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CHIRAL INVERSION OF DRUG: ROLE OF INTESTINAL BACTERIA IN THE STEREOSELECTIVE SULPHOXIDE REDUCTION OF FLOSEQUINAN

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Abstract—Chiral inversion at a sulphoxide position of flosequinan enantiomers [(±)-7-fluoro-1-methyl-3-methylsulphinyl-4-quinolone] occurred in conventional rats but not in either germ-free rats or rats treated with antibiotics after an oral administration of each enantiomer. Thus, it was postulated that the chiral inversion occurred by mechanisms mediated by intestinal bacteria. The intestinal content isolated from conventional rats reduced *R*(+)- and *S*(−)-flosequinan to produce the sulphide, while intestinal content from rats treated with antibiotics did not reduce the drug. Several strains of facultative anaerobes possessed a high flosequinan reducing activity. *Escherichia coli*, *Klebsiella oxytoca* and *Klebsiella pneumoniae* reduced *R*(+)-flosequinan to the sulphide stereoselectively. On the other hand, *Enterobacter aerogenes* and *Micrococcus agilis* exclusively reduced *S*(−)-flosequinan. The sulphide, which could be produced by intestinal bacteria from *R*(+)- and *S*(−)-flosequinan, was readily absorbed upon an oral administration to rats, and was oxidized fairly rapidly to *R*(+)- and *S*(−)-flosequinan and further to the sulphone form. Based on these data, it has been confirmed that chiral inversion at the sulphoxide position of flosequinan enantiomers occur via stereoselective reduction of sulphoxide by intestinal bacteria to form the sulphide, followed by oxidation of the sulphide in the body to produce *R*(+)- and *S*(−)-flosequinan.

Key words: chiral conversion; intestinal bacteria; stereoselective reduction; flosequinan

Intestinal bacteria have been known to play important roles in the metabolism of many drugs and carcinogens. Drugs administered orally are exposed to intestinal bacteria before absorption. Intestinal bacteria are generally involved in the reduction of drugs. In fact, intestinal bacteria are reported to reduce a variety of compounds possessing double bonds, aldehyde, ketone, alcohol, nitro, *N*-oxide, and azo groups [1, 2]. In addition, the intestinal bacteria reduced sulphoxide containing drugs [3, 4].

Flosequinan [(±)-7-fluoro-1-methyl-3-methylsulphinyl-4-quinolone], a peripheral vasodilator with effects on both arterial and vascular beds, is given to patients orally [5, 6]. This compound has a chiral sulphur yielding two enantiomers, *R*(+)- and *S*(−)-flosequinan. The enantiomers of flosequinan are metabolized to the sulphone as a major metabolite, and to the sulphide as a minor metabolite [5–8]. The pharmacokinetic behaviour of each enantiomer is different and chiral inversion is observed in rats. The chiral inversion is assumed to occur via the sulphide form [8], since that form is oxidized to flosequinan by cytochrome P450 and flavin-containing monooxygenase (unpublished observations). Supporting the concept of the mechanism of chiral inversion, it was found that the extent of chiral inversion was greater when each enantiomer was given to rats orally: only a low level of chiral inversion was seen when given intravenously [8].

To confirm the mechanism responsible for the chiral inversion evidence that the intestinal bacteria can reduce flosequinan to yield the sulphide is presented in this paper. Also reported is that strains of intestinal bacteria contain enzymes able to reduce flosequinan enantiomers stereoselectively.

MATERIALS AND METHODS

Materials. Racemic flosequinan, flosequinan sulphone (7-fluoro-1-methyl-3-methylsulphonyl-4-quinolone) and flosequinan sulphide (7-fluoro-1-methyl-3-methylthio-4-quinolone) was supplied by the Boots Company PLC (Nottingham, U.K.). The enantiomers of flosequinan were prepared in the Research Laboratory of Otsuka Pharmaceutical (Tokushima, Japan). $NADP^+$, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were obtained from Oriental Yeast (Tokyo, Japan). Dithiothreitol, neomycin, bacitracin chloramphenicol, nystatin and erythromycin were obtained from Wako Pure Chemicals (Osaka, Japan). Penicillin and streptomycin were obtained from Meiji Confectionery (Tokyo, Japan). Benzyl viologen was obtained from Tokyo Chemical Industry (Tokyo, Japan). All other chemicals and solvents were of the highest grade commercially available.

Animals and treatments. Sprague–Dawley male

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rats (6–7 weeks old) were purchased from Japan SLC (Shizuoka, Japan) and maintained on a commercial chow (MF; Oriental Yeast) and tap water *ad lib.* When necessary, rats were treated with antibiotics orally every 12 hr for 3 days before and throughout the period of experiments. For experiment 1 the mixture of antibiotics, which consists of neomycin, streptomycin and bacitracin, was given to rats at a dose of 200 mg/kg for each drug [9]. For experiment 2 the mixture of antibiotics, consisting of chloramphenicol (87.5 mg/kg), nystatin (25,000 units/kg), streptomycin (100 mg/kg), erythromycin (100 mg/kg) and penicillin (10,000 units/kg), was given to rats [10]. Germ-free rats purchased from Nippon Clea (Tokyo, Japan) were kept in a clean room and maintained on sterilized chow and water *ad lib.* *R*(+)- and *S*(−)-flosequinan and flosequinan sulphide were suspended with distilled water and administered orally at a dose of 10 mg/kg. Blood samples were collected from the tail vein and were centrifuged to obtain plasma. Urine samples excreted between 24 and 48 hr after administration were collected. The plasma and urine samples were stored at −20° until analysis.

Preparation of intestinal content. The intestinal content of rats was prepared according to the method of Ingebrigtsen and Froslie [11]. Rats were killed and the content of the cecum was removed anaerobically under a stream of carbon dioxide gas. The content of the cecum was homogenized by bubbling with carbon dioxide gas in 4 vol. of GAM medium (Nissui Pharmaceutical, Tokyo, Japan) and centrifuged at 500 rpm for 10 min. The upper phase was used immediately after preparation.

Culture and preparation of crude extracts of bacteria. Twenty-eight strains of bacteria were obtained from the Japan Collection of Microorganisms, Institute of Physical and Chemical Research (Saitama, Japan). Bacteria were grown in GAM medium anaerobically using the Gas Pak system (Becton Dickinson and Company, MD, U.S.A.) or aerobically with shaking. Crude extracts of bacteria were prepared according to the method of Kinouchi *et al.* [12]. Briefly, cells were harvested, washed and suspended in a PBS (pH 7.4). The suspension was treated with sonic oscillation (Model T-A-4280, Kaijo Denki, Tokyo, Japan) at 2.5 A for 5 min, and then centrifuged at 9000 *g* for 20 min. The supernatant fraction was stored at −80° after freezing in liquid nitrogen until use. The protein contents were determined by the method of Lowry *et al.* [13] using BSA as a standard.

Incubation of flosequinan with intestinal content and bacteria. A reaction mixture containing *R*(+)- or *S*(−)-flosequinan at a final concentration of 100 μM or 3 mM and intestinal content or bacterial culture was incubated at 37° (in the case of *Micrococcus agilis* at 30°) anaerobically or aerobically. Heat-treatment of the intestinal content was carried out at 100° for 5 min.

HPLC analysis. The concentrations of *R*(+)- and *S*(−)-flosequinan and their metabolites (flosequinan sulphide and flosequinan sulphone) were determined using a stereoselective HPLC. The materials in biological samples were extracted with chloroform (3 mL). The chloroform layer (2 mL) was evaporated to dryness under the stream of nitrogen at 40°. The residue was dissolved in the mobile phase described below and applied to HPLC equipped with an HLC-803D system (Tosoh, Tokyo, Japan), analytical column Chiralcel OD (4.6 mm i.d. × 250 mm; Daicel Chemical Industries, Tokyo, Japan), UV-8000 UV–visible absorbance detector (Tosoh), RF-530 fluorescence detector (Shimadzu, Kyoto, Japan) and C-R6A integrator (Shimadzu). The mobile phase consisting of ethanol–methanol (22:78 v/v) was delivered at a flow rate of 0.7 mL/min. *R*(+)- and *S*(−)-flosequinan and flosequinan sulphone were

Table 1. Urinary excretions after oral administration of *R*(+)- and *S*(−)-flosequinan at a dose of 10 mg/kg to germ-free rats and rats treated with antibiotics

		Urinary excretion (% of dose)							
		R(+)-Flosequinan				*S*(−)-Flosequinan			
Rats	Time (hr)	*R*-FSO	*S*-FSO	FS	FSO_2	*R*-FSO	*S*-FSO	FS	FSO_2
Control	0–24	2.47 ± 0.26	0.14 ± 0.01	ND	63.6 ± 11.9	0.79 ± 0.17	9.28 ± 1.58	ND	62.8 + 8.4
	24–48	0.13 ± 0.01	ND	ND	8.0 ± 2.0	0.01 ± 0.01	0.24 ± 0.06	ND	7.9 ± 1.0
Germ-free	0–24	1.26 ± 0.06	ND	ND	58.4 ± 2.9	ND	6.32 ± 1.69	ND	59.3 ± 18.5
	24–48	0.03 ± 0.02	ND	ND	8.6 ± 3.6	ND	ND	ND	11.2 ± 4.6
Antibiotics Experiment 1	0–24	4.22 ± 0.16	ND	ND	55.7 ± 2.2	ND	11.20 ± 1.77	ND	47.4 ± 3.3
	24–48	0.04 ± 0.04	ND	ND	6.9 ± 0.7	ND	0.22 ± 0.06	ND	6.6 ± 1.4
Antibiotics Experiment 2	0–24	4.93 ± 0.93	ND	ND	51.6 ± 1.2	ND	11.86 ± 0.69	ND	45.5 ± 1.1
	24–48	0.15 ± 0.08	ND	ND	10.4 ± 0.5	ND	0.56 ± 0.10	ND	10.3 ± 0.5

The results are the means ± SD from three rats.

R-FSO, *R*(+)-flosequinan; *S*-FSO, *S*(−)-flosequinan; FS, sulphide metabolite; FSO_2, sulphone metabolite; ND, not detected (<0.01%).

detected by their absorbance at 320 nm. Flosequinan sulphide was determined fluorometrically with excitation at 370 nm and emission at 430 nm.

Flosequinan reducing activity. The activities of *R*(+)- and *S*(−)-flosequinan reductase were calculated by measuring the production of the sulphide. Since flosequinan reducing activities in crude extracts of bacteria were scarcely detected using NADPH and NADH instead of benzyl viologen as electron donors, benzyl viologen was used in the present study. This method was reported by Yoshihara and Tatsumi [14] in the assay of aldehyde oxidase and sulphoxide reductase. A typical incubation mixture consisted of 100 mM Na–K phosphate buffer (pH 6.8), 1 mM benzyl viologen, 0.1 mM dithothreitol, 5 μg protein of crude extract of bacteria and a substrate [0.5 mM *R*(+)- or *S*(−)-flosequinan]. The reaction was started by the addition of 10 mM dithionite in a final volume of 0.1 mL. After incubation for 15 min at 37° the reaction was terminated by the addition of 5% (w/v) trichloroacetic acid (100 μL). After centrifugation the resulting supernatant fraction (100 μL) was mixed with 0.5 M Na–K phosphate buffer (2 mL, pH 6.8), and fluorescence measured using a 650-10LC fluorescence spectrophotometer (Hitachi, Tokyo, Japan) with excitation at 370 nm and emission at 430 nm.

RESULTS

Chiral inversion in germ-free rats and treated with antibiotics

To determine the role of intestinal bacteria in the metabolism of flosequinan, *R*(+)- and *S*(−)-flosequinan were given orally to germ-free rats and rats treated with antibiotics at a dose of 10 mg/kg. The urinary excretions of *R*(+)- and *S*(−)-flosequinan, flosequinan sulphide and sulphone are shown in Table 1. When *R*(+)- and *S*(−)-flosequinan were administered, more than 50% of the administered compounds were excreted as the sulphone form. The sulphide was not detected in urine. In conventional rats a respective optical antipode was detected separately in urine after the oral administration of *R*(+)- and *S*(−)-flosequinan. On the other hand, the optical antipode was not detected in the germ-free rats and the rats pretreated with antibiotics. From the fact that chiral inversion occurred in only conventional rats, it was assumed that the intestinal bacteria played a key role in the chiral inversion of flosequinan.

Reduction of R(+)- *and* S(−)-*flosequinan by rat intestinal bacteria*

To further clarify the metabolic pathways of flosequinan, in which intestinal bacteria are involved, the metabolism was investigated by intestinal content *in vitro*. *R*(+)- and *S*(−)-flosequinan, sulphide and sulphone forms were added to the incubation mixture containing intestinal content and incubated under an anaerobic condition. Figure 1 shows the time-dependent reduction of *R*(+)- and *S*(−)-flosequinan metabolism by 1 and 5% (w/v) intestinal content. Only the sulphide metabolite appeared when *R*(+)- and *S*(−)-flosequinan were added to the incubation mixture containing intestinal content. *R*(+)- and *S*(−)-flosequinan decreased according to the production of the sulphide form, which was also dependent on the concentration of intestinal content added to the mixture. The *R*(+)-flosequinan was a better substrate than was the *S*(−)-flosequinan for a reductase(s) in bacteria in intestinal content. Heat-

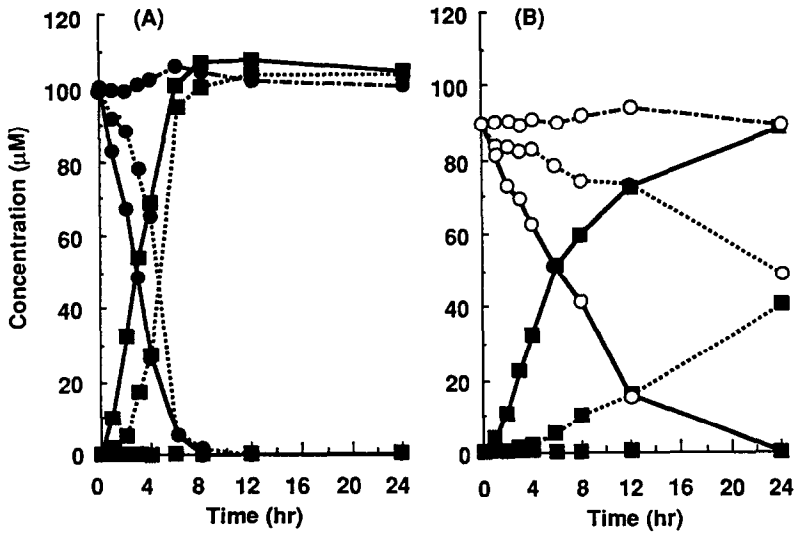

Fig. 1. Time-dependent reduction of *R*(+)- and *S*(−)-flosequinan to form the sulphide by intestinal content from untreated rats. *R*(+)- (A) and *S*(−)-flosequinan (B) were incubated anaerobically at 37° with 1% (dashed lines) and 5% (solid lines) intestinal content and with heat-inactivated intestinal content (5%, dashed and dotted lines) in GAM medium. ●, *R*(+)-flosequinan; ○, *S*(−)-flosequinan; ■, flosequinan sulphide.

Table 2. Effects of treatment of rats with antibiotics and anaerobicity on the reduction by intestinal content of *R*(+)- and *S*(−)-flosequinan to produce the sulphide

Time (hr)	*R*(+)-Flosequinan				*S*(−)-Flosequinan			
	Control*	Antibiotics Expt 1*	Antibiotics Expt 2*	Aerobic condition	Control*	Antibiotics Expt 1*	Antibiotics Expt 2*	Aerobic condition
	(% of reduction to sulfide)							
2	16.9	ND	ND	13.3	4.5	ND	ND	ND
4	41.5	ND	ND	22.1	12.3	ND	ND	4.1
8	76.1	ND	ND	37.8	22.6	ND	ND	10.1
12	85.9	ND	ND	47.7	30.2	ND	ND	12.6
24	90.2	ND	36.0	76.4	53.4	ND	41.1	39.5

The means of the experiments using the intestinal flora from three rats.
ND, not detectable (<0.1%).
* Under anaerobic condition.

Table 3. Reduction of *R*(+)- and *S*(−)-flosequinan by crude extracts prepared from bacteria

Bacteria strains	Reductase activity (nmol/min/mg)	
	R(+)-flosequinan	*S*(−)-flosequinan
Oligate anaerobes		
Bacteroides bivius	ND	ND
Bacteroides distasonis	0.29	ND
Bacteroides pyogenes	ND	ND
Bacteroides vulgatus	ND	ND
Bifidobacterium adolescentis	ND	ND
Bifidobacterium bifidum	ND	ND
Bifidobacterium longum	ND	ND
Clostridium innocuum	ND	ND
Eubacterium limosum	ND	ND
Eubacterium nitritogenes	ND	ND
Fusobacterium necrophorum	ND	ND
Megashaera elsdenii	ND	ND
Mitsuokella multiacida	ND	ND
Peptostreptococcus prevotii	ND	ND
Propionibacterium acnes	ND	ND
Selenomonas sputigena	ND	ND
Facultative anaerobes		
Bacillus alcalophilus	ND	ND
Enterobacter aerogenes	ND	5.82
Enterococcus faecium	ND	ND
Escherichia coli	5.6	ND
Klebsiella oxytoca	19.5	ND
Klebsiella pneumoniae	20.3	ND
Lactobacillus acidophilus	ND	ND
Lactobacillus brevis	ND	ND
Lactobacillus casei	ND	ND
Micrococcus agilis	ND	7.11
Morganella morganii	27.6	4.17
Staphylococcus aureus	ND	ND

ND, not detectable (<0.01 nmol/min/mg).

treated intestinal content did not decrease the concentration of *R*(+)- and *S*(−)-flosequinan added to the incubations. On the other hand, when the sulphide and sulphone forms were added to the incubation mixture containing intestinal bacteria, no change occurred in the concentrations of sulphide and sulphone forms (data not shown).

To confirm that intestinal bacteria in intestinal content were involved in the reduction of *R*(+)- and *S*(−)-flosequinan, the reduction of *R*(+)- and *S*(−)-flosequinan was examined by intestinal content from rats treated with antibiotics. When *R*(+)- and *S*(−)-flosequinan were incubated with intestinal content (2.5%) from conventional rats (control) anaerobically, the production of the sulphide form was observed (Table 2). The intestinal content from rats

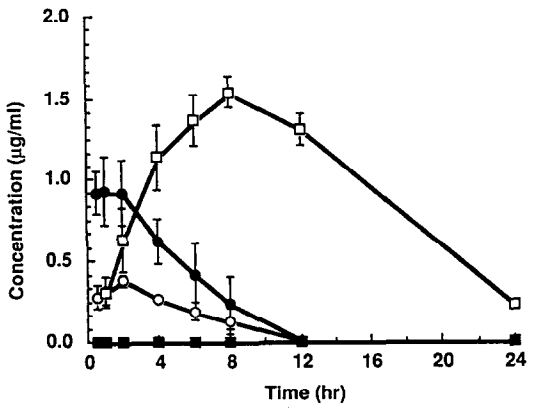

Fig. 2. Time-dependent changes in the plasma concentrations of $R(+)$- and $S(-)$-flosequinan, sulphone and sulphide forms after an oral administration of flosequinan sulphide at a dose of 10 mg/kg to male rats. Each point represents the mean ± SD from three rats. ●, $R(+)$-flosequinan; ○, $S(-)$-flosequinan; □, flosequinan sulphone; ■, flosequinan sulphide.

treated with antibiotics (experiment 1) did not produce the sulphide form even after a 24 hr incubation. No sulphide production was seen with the intestinal content from the rats treated with other antibiotics until 12 hr incubation, but was seen after 24 hr incubation in experiment 2. These data indicate that intestinal bacteria were involved in the reduction of flosequinan.

Intestinal bacteria were divided into facultative anaerobes and obligate anaerobes. Facultative anaerobes grow both under anaerobic and aerobic conditions, whereas obligate anaerobes do not grow under an aerobic condition. To confirm which bacteria in the intestine were involved in the reduction of $R(+)$- and $S(-)$-flosequinan the incubation was carried out both aerobically and anaerobically. The reduction of $R(+)$- and $S(-)$-flosequinan to form the sulphide occurred when incubated with the intestinal content under aerobic and anaerobic conditions (Table 2). The rate of reduction under anaerobic conditions was approximately 1.5–2.0 times faster than that under aerobic conditions.

Examination for stereoselective reduction in bacteria

In order to examine the reductase in intestinal bacteria able to produce the sulphide form from $R(+)$- and $S(-)$-flosequinan, crude extracts of 16 and 12 strains of obligate and facultative anaerobes, respectively, were prepared and incubated with $R(+)$- and $S(-)$-flosequinan. Reductase activities in bacteria are shown in Table 3. Almost all obligate anaerobes, except for *Bacteroides distasonis*, showed no reductase activity. Some facultative anaerobes showed relatively high reductase activity. *Escherichia coli*, *Klebsiella oxytoca* and *Klebsiella pneumoniae* showed high reductase activity specific to $R(+)$-flosequinan. Another two facultative anaerobes, *Enterobacter aerogenes* and *Micrococcus agilis*, showed high reductase activity specific to $S(-)$-flosequinan. On the other hand, *Morganella morganii* showed reductase activities both of $R(+)$- and $S(-)$-flosequinan.

Lower parts of the intestinal tract are usually kept anaerobic. Thus, it was examined whether the reductase activities of the facultative anaerobes were observed when cultured under anaerobic conditions. The above-mentioned six facultative anaerobes, which showed high reductase activity, also reduced $R(+)$- and/or $S(-)$-flosequinan. In addition, the stereoselectivity of the reductase remained unchanged (Table 3). These results indicate that anaerobicity is not essential for flosequinan reductase activities in these facultative anaerobes (Table 3).

Absorption and metabolism of the flosequinan sulphide

If chiral inversion occurred via the formation of the sulphide produced by bacteria in the intestine, the sulphide form must be absorbed and subsequently oxidized to $R(+)$- and $S(-)$-flosequinan by an enzyme(s) in rats. This possibility was examined by measuring the plasma concentrations of metabolites after oral administration of the sulphide to rats; results are shown in Fig. 2. The sulphide was not detectable even after the administration of the sulphide: $R(+)$- and $S(-)$-flosequinan and the sulphone appeared in the plasma. The concentration of $R(+)$- and $S(-)$-flosequinan reached maximum levels (0.93 and 0.38 μg/mL) in plasma 1 and 2 hr after the administration, respectively. The concentration of the sulphone form reached a plateau level (~1.5 μg/mL) 8 hr after the administration. AUCs of $R(+)$- and $S(-)$-flosequinan and the sulphone were calculated to be 4.89, 1.99 and 22.6 μg · hr/mL, respectively. It was indicated that the sulphide was absorbed and rapidly oxidized to yield the sulphoxides and develop further to the sulphone form.

DISCUSSION

Compounds containing a sulphoxide group are reduced to the sulphide form in animals [8, 15, 16]. The reduction may occur *in vivo* in either tissues or the intestinal bacteria. It has been reported that thioredoxin-linked enzyme systems [17], such as methionine sulphoxide reductase [18], sulindac reductase [19] and dimethyl sulphoxide reductase [20], catalysed the reduction to the sulphide form in liver and kidneys. Aldehyde oxidase has also been reported to reduce the sulphoxide compounds under aerobic conditions [14]. In the case of flosequinan, it was suggested that this drug was reduced by the cytosolic enzymes from the liver and kidneys, such as the aldehyde oxidase and the thioredoxin-linked enzyme systems *in vitro* (unpublished observations). However, it was also assumed that intestinal bacteria were involved in the reduction of flosequinan, since the extents of chiral inversion which occurred via the formation of the sulphide when given orally was greater than that seen when given intravenously [8]. Therefore, the role of intestinal bacteria in the sulphoxide reduction of flosequinan was investigated in the present study.

In this study, it was clarified that intestinal bacteria were capable of reducing *R*(+)- and *S*(−)-flosequinan. The intestinal content from rats treated with antibiotics was devoid of the reducing activities. However, the reducing activities returned after 24 hr incubation in experiment 2 due to re-growth of the intestinal bacteria. It can be considered that decreased bacteria by antibiotics regrew with reducing capabilities. The reductase in intestinal bacteria rather than enzymes in the body may be a major enzyme responsible for the reduction of flosequinan, since the chiral inversion was not observed in germ-free rats and rats treated with antibiotics. The authors have determined that flavin-containing mono-oxygenase and cytochrome P450 catalysed the stereoselective oxidation of the sulphide to produce *R*(+)- and *S*(−)-flosequinan (unpublished observations). Based on these data, it is confirmed that chiral inversion occurs via the reduction of the sulphoxides in the intestine by intestinal bacteria to form the sulphide, followed by oxidation of the sulphide in the body by flavin-containing mono-oxygenase and cytochrome P450 to produce *R*(+)- and *S*(−)-flosequinan.

It is known that the composition of intestinal bacteria is influenced by diet, age and disease [21]. In addition, treatment of animals with foreign compounds, especially antibacterial drugs, affects the intestinal bacteria. These compounds have been widely used clinically. Therefore, chiral inversion of flosequinan may also be influenced by diet, age, disease and foreign compounds.

The flosequinan reductase activity in the bacterial culture was observed using the GAM medium (Fig. 2), while the activity was not detected using LB* or NB medium. However, the crude extracts of the cells grown in the LB or NB medium showed a reductase activity comparable to that cultured in the GAM medium using reduced benzyl viologen and methyl viologen as an electron donor (data not shown). These results suggest that the flosequinan reductase is expressed in growing cells cultured in the LB or NB medium. These results may be accounted for by assuming that an electron donor(s) or proteins related to electron transfer are not present in growing cells cultured in the LB or NB medium. The GAM medium contains many kinds of nutritional factors: meat extract, liver extract, etc. It is not clear which one of the compositions in the GAM medium relates to the electron transfer system. Despite these experimental results, it is assumed that the reductase in intestinal flora can reduce flosequinan, since there are various kinds of nutrients and compounds in the intestine.

It is of interest that the high flosequinan reductase activity was seen in facultative anaerobes, among the investigated strains of intestinal bacteria. Although only small amounts (less than 1% of obligate anaerobes) are reported to grow in the intestine [21], the reduction capacity of intestinal content seen after aerobic incubations was more than half the level seen after anaerobic incubations.

Escherichia coli showed a high flosequinan reductase activity. Dimethyl sulphoxide reductase [22, 23], biotin sulphoxide reductase [24] and trimethylamine *N*-oxide reductase [25] were purified and cloned from *Escherichia coli* and other bacteria. These enzymes can serve as terminal electron transfer enzymes and reduce sulphoxide compounds with low specificity. However, the stereoselectivity of the reductase has not, to our knowledge, been reported. The reductase activities of these enzymes were present in cells grown anaerobically but repressed in aerobic growth [26, 27]. The present data indicate that flosequinan reductase activity in cells grown aerobically is higher than that in cells grown anaerobically, suggesting that flosequinan reductases differ from purified enzymes and those cloned from bacteria.

It was most interesting that the stereoselectivity was seen in the flosequinan reductase. The reductase may be a useful tool for the analysis of the three-dimensional structure of active sites and stereoselectivity in future studies. The purified or cloned enzymes with stereoselective sulphoxide reductase activity have not been previously reported. This group is currently purifying the stereoselective flosequinan reductase in order to compare these properties and structures.

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* Abbreviations: LB, Luria-Bertani; NB, Nutrient-Broth.

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