Production and Characterization of Monoclonal Antibodies against Sarafloxacin and Cross-Reactivity Studies of Related Fluoroquinolones

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Monoclonal antibodies were developed that bind sarafloxacin, a fluoroquinolone approved by the Food and Drug Administration for use against *Escherichia coli* in poultry. Splenocytes from mice immunized with a bovine serum albumin−sarafloxacin conjugate were fused with SP2/0 myeloma cells, and hybridomas secreting antibodies against sarafloxacin were selected and cloned. An enzyme-linked immunoassay was developed, and 50% inhibition of control values ranged from 7.3 to 48.3 ppb using sarafloxacin as the competitor. Tissue samples were spiked with sarafloxacin, and the average percent recoveries at 10, 50, and 100 ppb were 132, 78, and 81%, respectively. Monoclonal antibodies exhibiting high relative affinity for sarafloxacin were also characterized for their ability to detect five structurally related quinolones. The specificity and cross-reactivities of these antibodies are discussed in relation to three-dimensional, computer-generated molecular models of the fluoroquinolones.

Keywords: *Sarafloxacin; fluoroquinolone; ELISA; immunoassay*

INTRODUCTION

Fluoroquinolones (Figure 1) are bactericidal antibiotics that have been increasingly used in veterinary medicine to treat microbial infections. They are congeners of nalidixic acid and exert their bactericidal effects by inhibiting DNA gyrase within susceptible bacteria (Spoo and Riviere, 1995). Recently, sarafloxacin became the first fluoroquinolone to be approved by the U.S. Food and Drug Administration (FDA) for use in food animals, and the only approved use is in day-old broiler chickens to control early mortality associated with *Escherichia coli*.

To preserve the usefulness of this valuable class of antibiotics, steps must be taken to minimize the potential for developing resistant pathogens. These steps include monitoring test samples of bacteria to measure the emergence of any resistant pathogens and detecting extra-label use in other major food-producing animal species by screening for fluoroquinolone residues. Conventional methods for detection of fluoroquinolones include a spectrofluorometric assay (Waggoner and Bowman, 1987) and high-performance liquid chromatography (HPLC) assays (Gau et al., 1985; Lyon et al., 1994; Morton et al., 1986; Vree et al., 1985). These methods are labor intensive and require expensive equipment; therefore, they cannot be used for routine screening of large numbers of samples.

Immunoassay screening methods have been successfully developed as alternatives to the conventional microbiological or chemical methods for detecting pesticides (insecticides and herbicides), drug residues, and undesirable natural products (Azcona-Olivera et al., 1992; Candlish et al., 1988; Degand et al., 1992; Groopman et al., 1984; Hu et al., 1984; Jung et al., 1989; Plhak and Sporns, 1992; Roseman et al., 1992; Shelby and Kelley, 1992; Wong and Ahmed, 1992; Woychik et al., 1984; Xu et al., 1988). In contrast to microbiological assays, immunoassays are highly specific (Stanker et al., 1987; Vanderlaan et al., 1988; Van Emon et al., 1986), and unlike conventional chemical assays, they require minimal sample preparation procedures (Monroe, 1984).

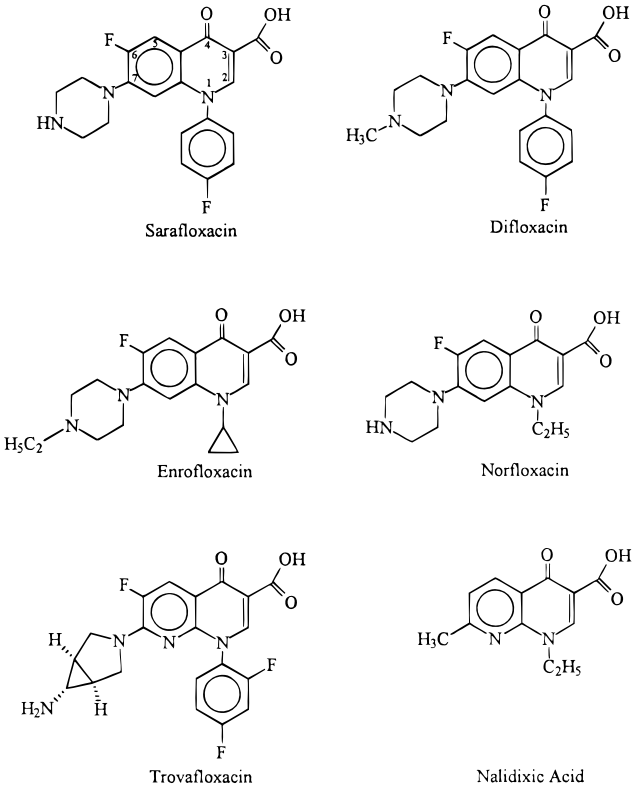

Figure 1. Structures of sarafloxacin and related fluoroquinolones evaluated in this study.

We report here the development and characterization of monoclonal antibodies (Mabs) to sarafloxacin, as well as the development of a competitive indirect enzyme-

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linked immunosorbent assay (ci-ELISA). The cross-reactivities of other structurally related fluoroquinolones with the anti-sarafloxacin antibodies were determined, and the competitors were then modeled using molecular mechanical and quantum mechanical methods in an effort to better explain the observed cross-reactivities. The results of this study indicate that the antibodies against sarafloxacin may be used as the basis for an immunoassay to rapidly screen samples for the presence of fluoroquinolones in tissues or body fluids.

MATERIALS AND METHODS

Chemicals and Reagents. Sarafloxacin and difloxacin (Abbott Laboratories, North Chicago, IL), enrofloxacin (Bayer, Kansas City, MO), and trovafloxacin (Pfizer Inc.; Groton, CT) were gifts from their respective manufacturers. Norfloxacin, nalidixic acid, bovine serum albumin (BSA), ovalbumin (OVA), goat anti-mouse immunoglobulin G conjugated to horseradish peroxidase (IgG−peroxidase), ethylenediamine, poly(ethylene glycol) (PEG 4000), hypoxanthine, aminopterine, thymidine, and polyoxyethylene sorbitan monolaurate (Tween 20) were purchased from Sigma Chemical Co. (St. Louis, MO). 2,6,10,-14-Tetramethylpentadecane (pristane) was purchased from Aldrich Chemical Co. (Milwaukee, WI). RIBI adjuvant was purchased from RIBI ImmunoChem Research, Inc. (Hamilton, MT). K-Blue peroxidase substrate was purchased from ELISA Technologies (Lexington, KY). BupH phosphate-buffered saline (PBS) packs, 10 000 MWCO Slide-A-Lyzer dialysis cassettes, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC), and *N*-hydroxysulfosuccinimide (NHS) were purchased from Pierce (Rockford, IL). Iscove's media, fetal bovine serum, and penicillin/streptomycin solution were obtained from Gibco (Grand Island, NY). Tissue culture plasticware and microtiter plates were obtained from Intermountain Scientific Corp. (Bountiful, UT). BALB/c mice were obtained from Harlan/Sprague Dawley (Houston, TX).

Hapten Conjugation. To convert carboxylic acid groups on the carrier proteins to primary amines, BSA and OVA were treated with an excess of ethylenediamine (EDA), as described previously (Kamps-Holtzapple et al., 1993). The cationized (EDA-treated) carrier proteins were dialyzed against PBS (pH 7) to remove free EDA. To 6 mg of each EDA-treated carrier protein (in 1.5 mL of 33% DMF/0.1 M sodium phosphate, pH 8) was added 3 mg of sarafloxacin, 10 mg of NHS, and 10 mg of EDC. The solutions were incubated at room temperature with stirring and then exhaustively dialyzed against PBS (pH 7). The sarafloxacin−protein conjugates were designated cBSA−Saraflox and cOVA−Saraflox, with the "c" indicating that the proteins have been cationized with EDA.

Monoclonal Antibody Production. Five female BALB/c mice were immunized intraperitoneally on days 1, 14, and 35 with 100 μg of cBSA−Saraflox in 0.2 mL of physiological saline with RIBI adjuvant (prepared according to the manufacturer's instructions). On day 21, serum titers were determined by indirect ELISA using cOVA−Saraflox as the plate-coating antigen. The sera were then tested using a ci-ELISA to determine which mice were producing antibodies against sarafloxacin. After a rest period of 1 month, the mouse exhibiting the most sensitive antibodies against sarafloxacin was immunized a final time with 100 μg of cBSA−Saraflox in 0.2 mL of physiological saline (without RIBI adjuvant). Four days later, the mouse was sacrificed and splenocytes were obtained.

Hybridoma Production. Splenocytes from an immunized mouse were fused with SP2/0 myeloma cells using PEG (Stanker et al., 1987). Fused cells were resuspended in HAT medium (Iscove's modified Dulbecco's medium containing 36 mM $NaHCO_3$, 2 mM glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, 5% fetal bovine serum, 100 mM hypoxanthine, 0.2 mM aminopterin, and 8 mM thymidine) and pipetted into the wells of 96-well plates containing a feeder layer of mouse macrophages. After 10 days, hybridoma supernatants were analyzed by ci-ELISA for the presence of antibodies against sarafloxacin. Cells in fusion wells with supernatants exhibiting inhibition were transferred from the fusion plates to 24-well plates. Cultures showing the highest percentage of inhibition were cloned twice by limiting dilution. For each cloning procedure, the cells were plated in HT medium on a layer of macrophage feeder cells, and final clones were obtained from rows that had been seeded with one (or fewer) cell per well.

Indirect ELISA. An indirect ELISA, described by Stanker et al. (1993), was used to determine the titers of the mouse sera and for the initial screening of hybridomas. Wells of polystyrene microtiter plates were coated with 100 μL of cOVA−Saraflox (2 μg/mL) and air-dried overnight at 40 °C. The cOVA conjugate was used as the solid-phase antigen to avoid detection of antibodies to the cBSA carrier used to immunize the animals. Nonspecific binding was decreased by blocking the wells with 3% (w/v) nonfat milk solution in deionized water for 30 min at 37 °C. After five washes with 0.05% Tween 20 in distilled water, 100 μL of the appropriate cell culture supernatant dilutions was allowed to bind to the coated microwells for 60 min at 37 °C. Unbound antibody was removed by washing five times with the 0.05% Tween 20−water solution. Next, 100 μL of goat anti-mouse IgG−peroxidase conjugate (1:1000 dilution) was added to each well. After a 60 min incubation at 37 °C, the plates were washed 10 times with 0.05% Tween 20, and bound antibody−peroxidase conjugate was determined using K-Blue, a colorimetric substrate. Absorbance was read at 655 nm after 20 min.

Competitive Indirect ELISA. A ci-ELISA was used to determine the sensitivity and specificity of the mouse sera and monoclonal antibodies. Microtiter plates were coated and blocked as described above. After five washes with 0.05% Tween 20 in distilled water, 100 μL of sarafloxacin standard in assay buffer (0.1 M Tris, 0.15 M NaCl, 0.05% Tween 20, 1% BSA, pH 7.5) and 100 μL of cell supernatant (diluted 1:200 in assay buffer) were added to each well and incubated for 60 min at 37 °C. After five washes with 0.05% Tween 20, the assay was completed as described above.

In each experiment, control wells containing all components except the competitor were prepared and the activity (color reaction) in these wells was taken to represent 100% activity. The test wells, containing different amounts of competitor, were normalized to the 100% activity wells, and percent inhibition was calculated as

$$\% \text{ inhibition} = [1 - (A_{655} \text{ of test}/A_{655} \text{ of control})] \times 100$$

Liver Extract Preparation. Minced samples (1 g) of sarafloxacin-free chicken liver were spiked with sarafloxacin at concentrations of 10, 50, or 100 ppb (ng/g). The spiked samples were diluted with 5 mL of assay buffer, vortexed for 1 min, and centrifuged at 14000*g* for 45 min at 4 °C. The supernatants were added directly to the microtiter plates and analyzed by ci-ELISA.

Molecular Modeling Studies. *Determination of Minimum Energy Conformations.* Molecular modeling studies were performed using a CAChe WorkSystem running on a Macintosh Quadra 700 equipped with a RP88 coprocessor board and a CAChe stereoscopic display (CAChe Scientific, Inc., Beaverton, OR). Minimum energy conformations of all of the compounds were calculated using Allinger's standard MM2 force field (Allinger, 1977) augmented to contain force field parameters for cases not addressed by MM2 (CAChe Scientific). Following the initial optimization, a sequential search for low-energy conformations was performed by rotating dihedral angles through 360° in 60° increments. The structures resulting from the computations were viewed and superimposed using the CAChe Visualizer+ application.

Determination of Electronic Properties. The electronic wavefunction for each compound was calculated by solving the Schrödinger equation using the extended Hückel approximation (Hoffmann, 1963). The wavefunction data were converted into three-dimensional coordinates for visualizing electron densities and electrostatic potentials using the CAChe Tabulator application. The electron probability density value was

set at 0.01 electron/Å^3 for all calculations. Electrostatic potentials were calculated in reference to an incoming positive charge.

RESULTS

Hapten Conjugation. Sarafloxacin is a small molecule (MW < 1000) that must be conjugated to a carrier protein to elicit an immune response. Using the free carboxylic acid on sarafloxacin, the drug was linked to cationized BSA and OVA using carbodiimide as described previously (Holtzapple et al., 1997). This results in an amide bond between sarafloxacin and the derivatized carrier proteins. Nondenaturing gel electrophoresis (Kamps-Holtzapple et al., 1993) was performed to demonstrate that sarafloxacin had been successfully conjugated.

Serum Titer Determination. Mice immunized with the cBSA−Saraflox conjugate resulted in antibody serum titers >250 000 as determined by indirect ELISA, with titer being defined as the reciprocal of the dilution that results in an absorbance value that is twice that of background (data not shown). A ci-ELISA was used to determine that all five mice were producing specific antibodies against sarafloxacin.

Hybridoma Production. Splenocytes from a BALB/c mouse immunized with cBSA−Saraflox and expressing specific antibodies to sarafloxacin were fused with SP2/0 myeloma cells. The resulting hybridomas were cultured in 30 96-well culture plates, and growing hybridomas were observed in >90% of the wells 10 days after fusion. The supernatant from each well was screened for the presence of antibodies against the immunogen using an indirect ELISA. Hybridoma cells from 150 wells that were positive in the above ELISA were expanded, and the supernatants were analyzed by ci-ELISA for antibodies capable of binding free sarafloxacin. Of these 150 picks, only 15 resulted in stable hybridomas secreting antibodies against free sarafloxacin. Cells from these 15 were subcloned, and from these, 6 monoclonal cell lines secreting anti-sarafloxacin antibodies were established and used for subsequent studies. All of the Mabs were IgG1 antibodies with κ light chains.

Antibody Characterization. Representative ci-ELISA curves for sarafloxacin obtained using monoclonal antibodies Sara-5, Sara-55, and Sara-95 are shown in Figure 2. Similar curves were generated for each Mab, and the 50% inhibition of control values (IC_{50}) for each are shown in Table 1. Each point represents the average of nine replicates. Sara-96 exhibited the highest relative affinity for sarafloxacin with an IC_{50} of 7.3 ppb (ng/mL) followed by Sara-95, Sara-131, Sara-67, Sara-55, and Sara-5 with IC_{50} values of 8.2, 9.0, 10.0, 12.3, and 48.3 ppb, respectively. For the most sensitive antibody, Sara-96, the mean intraassay IC_{50} coefficient of variation (CV) was 11.0% (n = 3), and the mean interassay IC_{50} CV was 10.8% (n = 3). The detection limit of the assay (defined as the lowest concentration of sarafloxacin exhibiting OD readings that were lower than that of the zero standard by twice the standard deviation of the zero standard) was 2 ppb.

Each antibody was evaluated for its ability to bind to other fluoroquinolones and to nalidixic acid. Representative ci-ELISA curves for the fluoroquinolones using Mab Sara-96 are shown in Figure 3, and the IC_{50} values obtained from these experiments are summarized in Table 1. Five of the Mabs (Sara-5, Sara-55, Sara-67, Sara-95, and Sara-96) bind sarafloxacin, difloxacin, and trovafloxacin with nearly equal relative affinity. It is not surprising that the Mabs exhibit similar relative affinities for difloxacin since the chemical structure of this compound is similar to that of sarafloxacin; however, it is surprising that trovafloxacin is such a good competitor despite significant structural changes in the 7-(1-piperazinyl) ring that is distal to the site used to

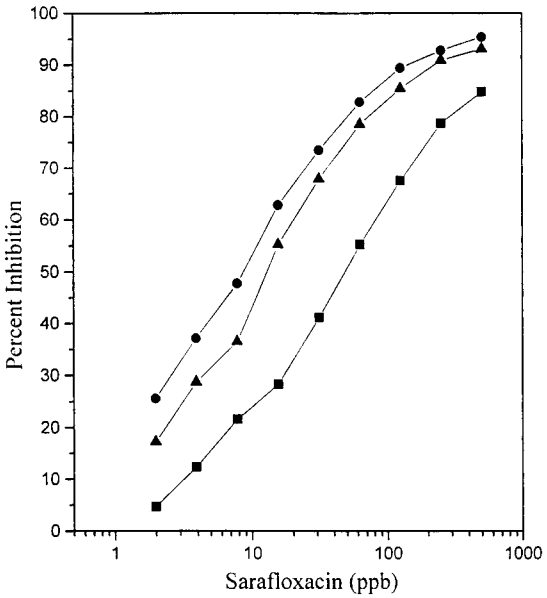

Figure 2. Representative inhibition curves for monoclonal antibodies Sara-5 (■), Sara-55 (▲), and Sara-95 (●) using sarafloxacin as the competitor.

Table 1. Cross-Reactivity of the Monoclonal Antibodies against Sarafloxacin: IC_{50} Values[a]

compound	Sara-5	Sara-55	Sara-67	Sara-95	Sara-96	Sara-131
sarafloxacin	48.3	12.3	10.0	8.2	7.3	9.0
difloxacin	22.4	6.6	6.9	5.7	5.8	7.0
trovafloxacin	52.5	21.1	8.7	9.6	9.8	123
enrofloxacin	58.2	34.0	141	154	248	3307
norfloxacin	181	115	163	154	253	921
nalidixic acid	312	300	704	625	698	1038

[a] IC_{50} values in ppb.

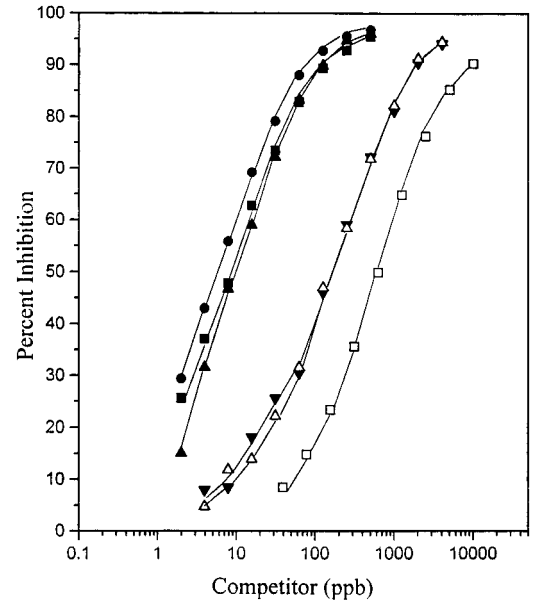

Figure 3. ci-ELISA curves for monoclonal antibody Sara-96 using sarafloxacin (■), difloxacin (●), trovafloxacin (▲), enrofloxacin (▼), norfloxacin (△), and nalidixic acid (□) as competitors.

Table 2. Recovery of Sarafloxacin from Spiked Liver by ci-ELISA

sarafloxacin added, ppb (ng/g)	sample[a]	recovery ppb[b] (ng/g)	recovery %[c]
0	A	nd[d]	
0	B	nd	
0	C	nd	
10	A	14.5 ± 1.5	145
10	B	12.5 ± 2.3	125
10	C	12.5 ± 0.9	125
50	A	40.5 ± 3.1	81
50	B	45.7 ± 3.6	91
50	C	31.3 ± 3.4	63
100	A	82.1 ± 6.1	82
100	B	81.0 ± 4.0	81
100	C	80.2 ± 7.4	80

[a] Samples were spiked separately in triplicate and assayed in separate experiments (A−C). [b] Mean recoveries ($n = 3$) for samples containing 10, 50, or 100 ppb sarafloxacin were 13.2, 39.2, and 81.1 ppb, respectively. [c] Mean percent recoveries ($n = 3$) for 10, 50, and 100 ppb were 132, 78, and 81%, respectively. [d] None detected (<2 ppb, the detection limit of this assay).

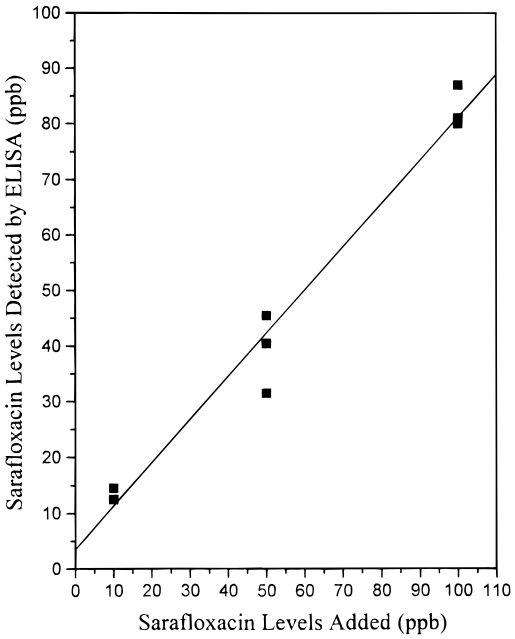

Figure 4. Correlation between the spike level of sarafloxacin and the level detected by ELISA. ELISA value = 0.776 × spike value + 3.6; $R = 0.988$.

attach sarafloxacin to the carrier protein. The factors affecting the cross-reactivity of trovafloxacin in the immunoassays were further investigated using molecular modeling techniques.

Detection of Sarafloxacin in Chicken Liver. The ci-ELISA was used to detect sarafloxacin in chicken liver extracts. Liver samples spiked at 10, 50, and 100 ppb (ng/g) gave recoveries of 132, 78, and 81%, respectively (Table 2). The precision (% CV) within the assays ranged from 5% to 18%, and the precision between analyses ranged from 2% to 18% with an overall precision of 9.5%. Figure 4 demonstrates the correlation between the spike level of sarafloxacin and the level detected by ELISA.

Molecular Modeling Studies. *Determination of Lowest Energy Conformations.* Computer modeling studies were performed on the compounds shown in Figure 1 in an effort to correlate structural and electronic properties of each compound with the ability to inhibit antibody binding to cOVA−Saraflox. The global minimum energy conformations of the compounds were obtained by multiple sequential searches for low-energy conformations and are displayed in Figure 5A. In this view, the planar quinolone rings lie perpendicular to the plane of the page and the 1-piperazinyl ring at position 7 (or bicyclic ring system in trovafloxacin) lies on the left side of the structures. This view demonstrates that substitution of the 7-(1-piperazinyl) ring in sarafloxacin with the bicyclic ring of trovafloxacin does not drastically change the general conformational structure. Even though the chemical structure is quite different, mimicry of the conformational structure of sarafloxacin may explain why five of the monoclonal antibodies bind sarafloxacin and trovafloxacin with nearly equal relative affinity.

In contrast, the smaller groups substituted for the fluorophenyl ring at the 1-position do not chemically or conformationally mimic that of the original ring in sarafloxacin. The lower binding affinity exhibited by the antibodies for enrofloxacin, norfloxacin, and nalidixic acid may reflect both loss of binding contacts and introduction of steric hindrance at this position.

Determination of Electronic Properties. The electrostatic potential calculations displayed on the electron density surfaces of the global minimum energy conformations for the quinolones are shown in Figure 5B [with structures oriented as in (A)]. The electron density isosurfaces give information about the volume and shape of the molecules, whereas the electrostatic potential describes the potential energy of a proton placed at a point near the molecule. The more positive potential energy is represented by white and red regions (generally designating carbons and hydrogens), and these areas are repulsive to a proton. The more negative potential energy is represented by violet and charcoal regions (generally designating fluorines, oxygens, and nitrogens), and these areas are attractive to a proton. Negative areas are noted in the figure using asterisks (designating the fluorine in the 4-fluorophenyl ring) and arrows (designating the ring nitrogen in the 1-piperazinyl ring).

In comparison to the ball-and-stick models depicted in Figure 5A, the space-filling models in Figure 5B show more clearly the shape of the molecules and also show how changes in the chemical structure affect the electronic nature of the fluoroquinolones. It is interesting to note the electronic differences between sarafloxacin and trovafloxacin due to the differences in the ring structures at position 7. In trovafloxacin, the nitrogen (along with its negative electrostatic potential) in the bicyclic ring system is rotated up compared to the position of the nitrogen in the 1-piperazinyl ring of sarafloxacin (arrows mark the position of the nitrogens). For five of the antibodies, the relative binding affinities of trovafloxacin and sarafloxacin are similar despite this electronic difference, but in Sara-131, this difference may contribute to the 10-fold decrease in relative affinity for trovafloxacin.

The differences in structure that occur in enrofloxacin, norfloxacin, and nalidixic acid due to substitution of the fluorophenyl ring with other groups also can be seen more clearly using the space-filling models. In addition to possible steric hindrance introduced by the cyclopropyl (enrofloxacin) and ethyl (norfloxacin and nalidixic acid) groups, the electronegative fluorine that is present in sarafloxacin (marked with an asterisk) is missing in these compounds. The adverse steric and electronic effects are reflected in the lower relative affinities.

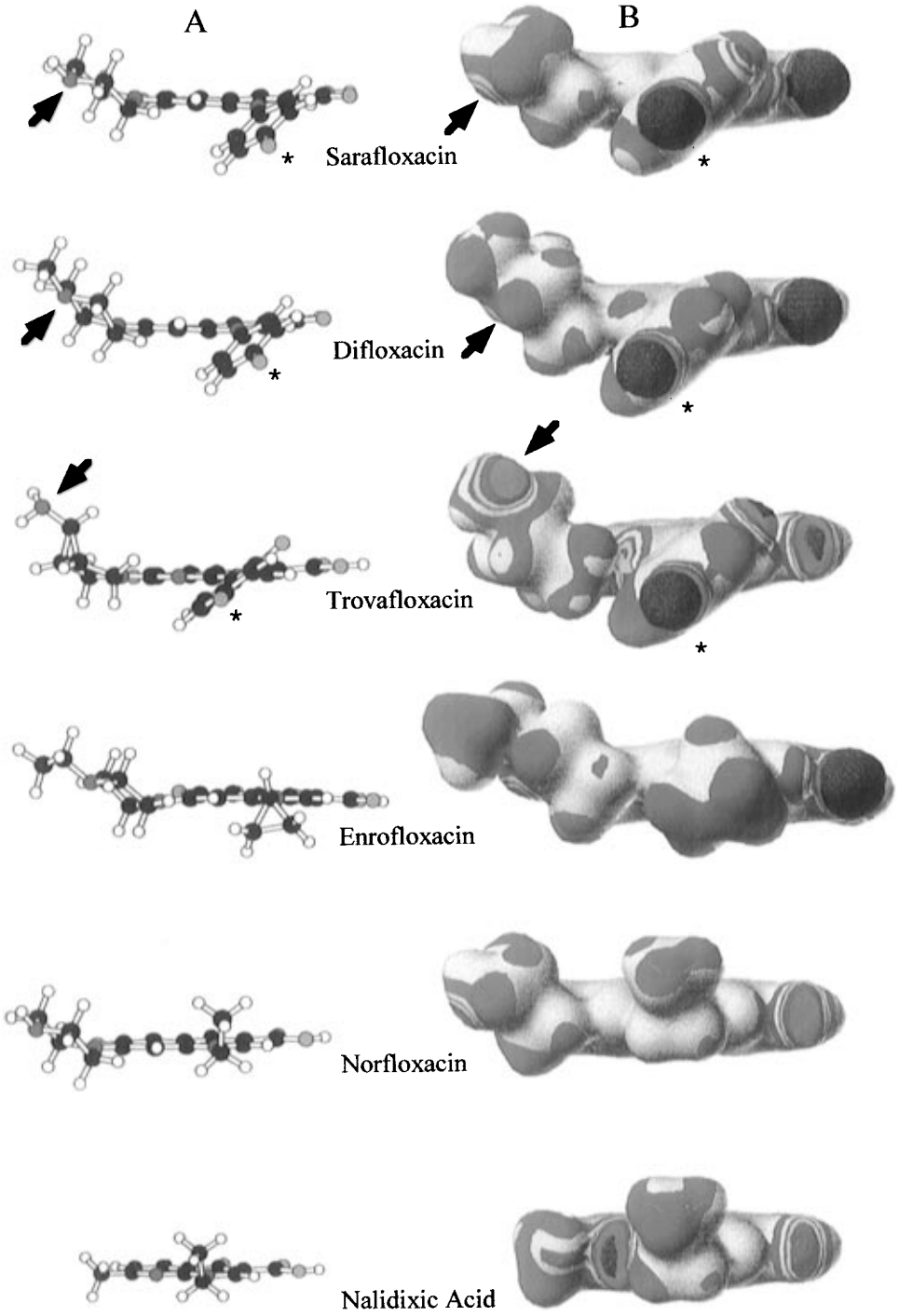

Figure 5. (A) Ball-and-stick models of the minimum energy conformations of the fluoroquinolones and nalidixic acid. The elements are represented in the following manner: black, carbon; white, hydrogen; red, nitrogen; blue, oxygen; yellow, fluorine. The asterisks are placed next to the fluorine in the 4-fluorophenyl ring, and the arrows point to the secondary amine in the 1-piperazinyl ring. (B) Electron density isosurfaces of the minimum energy conformations of the fluoroquinolones. The asterisks and arrows are used in the same manner as in (A). The energy values in atomic units (1 au = 627.503 kcal/mol) of the color boundaries are as follows: white−red, +0.09; red−yellow, +0.03; yellow−green, +0.01; green−cyan, 0.00; cyan−blue, −0.01; blue−violet, −0.03; and violet−charcoal, −0.06.

DISCUSSION

In this study, six monoclonal antibodies were isolated using cBSA−Saraflox as the immunogen. The relative affinities of these six Mabs for other structurally related quinolones were determined and expressed in terms of IC_{50} values. The results demonstrate that (a) the four-atom linker (ethylenediamine) that was used to attach the carboxylic acid group of sarafloxacin to the carrier protein allowed production of antibodies with high relative affinity for the hapten; (b) all of the Mabs recognized sarafloxacin and the five structurally related compounds shown in Figure 1; (c) five of the Mabs exhibited similar relative affinities for sarafloxacin, difloxacin, and trovafloxacin; and (d) substitution of a cyclopropyl ring in enrofloxacin for the fluorophenyl ring in sarafloxacin was not as detrimental to antibody binding of Mabs Sara-5 and Sara-55 as for other Mabs.

To determine which structural features of the molecules are important to antibody binding, both cross-reactivity and molecular modeling data were analyzed.

It is evident from the cross-reactivity data that fluoroquinolones which retain the 4-fluorophenyl ring at position 1 are good competitors even with the presence of major structural differences at position 7 (e.g., site of attachment of the 1-piperazinyl ring on sarafloxacin). This was unexpected since conjugation to the carrier protein was via the carboxyl moiety at position 3. We would expect that molecular substitutions distal to the point of attachment would greatly influence antibody binding; however, substitution of the piperazinyl ring in sarafloxacin by the bicyclic ring system in trovafloxacin was not detrimental to binding for most of the antibodies. It may be that the antibodies with higher relative affinities interact predominantly with the large fluorophenyl ring rather than the piperazinyl ring. A comparison of the cross-reactivity data of sarafloxacin, norfloxacin, and nalidixic acid would suggest that the group at position 7 contributes to antibody binding, but to a lesser extent than does the fluorophenyl ring. Loss of the fluorophenyl group (e.g., comparing norfloxacin to sarafloxacin) results in a 10−20-fold decrease in antibody binding for Mabs Sara-55, Sara-67, Sara-95, and Sara-96. For these same antibodies, loss of the piperazinyl ring and adjacent fluorine (e.g., comparing nalidixic acid to norfloxacin) was not as detrimental to antibody binding, resulting in a 2−4-fold decrease in relative affinity.

Since cross-reactivity data demonstrated that the piperazinyl ring contributes to antibody binding, it was puzzling why substitution of the bicyclic ring system in trovafloxacin for the piperazinyl ring in sarafloxacin was not detrimental to antibody binding for five of the Mabs. Molecular modeling data provided structural clues to explain the observed cross-reactivity data. Ball-and-stick models as well as space-filling models of trovafloxacin and sarafloxacin demonstrated that although the chemical structure of the trovafloxacin ring system at position 7 was vastly different from the sarafloxacin piperazinyl ring, the conformational structures of the two were similar. This mimicry at the level of conformational structure allowed the Mabs to bind trovafloxacin with greater relative affinity than would have been expected given the differences in chemical structure in this portion of the molecule. Computer-generated molecular modeling studies, therefore, can aid in understanding what structural and electronic features are important for antibody binding and can give reasons for unexpected cross-reactivity.

In conclusion, monoclonal antibodies recognizing sarafloxacin and other fluoroquinolones were developed and used in a simple ci-ELISA format. A sensitive assay was produced, and preliminary studies demonstrate that it is capable of detecting sarafloxacin in chicken liver in the low parts per billion range. The assay has the potential to be incorporated into a residue monitoring program as a rapid initial screen to detect sarafloxacin in chicken and other food animal tissues.

ABBREVIATIONS USED

BSA, bovine serum albumin; cBSA, cationized bovine serum albumin; OVA, ovalbumin; cOVA, cationized ovalbumin; PBS, phosphate-buffered saline; EDTA, ethylenediaminetetraacetic acid; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride; EDA, ethylenediamine; DMF, dimethylformamide; NHS, *N*-hydroxysulfosuccinimide; HAT, hypoxanthine/aminopterin/thymidine; MWCO, molecular weight cutoff.

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