interaction from the binding of the antibiotic to an activated site in SF. It is known that SF dissociates the hydroxyl groups in an acidic condition to neutralize the system. Dissociation of the hydroxyl groups from SF promotes positively charged aluminum moieties. Dissociation of the aluminum moieties resulting from further neutralizing of the acid promotes negatively charged SOS groups. Thus, SF has two oppositely charged sites in the molecule at the time it is responding to the acid, shown schematically in Fig. 3.[20,21] The interaction rate of TC or DC with SF increased under an acidic condition, which implies that a greater chemical interaction has occurred and TC or DC could possibly bind to either of the sites in SF. However, the binding of an excessive amount of antibiotic with these activated sites of SF possibly affects paste forming or the mucoadhesion property of CO because the activated sites also play a vital role in paste formation and mucoadhesion resulting from binding to the protein in the mucus layer.

Another possible interaction mechanism is the physical trapping of the paste structure of SF in the network. It is known that the paste formation of SF is caused by the interaction between the positively charged aluminum moieties and the negatively charged SOS moieties of the SF molecules described above. However, the non-interactive property of MNZ or AMPC suggests that antibiotics are not physically trapped in the network of the paste structure of SF.

It may be relevant to the structural property of SF that a small amount of TC or DC is adsorbed to SF in the non-acidic aqueous dispersion system. The amount of aluminum in the SF molecule is not so constant that it is expressed within a known range because of the synthetic process of SF. SF in its original structure has some partly free SOS moieties or surplus aluminum moieties to which TC or DC possibly bond. A small amount of TC or DC may bond to the moieties even under non-acidic conditions.

After assaying the four antibiotics, TC was selected as the model drug because of its high rate of interaction with SF and its general use for the eradication of *H. pylori*.

Determination of TC in the CO The TC content in the

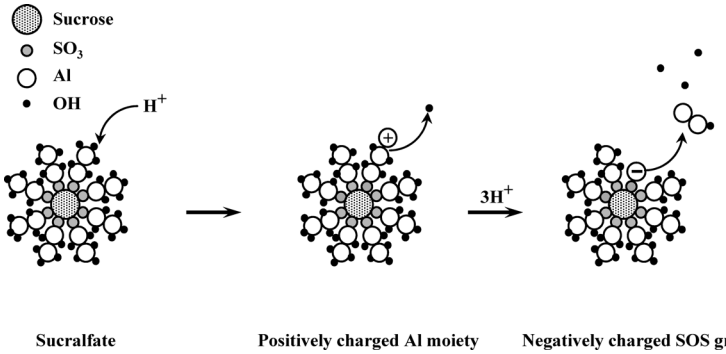

Fig. 3. Mechanisms Resulting from the Acid Consumption of SF of Positively Charged Aluminum Moieties Followed by Negatively Charged SOS-Groups

CO was about 11.7% with a water content measured by LOD of about 4.6%. Correcting for the water content, about 70% of the TC from the complexation bonded to the SF, almost identical to the rates under acidic and non-acidic conditions shown in Table 1.

TC Release from the CO TC release from the CO and from the PM in water or acidic medium (No. 1 medium) was evaluated using the paddle method.

Over 90% of the TC was dissolved immediately in the PM in water. Even after repeated washing of the CO with water during the preparation to remove the free TC, 20% of the TC was released from the CO in water. Possibly the bonds between the TC and SF were partly broken during lyophilization, the final step of the preparation. The gradual decrease in the concentration of TC following release from the CO or PM seemed to be caused by interaction with SF under a non-acidic condition.

The gradual release of TC from the CO after an immediate release of about 70% reached a maximum of about 90% at 1 h. On the other hand, almost all of the TC in the PM was dissolved as soon as the PM was introduced into the No. 1 medium.

The CO paste that had adhered to the bottom of the test vessel a few minutes after introducing CO into the medium was gradually diminished from the release of TC. This finding suggests that the compositions prepared in this study have the potential to show mucoadhesion and that the paste formation of the CO as a result of SF in an acidic condition is associated with the gradual release of TC from the CO in No. 1 medium. The formation of paste caused a decrease in the surface area of the CO particles necessary for the release of TC. Sustained release of TC was a result of the smaller surface area of the paste formation and was maintained by the diminishing process of the dissolving paste. On the other hand, TC was immediately released from the PM, in which a paste formation was not observed. TC apparently dissolved in No. 1 medium as soon as it was introduced. SF apparently dispersed completely in the medium because of the large volume of medium and the action of mechanical mixing. As a result, SF also dissolved in the No. 1 medium without the paste-forming necessary for sustained release.

The paste formation was likely started by agglomeration among the SF particles. The mechanism of agglomeration requires two processes. The first is the dissociation of the aluminum moieties from the SF or CO and the resultant SOS groups, which are the driving force for the formation of the mucoadhesive paste (Fig. 3). The second process is the collision opportunities of the SF or CO particles, on which surface the SOS groups bond with the ionized aluminum moieties of the other SF or CO particles. In spite of reducing the collision opportunities with an excessive amount of medium, the particles agglomerated in the CO but not in the PM. This appears to be caused by the difference in the response to the acid in the first process. Some hydroxyl groups of the CO appeared to be dissociated in advance during preparation. As a result, the CO did not complete the first process and clearly formed the paste from the collision opportunities upon being introduced. The PM apparently lost the opportunity because of the delay in the production of the SOS groups.

The amount of TC released from the CO or PM in the No. 1 medium was not complete (about 90%) and was followed by a gradual decrease in the concentration, similar to the case in water. However, the mechanisms seem to be different. In water, the mechanism seemed to be re-adsorption to SF in a non-acidic aqueous dispersion system, as described above, but in the acidic condition such as the No. 1 medium, the rate of decrease of the TC concentration was similar to the decomposition rate of the original TC powder. This finding indicates that the incomplete release of TC at C_{max} and the gradual decrease that followed in the No. 1 medium are caused by decomposition of the TC released from the CO or its immediate dissolution from the PM.

Evaluation of TC Release from the CO Using the Flow-Through Cell Method As shown in Fig. 4, with the flow-through cell method of gradual introduction of the acidic medium, there was no immediate release and TC was released more gradually from the CO that had formed a paste. The gradual release period was extended to 3 h from 1 h using the paddle method. Interestingly, the released TC showed very little decomposition (*ca.* 90%) using the paddle method. The sustained release of TC in the PM was slight. The release rate of TC from CO at the slow flow rate (4 ml/min) was slower than that at the fast flow rate (8 ml/min) (data not shown).

The sustained release of TC from the CO was a result of paste formation, as described above. The testing conditions and the acidity of the test medium influence paste formation because paste is a result of collision and binding among the proton-consuming CO particles. Considering the difference between two methods (flow-through cell method and paddle method), two critical points are remarkable. One is the influence of mechanical mixing and the other is the amount of the

test medium required. The dissociation of the aluminum moieties from the CO, the first process of agglomeration, appeared to be accelerated by paddle mixing. The continued mechanical action caused rapid dissolution of the CO particles before the opportunity for collision decreased by the large amount of test medium. These results clarified that a paste formation for sustained release is better achieved using a smaller amount of acid with gentle action.

Administration with fasting should result in greater mucoadhesion of a CO because of the small amount of acid and of movement in a fasted state stomach. Thus, the flow-through cell method better simulates the release of a CO when administered in human under fasted state conditions. Another interesting result was the complete release of the TC from the CO without decomposition from the acidic medium. The concentration of released TC was measured at each sampling point as soon as it was recovered and before decomposition by the acid. The results suggested that the TC in the CO is chemically stable in spite of an acidic condition, possible because of neutralization by the SF, and that the paste formation of the CO contributed to the long-term release of TC by protecting the TC from decomposition.

The ability of the CO to quickly form a paste suggests an advantage for a stable delivery of TC over time. In the case of the PM, an acid introduced into the system seemed to initiate production of the CO but not as a paste. Consequently, the use of acid delays the formation of a paste, which is superseded by the dissolution of TC and SF.

Releasing Mechanism of TC from CO Measurement of the change in the zeta potential was conducted using the CO particles. The zeta potential of the CO particles when dispersed in water showed a negative charge. However, the charge changed immediately to positive after the addition of the acid, reaching a maximum 30 min later and followed by a gradual decrease as shown in Fig. 5A. The pH of the system affected the change in the zeta potential and was lowered by the addition of the acid, followed by gradual increase.

These mechanisms are illustrated in Fig. 6. The aluminum moieties of the CO seemed to partly bond to the TC. The ad-

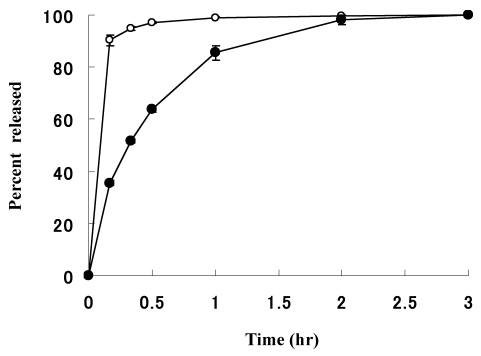

Fig. 4. Dissolution Profiles of TC from the CO (●) and the Physical Mixture (○) in No. 1 Disintegration Fluid Specific JP (pH 1.2) Evaluated Using the Flow-Through Cell Method

Data are expressed as mean±S.D. of three runs.

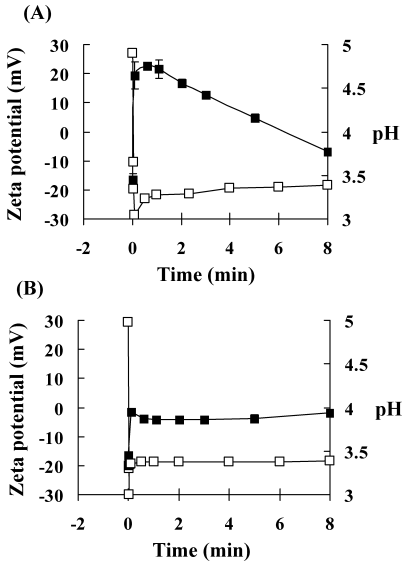

Fig. 5. Change in the Zeta Potential of the CO (■) and pH of the System (□) of the CO Alone (A) and with the Presence of Free Tetracycline (B)

Data are expressed as mean±S.D. of three runs.

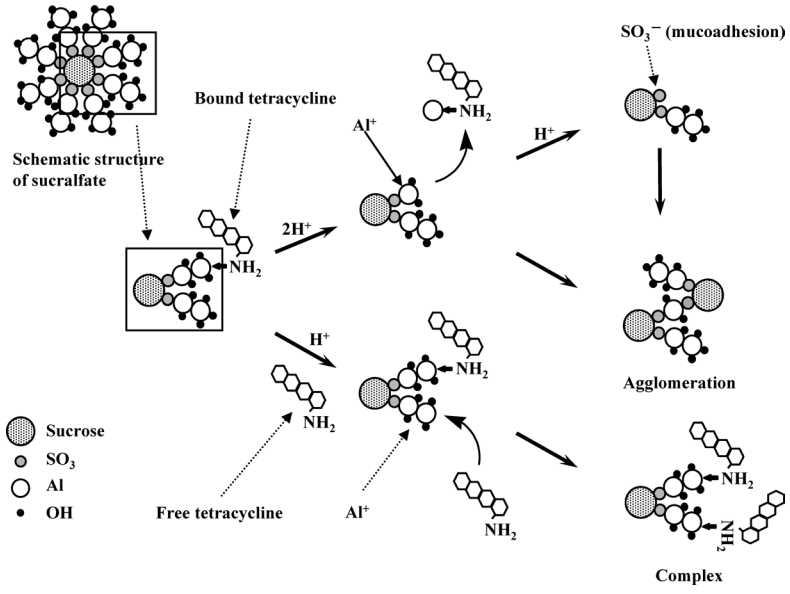

Fig. 6. Acid Consuming Process of the CO and Resultant Charged Surfaces of the Particles

dition of the acid dissociated the TC and the hydroxyl groups from the other aluminum moieties of the SF. The resultant positive charge of fresh aluminum moieties caused a positive change in the zeta potential. At this point, aluminum moieties of SF are released with the TC because of the strong coordinate bond between them. Concerning the structure of SF, the positive charge resulting from the release of the TC with Al indicates that it came from the inner aluminum moieties. In other words, the TC in the CO was bonding with the outer aluminum moieties of the SF. After release of the TC, the CO continued to consume the added acid completely to neutralize the system. As a result, the positively charged new aluminum moieties were also dissociated and negatively charged SOS groups were produced. The opposite charged sites were bound to each other to form the agglomeration of the CO. In this way, the positive charge of the particles at first was neutralized gradually from the agglomeration of the CO. This process appears to be closely related to the gradual release mechanisms of the TC, essential for production of the SOS groups that function in the agglomeration process, followed by the paste formation of the CO. From the test of TC release, we found that the CO formed a paste from the immediate release of TC and thus produced SOS groups. The surface area of the CO particles was reduced from the agglomeration and the resultant paste formation. The TC release rate apparently decreased as a result of reduction in the surface area.

With the presence of free TC in the system, the zeta potential did not become positive but remained neutral as shown in Fig. 5B. The pH was also constant. Apparently the free TC bonded to the positively charged new aluminum moieties produced by the addition of acid as shown in Fig. 6. Negatively charged SOS groups were not produced because the free TC bonded to the aluminum moieties before their dissociation. Possibly acid consumption was quickly accomplished by the dissociation of the hydroxyl groups from the aluminum moieties.

The zeta potential expressed the electrical characteristics of the particle surface of the CO and suggests that TC binds on the surface of the SF particles. Furthermore, TC appeared to bind to the outer aluminum moieties of SF.

Conclusion

Some antibiotics, such as the tetracycline family, strongly interact with SF under an acidic condition. It was demonstrated that the acidic complexation of TC with SF gradually released TC in an acidic condition. From the results, the flow-through cell method showed better sustained release profile than the paddle method because of its smaller amount of test medium and condition of no mixing. It suggested that the oral administration with fasting is more appropriate for CO. Furthermore, decomposition of TC in the acidic medium may be protected by paste formation. Zeta potential studies of the CO particles provided useful information on the release mechanism. The immediate release of the TC appeared to play a vital role in the paste formation. These findings are relevant to the eradication of *H. pylori*, which thrives under acidic conditions in the stomach. In our previous paper, the mucoadhesive property of the CO was demonstrated to be excellent. Here, we demonstrate that the gradual release of TC from the CO would directly attack *H. pylori* over a long period of time, suggesting possible eradication of *H. pylori* infection. The benefits of the mucoadhesive formulation of antibiotics presented in this study has potential to be the foundation for a new therapy of treatment for *H. pylori* infection with a high eradication rate, minimal side effects, easy compliance, and less risk of resistance. The next step is further study to clarify the actual antibiotic activities.

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