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Development of a highly sensitive and specific immunoassay for enrofloxacin based on heterologous coating haptens

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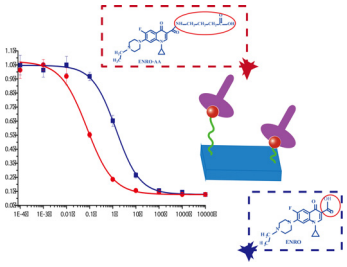
HIGHLIGHTS

- A new derivative of enrofloxacin was synthesized and used as coating hapten.
- The effect of coating hapten on the sensitivity and specificity of ELISA was studied.
- A highly sensitive and specific immunoassay for enrofloxacin was developed.

GRAPHICAL ABSTRACT



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ABSTRACT

In the paper, an enzyme-linked immunosorbent immunoassay (ELISA) for detection of enrofloxacin was described using one new derivative of enrofloxacin as coating hapten, resulting in surprisingly high sensitivity and specificity. Incorporation of aminobutyric acid (AA) in the new derivative of enrofloxacin had decreased the IC_{50} of the ELISA for enrofloxacin from 1.3 $\mu g\,L^{-1}$ to as low as 0.07 $\mu g\,L^{-1}$. The assay showed neglect cross-reactivity for other fluoroquinolones but ofloxacin (8.23%), marbofloxacin (8.97%) and pefloxacin (7.29%). Analysis of enrofloxacin fortified chicken muscle showed average recoveries from 81 to 115%. The high sensitivity and specificity of the assay makes it a suitable screening method for the determination of low levels of enrofloxacin in chicken muscle without clean-up step.

1. Introduction

Enrofloxacin (Fig. 1), one member of fluoroquinolones, is a high-potency effective against enteric Gram-negative bacteria, and it has been widely used only in veterinary clinical practice [1]. According to one WHO report in 1998, enrofloxacin is globally the most extensively approved antibiotic of the fluoroquinolones for livestock and aquiculture [2,3]. Thus, its residues may persist in edible tissue or product of food-producing animal, and also may result in the development of drug-resistant bacterial strains or allergies [4,5], for example, the USA FDA has withdrew the approval of enrofloxacin for poultry in 2005 with consideration of it causing the emergence of resistance in *Campylobacter* [3,6]. In order to protect consumers from potential contaminated food, the European Commission has established the maximum residue limit (MRL) for enrofloxacin at 100 $\mu g\,kg^{-1}$ in several edible animal tissues, while, Japan has set at 10 $\mu g\,kg^{-1}$ in chicken muscle [7,8]. In China, the species of animal, usage, dosage, and withdrawal period of enrofloxacin have been determined by the Ministry of Agriculture of the People' Republic of China (No. 235, 2002). These have created an urgent need for sensitive, rapid, robust, and accurate analytical methods to monitor enrofloxacin residues in the food supply of animal origin.

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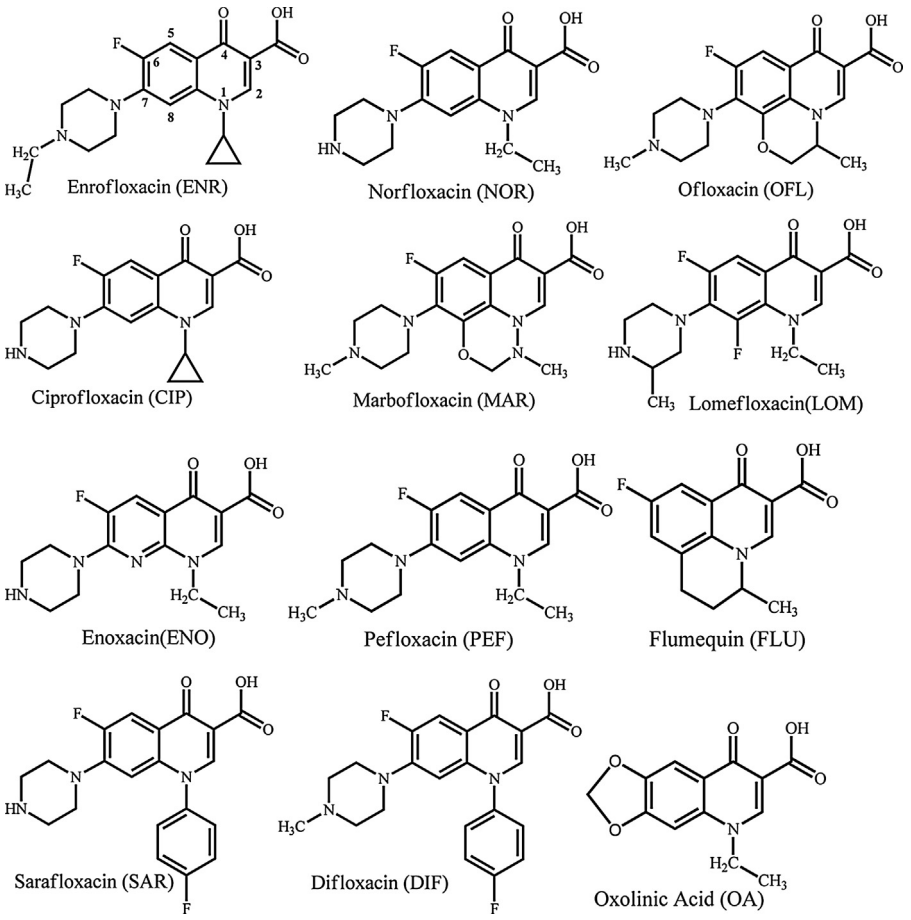

Fig. 1. Chemical structures of fluoroquinolones.

Some analytical methods have been developed for the determination of fluoroquinolons residues, mostly including enrofloxacin, in tissues or product of food-producing animal such as liquid chromatography [9,10] and some methods are special for enrofloxacin [11–13]. Although these methods are mostly sensitive and accurate, they are generally expensive and time consuming and not suitable for routine monitoring a large amount of samples. Recently, alternative methods such as immunoassay based on antigen–antibody recognition have been reported for screening enrofloxacin in animal tissues [14,15]. Our group has reported the liposome immune lysis assay and quantum dot-based fluoroimmunoassay for enrofloxacine in carp and chicken muscle [16,17] and also generic-ELISA for 12 fluroquinolones in different food matrices [18]. Several immunoassays for the determination of enrofloxacine in edible animal tissues and aquatic product based on poly- or monoclonal antibodies are also described in the past decade [14,15,19,20]. However, the sensitivity of these methods was not very high, or the sample pretreatment steps were quite complicated and time-consuming, thus they could not meet the requirement of strictly regulation for enrofloxacin. Normally, the heterologous enzyme immunoassay systems with different bridge length linkers between the hapten and carrier protein presents a good alternative to homologous assays in achieving higher sensitivity of ELISA [2,5], whereas, this strategy is not, so far, employed to develop ELISA for enrofloxacin to the author's knowledge, partly due to the easy realization of enrofloxacin coupling to carrier protein by using its carboxyl group at position 3 (Fig. 1).

In the paper we presented here, we have synthesized one new enrofloxacin derivative and used as coating hapten to develop ELISA for enrofloxacin in chicken muscle. When combining with one new prepared polyclonal antibody, the developed heterologous ELISA in bridge length showed high sensitivity and specificity for enrofloxacin.

2. Experimental

2.1. Regents and apparatus

Bovine serum albumin (BSA), ovalbumin (OVA), 4-aminobutanoic acid, *N*-hydroxysuccinimide (NHS), and 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (EDC), isobutyl chloroformate, tributylamine, ofloxacin, lomefloxacin, enoxacin, danofloxacin, oxolinic acid, and marbofloxacin were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Enrofloxacin, ciprofloxacin, norfloxacin, flumequine, pefloxacin, sarafloxacin, and difloxacin were purchased from the China Institute of Veterinary Drug Control (Beijing, P.R. China). The peroxidase-conjugated Goat anti-Rabbit IgG were acquired from Huamei Biotech Co. (Beijing, P.R. China). Incomplete Freund's adjuvant (IFA), complete Freund's adjuvant (CFA) was obtained from Gibco BRL (Carlsbad, CA, USA). Deionized water was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA). Polystyrene microtiter plates were purchased from Costar (Costar Inc., Cambridge, MA, USA). The NanoDrop ND-1000 spectrophotometer was purchased from Gene Company Ltd. (Hong Kong, P.R. China). The ELISA plate reader was obtained from TECAN Inc. (Durham, NC, USA). Hapten was confirmed by high performance liquid chromatography–mass spectrometry (HPLC–MS/MS). Chromatography was performed on a Waters Alliance 2690 LC system (Waters Corp., Milford, MA, USA) and the Quattro LC triple-quadrupole