Separation of Basic Drug Enantiomers by Capillary Electrophoresis with New Glycosaminoglycan

Tsuyoshi Tsukamoto,* Takanori Ushio, and Jun Haginaka*†
Pharmaceutical Research LAB., Taiho Pharmaceutical CO., LTD., 224-2 Ebisuno, Hiraishi, Kawauchi-cho, Tokushima 771-01
†*Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11-68, Koshien Kyuban-cho, Nishinomiya, Hyogo 663*

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A new fucose-containing glycosaminoglycan was investigated as the chiral additive for the separation of basic drug enantiomers by capillary electrophoresis. Tolperisone and eperisone enantiomers were not separated with α- or β-cyclodextrin, or heparin as the chiral additive, but were separated with the new fucose-containing glycosaminoglycan. A variety of basic drug enantiomers were resolved using 2-5%(w/w) solution of the glycosaminoglycan in 10 mM phosphate buffer (final pH 5.0).

The separation and analysis of enantiomers are an important subject in the development of a bioactive compound. Various kinds of chromatographic methods have been developed for resolution of enantiomeric compounds. Among those, HPLC methods have played an important role in analytical and preparative purposes.[1] Capillary electrophoresis (CE) is a new separation technique with high resolving powers and is used in the biochemical and analytical fields. Recently, various CE methods have been developed for the separations of drug enantiomers using chiral selectors as the running buffer additives.[2] The chiral additives so far employed have included polysaccharides, proteins, bile salts and chiral mixed micelles. Glycosaminoglycans such as heparin[3] and chondroitin sulfate C[4] were successfully used as the additive for chiral resolution of basic and neutral drugs. Chondroitin sulfate C was successfully applied to test the optical purity of a drug.

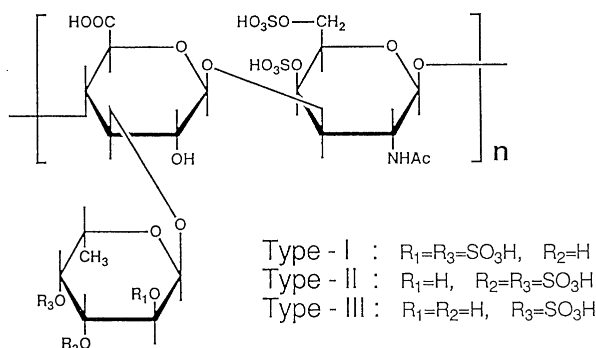

Figure 1. Structure of FGAG and DHG.

Recently, fucose-containing glycosaminoglycan (FGAG)[5] was isolated from the body wall of sea cucumber, *Stichopus japonicus*. By oxidative depolymerization of FGAG with hydrogen peroxide, depolymerized holothurian (which means sea cucumber) glycosaminoglycan (DHG) was obtained. As shown in Figure 1, FGAG and DHG were a mixture of type-I, -II and -III in the ratio 5:3:1. DHG is now in clinical trials as anticoagulant for human use.[6,7] The average molecular weights of FGAG and DHG were estimated to be about 67000 and 13000, respectively, by size-exclusion chromatographic system with low-angle laser light-scattering detection. In this study, we investigated new glycosaminoglycans, FGAG and DHG, as the chiral additives for the separations of basic drug enantiomers, whose structures are shown in Figure 2, by CE. Since FGAG and DHG contained fucose in the molecule, it was expected that chiral recognition properties of FGAG and DHG could be different from those of other glycosaminoglycans such as heparin and chondroitin sulfate C.

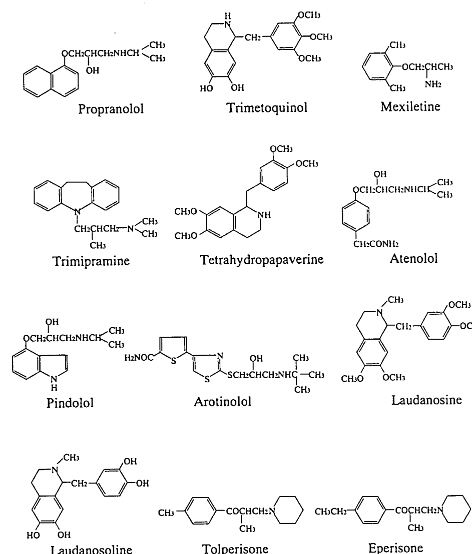

Figure 2. Structures of Basic Drugs.

Figure 3 shows typical electropherograms of tolperisone using FGAG and DHG as the buffer additives. Tolperisone enantiomers were resolved within 20 min. The CE system was operated in the conventional mode with the cathode at the detector end of the capillary under an applied voltage of 12 kV. Two %(w/w) FGAG or DHG solution of pH 5.0 in 10 mM phosphate buffer was used as the buffer additive. The fused silica capillary columns were 75 μm i.d with a column length of 57 cm (effective length, 50 cm). Tolperisone was introduced with pressure injection mode. Similar chiral recognition of eperisone enantiomers was obtained with FGAG or DHG.

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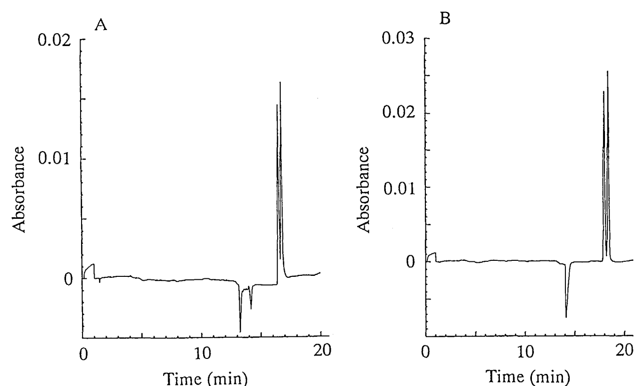

Figure 3. Electropherograms of tolperisone in 10 mM phosphate buffer of pH 5.0 containing 2%(w/w) FGAG (A) or DHG (B).

The chiral recognition abilities of FGAG and DHG were compared with those of heparin, and α- and β-cyclodextrins (CDs). Two %(w/w) heparin solution of pH 4.5 in 10 mM phosphate buffer and 20 mM α- or β-CD solution of pH 2.7 in 25 mM phosphate buffer containing 2 M urea were used as the running buffer. The enantioseparation factor (t_2/t_1) was calculated based on the migration times of the first and second migrated enantiomers (t_1 and t_2). The results obtained were summarized in Table 1. Tolperisone and eperisone enantiomers were not separated with α- or β-CD, or heparin as the chiral additive despite optimization of separation conditions, but completely separated with FGAG or DHG.

Table 1. Comparision of FGAG, DHG, heparin, α-CD and β-CD with respect to the chiral resolution of tolperisone and eperisone

	Enatioseparation factor	
Chiral selector	Tolperisone	Eperisone
FGAG[a]	1.016	1.014
DHG[a]	1.023	1.013
heparin[b]	1.000	1.000
α-CD[c]	1.000	1.000
β-CD[c]	1.000	1.000

[a]running buffer, 10 mM phosphate buffer of pH 5.0 containing 2%(w/w) FGAG or DHG; applied voltage 12 kV. [b]running buffer, 10 mM phosphate buffer of pH 5.0 containing 2%(w/w) heparin; applied voltage, 15 kV. [c]running buffer, 25 mM phosphate buffer of pH 2.7 containing 2 M urea and 20 mM α- or β-CD; applied voltage, 15 kV.

Chiral recognition abilities of FGAG for other drug enantiomers were further investigated. The effects of buffer concentration, buffer pH and concentration of FGAG on migrations and enantioseparations of basic drugs were examined. A variety of basic drug enantiomers were resolved using 2-5%(w/w) FGAG solution of pH 5.0 in 10 mM phosphate buffer. The enantioseparation factors of resolved drug enantiomers within 30 min were 1.017 for propranolol, 1.020 for trimetoquinol, 1.009 for mexiletine, 1.012 for trimipramine, 1.011 for tetrahydropapaverine, 1.017 for pindolol, 1.007 for atenolol, 1.010 for arotinolol, 1.003 for laudanosine and 1.003 for laudanosoline. In addition to ionic, hydrogen-bonding and hydrophobic interactions, inclusion within the FGAG or DHG super-structure might play an important role in chiral recognition of these drug enantiomers. When the 3%(w/w) FGAG and DHG solutions were used, FGAG gave the higher enantioseparation factor for the separation of propranolol than DHG. On the other hand, the FGAG and DHG gave similar enantioselectivity for the separations of tolperisone and eperisone. It is interesting that the effect of the molecular weight of the new glycosaminoglycan on the chiral recognition ability is observed, depending on a drug enantioseparated.

The results obtained above reveal that the new glycosaminoglycans (FGAG and DHG) are a promising candidate as the chiral additive for the separation of drug enantiomers by CE. The separation of drug enantiomers will be optimized by changing buffer concentration, buffer pH, and concentration and molecular weight of the new glycosaminoglycan. Further study on this point is in progress.

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