BIOACTIVATION AND COVALENT BINDING OF HYDROXYFLUPERLAPINE IN HUMAN NEUTROPHILS: IMPLICATIONS FOR FLUPERLAPINE-INDUCED AGRANULOCYTOSIS

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ABSTRACT:

The use of fluperlapine and the structurally related clozapine has been associated with the induction of agranulocytosis in humans. Unlike clozapine, fluperlapine is relatively resistant to chemical and biochemical oxidations. In this study we demonstrated that 7-hydroxyfluperlapine, the major metabolite of fluperlapine in humans, is oxidized to a reactive intermediate by HOCl and by myeloperoxidase in the presence of H₂O₂ and Cl⁻. This reactive intermediate was identified as an iminoquinone species with a M₁ ion at m/z 324 by mass spectrometry. The iminoquinone intermediate was trapped by N-acetyl-L-cysteine (NAC) as well as GSH. NMR spectra of the NAC adducts indicated that the NAC was bound to the 6 and 9 positions of the aromatic ring. This is the same orientation as the binding of nucleophiles to the reactive metabolite of clozapine. We were able to use an antibody against clozapine to demonstrate that 7-hydroxyfluperlapine, but not fluperlapine itself, covalently modifies human myeloperoxidase. Furthermore, we demonstrated that 7-hydroxyfluperlapine is metabolized by activated neutrophils to a reactive intermediate that covalently binds to neutrophils. In the presence of NAC or GSH, such covalent binding was inhibited and the NAC or GSH adducts were formed. Thus, the reactivity and even the orientation of the binding of the reactive metabolite of 7-hydroxyfluperlapine is very similar to that of clozapine. These results provide a mechanism for the formation of a reactive metabolite of fluperlapine similar to clozapine that may explain its ability to induce agranulocytosis.
MPO activity was defined as the amount of enzyme that decomposed 1 was determined spectrophotometrically (Hussarin et al., 1970). One unit of was purchased from Zymed (San Francisco, CA). The concentration of NaOCl IL). Horseradish peroxidase-conjugated goat anti-rabbit IgG (H tario, Canada). Enhanced chemiluminescence film was purchased from Am-

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Chemical Company (Milwaukee, WI). NaOCl was purchased from Aldrich Chemical (San Leandrow, CA). The chromatography columns were supplied by Phenomenex (Tor-

Japan). The chromatography columns were supplied by Phenomenex (Rockford, IL). Horseradish peroxidase-conjugated goat anti-rabbit IgG (H + L chains) was purchased from Zymed (San Francisco, CA). The concentration of NaOCl was determined spectrophotometrically (Hussarin et al., 1970). One unit of MPO activity was defined as the amount of enzyme that decomposed 1 μmol of H₂O₂ per min at 25°C and pH 6.

Experimental Procedures

Materials. Clozapine, fluperlapine, and 7-hydroxyfluperlapine were generous gifts from Novartis Pharmaceuticals. H₂O₂ was purchased from ACP Chemicals (Montreal, Québec, Canada). NaOCl was purchased from Aldrich Chemical Company (Milwaukee, WI). MPO was obtained from Cortex Biochemical (San Leandrow, CA). NAC, GSH, casein, and thimerosal were purchased from Sigma Chemical Co. (St. Louis, MO). Nitrocellulose and stock acrylamide solution (40%) were purchased from Bio-Rad (Mississauga, Onto-

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methanol fraction of the extraction was collected and concentrated. The products were additionally purified by HPLC with a mobile phase of water/ acetonitrile (85:15, v/v) with 5 mM ammonium acetate and a flow rate of 5 mL/min. The fractions with retention times of 17 and 40 min were collected separately. HPLC analysis showed the purity of the final products was more than 95%.

Human Leukocyte Isolation. Neutrophils and peripheral blood mononuclear cells were isolated from venous blood of healthy volunteers as described previously (Liu and Uetrecht, 1995). Trypan blue exclusion showed the initial viability to be more than 98% for all preparations. Cytospin slides were prepared and stained with Wright’s stain. Light microscopy confirmed that >90% of the cells had characteristic neutrophil morphology.

Incubation of Neutrophils with Fluperalpaine, 7-Hydroxyfluperalpaine, and Clozapine. Neutrophils, 5 x 10^5 in 1 ml of Hanks’ balanced salt solution (without phenol red), were incubated with various concentrations of fluperalpaine (0–50 μM), 7-hydroxyfluperalpaine (0–50 μM), and clozapine (0–8 μM) at 37°C for 1 h in a shaking water bath in the presence or absence of phospholipid 12-myristate-13-acetate (PMA; 40 ng/ml). Samples were then boiled at 100°C for 10 min before loading on the gel.

After the incubation of 7-hydroxyfluperalpaine (50 μM) and neutrophils (5 x 10^5) in the presence of NAC or GSH (5 mM), the supernatants were collected and solvents were removed by a stream of nitrogen at 25°C. The samples were redissolved in water and analyzed by LC/MS using selective ion monitoring (SIM) at m/z 487 for NAC adducts and at m/z 631 for GSH adducts. LC/MS/MS spectra of the NAC and GSH adducts were also obtained under appropriate collision-activated dissociation (CAD) conditions.

Covalent Binding of Fluperalpaine and 7-Hydroxyfluperalpaine to Human MPO. Fluperalpine or 7-hydroxyfluperalpine (50 μM; 0.1 ml) was incubated with MPO (1 or 5 U) in the presence of H_2O_2 (100 μM). After incubation for 30 min at 37°C, the reaction was stopped by cooling the sample on ice, and an equal volume of SDS-PAGE sample buffer containing dithiothreitol (6 mg/ml) was added. The samples were then boiled at 100°C for 10 min before analysis by SDS-PAGE.

Production of Antoclazapine-NAC-Keyhole Limpet Hemocyanin (KLH) Antiserum. The polyclonal antoclazapine-NAC-KLH antiserum used in these studies was raised as described previously (Gardner et al., 1998a). As well as recognizing clozapine, this antiserum has been shown by immunoblot inhibition experiments to recognize structural analogs of clozapine such as olanzapine. Similar hapten inhibition experiments (see below for details) were performed with fluperalpine and hydroxyfluperalpine to ensure that the antiserum would also recognize these two structural analogs.

Hapten Inhibition Experiments. To produce clozapine-modified neutrphils for use in the hapten inhibition experiments, the following method was used. Clozapine (20 μM in PBS pH 6.0) was reacted with NaOCl (18 μM) to generate clozapine-reactive metabolite and human neutrophils (5 x 10^5 in 1 ml of Hanks’ balanced salt solution without phenol red) were added immediately to the reactive metabolite solution as described previously (Gardner et al., 1998a). After incubation at 37°C for 15 min the neutrophils were collected by centrifugation and lysed in cell lysis buffer. A portion of the lysed sample was diluted with an equal volume of SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer containing dithiothreitol (6 mg/ml). The samples were then boiled at 100°C for 10 min before loading on the gel.

Electrophoretic transfer of resolved proteins to nitrocellulose was carried out using a buffer of 15.7 mM Tris, 120 mM glycine (pH 8.3) containing 20% (v/v) methanol, 10% (v/v) acetic acid and then destained using 70% (v/v) methanol, 2% (v/v) acetic acid or used for antibody development. Comprehensive details of the immunoblotting methods used have been published previously (Gardner et al., 1998a).

In experiments to determine the specificity of the anticlazapine-NAC-KLH antiserum, the antiserum (1:4,000 final dilution) was incubated with various concentrations (0–20 μM) of clozapine, fluperalpine, 7-hydroxyfluperalpine, mianserin, and procainamide (see Fig. 1 for structures) for 30 min before addition of the primary antiserum to the nitrocellulose membrane. Clozapine, fluperalpine, and procainamide were dissolved in PBS; mianserin was dissolved in H_2O and 7-hydroxyfluperalpine was dissolved in ethanol. Control samples received an equivalent volume of ethanol. The washing and subsequent development of the immunoblots were then performed as outlined previously (Gardner et al., 1998a).

Other Methods. Protein concentration was determined using a BCA protein assay kit (Pierce, Rockford, IL) with BSA as the standard.

Results

Oxidation of 7-Hydroxyfluperalpine by HOCI. 7-Hydroxyfluperalpine was easily oxidized by HOCI. On oxidation, a major reactive intermediate of 7-hydroxyfluperalpine was detected by mass spectrometry with a M + 1 ion at m/z 324. This is 2 mass units less than the M + 1 ion of the parent drug and is presumed to be the iminoquinone shown in Fig. 3A. The mass spectrum of the iminoquinone species of 7-hydroxyfluperalpine obtained under CAD condition gave fragment ions at m/z 58 [85%; (CH_3)_2NHCH_2C=], m/z 84 [70%; CH_3CHN(CH_2CH_2CH_3^+)], m/z 101 (100%; CH_3N(CH_2CH_2NH)_2^-), m/z 226 (50%; MH^+ - methylpiperazine), m/z 252 [50%; MH^- - (CH_2)_2N(CH_2CH_3)], and m/z 267 [75%; MH^- - (CH_2)_2N(CH_3)]. As well as the MH^- ion of the iminoquinone at m/z 324 (75%) (Fig. 3B).

On addition of equal equivalent of HOCl, 7-hydroxyfluperalpine there was an increase in absorptions at 308 and 420 nm, which then decreased rapidly (Fig. 4). Analyzed by LC/MS, the resultant iminoquinone species showed reactivity toward both water and chloride ion forming stable products with molecular ions of m/z 342 and 360, respectively. The stable product with m/z 360 contained the characteristic chloride isotope peak at m/z 362.

Oxidation of 7-Hydroxyfluperalpine by MPO Enzyme System and Formation of 7-Hydroxyfluperalpine-NAC Adducts. 7-Hydroxyfluperalpine was extensively oxidized by human MPO in the presence of H_2O_2 and Cl^- . When NAC was also present in the reaction mixture, the reactive iminoquinone intermediate preferentially reacted with NAC rather than water or chloride, and subsequently it could be detected as 7-hydroxyfluperalpine-NAC adducts. The LC/MS analysis of the reaction mixture showed two major peaks of the NAC adducts with the protonated molecular ions of m/z 487 and retention times of 5.0 and 6.0 min, respectively, whereas the stable water or chloride adducts were not detected (Fig. 5). Each HPLC peak of NAC adduct contained a pair of diastereomers of that NAC adduct, and they were not separable by HPLC. In the control experiments, when H_2O_2 was replaced by water, the NAC adducts were not detected.

The LC/MS/MS of the diastereomeric NAC adducts with a retention time of 5.0 min showed major CAD fragments at m/z 84 [25%;
of the 1 H NMR of the diastereomeric NAC adducts with a retention time of 5.0 min showed peaks at 6.83 ppm, which was due to H-9, whose coupling with the same 19 F doublet peak at 6.83 ppm was due to H-6, whose coupling with the 19 F doublet peak at 6.77 and 6.83 ppm that had the same coupling constant of 9.5 Hz, which was due to the coupling with H-9 and H-6, respectively. However, the 19 F in the diastereomeric NAC adducts with a retention time of 5.0 min contained two doublet peaks having the same coupling constant of JHF = 9.5 Hz at δ = 139.35 ppm and δ = 139.96 ppm, respectively; the 19 F in the diastereomeric NAC adducts with a retention time of 6.0 min contained two doublet peaks having the same coupling constant of JHF = 12.5 Hz at δ = 135.79 ppm and δ = 135.90 ppm, respectively. From 19 F NMR, the loss of coupling with H-9 and H-6 in the pair of diastereomers with a retention time of 5.0 min and the loss of coupling with H-9 in the diastereomers with a retention time of 6.0 min confirmed that NAC was bound to positions 6 and 9.

**Metabolism of 7-Hydroxyfluperlapine by Human Neutrophils in Presence of NAC or GSH.** 7-Hydroxyfluperlapine was metabolized by PMA-activated human neutrophils at pH 7.4. In the presence of NAC, two pairs of 7-hydroxyfluperlapine-NAC adducts with protonated molecular ions of m/z 487 were also detected using LC/MS in the SIM mode. Their LC/MS/MS spectra and HPLC retention times indicated that they were the same as those adducts obtained in the MPO enzyme system. The NAC adducts showed retention times at 5.0 and 6.0 min, with the approximate ratio of 1:2. The two diastereomeric adducts with NAC attached to position 6 remained dominant.

Similarly, the overlapping doublet peaks with the coupling constant of 12.5 Hz given by the two diastereomers of NAC adduct with a retention time of 6.0 min indicated that the NAC was bound to position 6 of 7-hydroxyfluperlapine. The assignment of binding positions of NAC in the NAC adducts was additionally supported by 19 F NMR. The 19 F in 7-hydroxyfluperlapine had a doublet of doublets at δ = 139.35 ppm with J' = 12.5 Hz and J'' = 9.5 Hz, which was due to the coupling with H-9 and H-6, respectively. However, the 19 F in the diastereomeric NAC adducts with a retention time of 5.0 min contained two doublet peaks having the same coupling constant of JHF = 9.5 Hz at δ = 130.75 ppm and δ = 130.96 ppm, respectively; the 19 F in the diastereomeric NAC adducts with a retention time of 6.0 min contained two doublet peaks having the same coupling constant of JHF = 12.5 Hz at δ = 135.79 ppm and δ = 135.90 ppm, respectively. From 19 F NMR, the loss of coupling with H-6 in the pair of diastereomers with a retention time of 5.0 min and the loss of coupling with H-9 in the diastereomers with a retention time of 6.0 min confirmed that NAC was bound to positions 6 and 9.

**Experimental Procedures.** The positions where NAC covalently modified hydroxyfluperlapine were demonstrated by NMR spectroscopy. In 1 H NMR, the aromatic region of the diastereomeric NAC adducts with a retention time of 5.0 min showed peaks at δ 7.28 to 7.46 ppm (4H, m), and two doublet peaks at δ 6.72 to 7.28 ppm that had the same coupling constant of 12.5 Hz) (Fig. 7). For 7-hydroxyfluperlapine, the doublet peak at 6.72 ppm was due to H-6, whose coupling with 19 F on its ortho position gave the coupling constant of JHF = 12.5 Hz. The two doublet peaks with the same coupling constants of 9.5 Hz given by the two diastereomers of NAC adduct with a retention time of 5.0 indicated that the NAC was bound to position 9 of 7-hydroxyfluperlapine.
LC/MS/MS of the adducts showed similar CAD fragments with major peaks at m/z 301 (40%; 7-hydroxyfluperlapine S-(CH₂)₂NCH₃H), m/z 358 (100%; 7-hydroxyfluperlapine S₁H), m/z 502 (20%; MH⁺ - C₅H₈O₃N), and the parent ion at m/z 631 (MH⁺; Fig. 6B).

Recognition of Fluperlapine by Anticlozapine-NAC-KLH Antiserum. Hapten inhibition studies demonstrated that, in immunoblot experiments, the recognition of the clozapine-reactive metabolite-modified neutrophil proteins by the antiserum could be completely inhibited by preincubation of the antiserum with clozapine, fluperlapine, and 7-hydroxyfluperlapine at concentrations of 2 (Fig. 8B) or 20 μM (Fig. 8A). Mianserin, in which the piperazine is fused to the dibenzoazepine ring, inhibited the antiserum binding completely at a concentration of 20 μM but only partially at the lower concentration (Fig. 8). Procainamide, which lacks the piperazine ring but still has a tertiary amine group, was a much weaker inhibitor of antibody recognition. Using a wider range of fluperlapine concentrations, the IC₅₀ for inhibition of the antiserum binding was determined to be approximately 10 nM (data not shown). This is similar to that reported previously for olanzapine (Gardner et al., 1998a).

Immunoblotting of Neutrophils Exposed to Fluperlapine, 7-Hydroxyfluperlapine, and Clozapine In Vitro. When neutrophils were incubated with 7-hydroxyfluperlapine (0–50 μM) in the presence of PMA, the metabolite became covalently bound to a number of neutrophil polypeptides (Fig. 9). At concentrations of 2, 5, or 10 μM, a number of 7-hydroxyfluperlapine-modified polypeptides became covalently modified. However, in the absence of PMA, this binding was not observed. When clozapine (0.5 or 5 μM) was incubated with human neutrophils in the presence of PMA, the major polypeptides modified also had molecular masses of between 45 and 116 kDa but the pattern was somewhat different (Fig. 9). Covalent binding of fluperlapine to human neutrophil polypeptides was not observed in either the presence or absence of PMA (Fig. 9). The recognition of clozapine and 7-hydroxyfluperlapine-modified polypeptides by the antiserum could be inhibited by preincubating the antiserum with clozapine or hydroxyfluperlapine (Fig. 10), indicating that this binding represented specific recognition of drug-modified polypeptides by the antiserum. Covalent binding of reactive metabolites of clozapine (Fig. 11A) and hydroxyfluperlapine (Fig. 11B) to PMA-activated human neutrophils in vitro could be inhibited by including the nucleophiles NAC or GSH in the incubation. No such inhibition was observed when N-acetyl-L-lysine was included in the incubation. In fact, N-acetyl-L-lysine appeared to increase the binding, which is difficult to explain.

Covalent Binding of Fluperlapine and 7-Hydroxyfluperlapine to Human MPO In Vitro. When human MPO was incubated with 7-hydroxyfluperlapine (0–50 μM) in the presence of H₂O₂ (100 μM), the metabolite became covalently bound to the protein in a concentration-dependent manner and was detected as a 58-kDa adduct by
immunoblotting (data not shown). In addition, some high molecular weight aggregates of 7-hydroxyfluperlapine-modified MPO were observed. No covalent binding was observed in the absence of drug or H₂O₂. No such binding was observed when parent fluperlapine was incubated under identical conditions (data not shown).

Discussion

As a potential replacement for the antipsychotic drug, fluperlapine shares many structural similarities with clozapine (Fig. 1). However, replacing the secondary amine at position 5 with a methylene carbon prevents fluperlapine from forming a nitrenium ion analogous to clozapine. The results from the current study demonstrate that although fluperlapine is unreactive, its major metabolite, 7-hydroxyfluperlapine, is readily oxidized to a reactive intermediate by HOCl, which is the major oxidant produced by activated neutrophils. The mass spectrum, UV spectrum, and identity of the adducts formed on addition of nucleophiles provide substantive evidence that the reactive intermediate is the iminoquinone shown in Fig. 12. Another possible structure for the reactive intermediate is a quinoneiminium ion involving the piperazine ring. Such a quinoneiminium ion would be expected to readily hydrolyze to give a quinone analog and the free piperazine. The hydrolysis of a similar intermediate of vesnarinone has been reported previously (Uetrecht et al., 1994). However, we failed to find any hydrolytic products of the proposed quinoneiminium ion or any GSH or NAC adduct of the hydrolytic product.

Neutrophils were incubated with varying concentrations of drugs in the presence (+) or absence (−) of PMA at 37°C for 1 h and then processed as described in Experimental Procedures. Protein from 0.5 × 10⁶ PMA-activated neutrophils (15 μg) was loaded in each lane. For immunoblotting primary antiserum (anticlozapine-NAC-KLH) was used at a dilution of 1:3000. This figure is representative of three individual experiments.
that was preincubated with clozapine (CLOZ; 50 μM) in the presence of PMA (40 ng/ml) for 60 min at 37°C. Control incubations (–) contained PMA alone but no drug. The blots were incubated with the anticlozapine-NAC-KLH antiserum alone (dilution 1:5000) or with antiserum that was preincubated with clozapine (CLOZ; 50 μM) or fluperlapine (FLUP; 50 μM) for 30 min before addition to the blot. Protein loading was 8.7 μg/lane.

structure. Due to the sp^3 hybridization on C-5 and the nontwisting π bond between N-10 and C-11, the seven-membered ring is bent and forces the two aromatic rings to the same side of the molecule. This configuration creates a chiral center. The two enantiomers of 7-hydroxyfluperlapine are probably not interchangeable because a completely planar and high-energy transition state would be required for such an interchange. The chirality of 7-hydroxyfluperlapine is demonstrated by its proton NMR spectrum in which the two diastereotopic protons on C-5 near the chiral center are split into a doublet of doublets (δ 3.4–3.6 ppm, J = 48.3 Hz, J = 13.0 Hz).

The iminoquinone species generated from 7-hydroxyfluperlapine is electrophilic and preferentially reactive toward sulfhydryl-containing nucleophiles. In the presence of NAC or GSH, the prechiral iminoquinone intermediate generates two pairs of diastereomeric NAC or GSH adducts. The CAD mass spectra of both NAC and GSH adducts show the common major fragment at m/z 358, which is generated from the loss of the NAC or GSH moiety with sulfur still attaching to 7-hydroxyfluperlapine. This suggests that NAC and GSH are bound to the aromatic ring (Fig. 6). The proton NMR spectra of the NAC adducts show that the binding of the substituents are to positions 6 and 9 (Fig. 7). The same set of NAC and GSH adducts were also obtained with MPO/H_2O_2/Cl^− enzyme system (Fig. 5A) and with PMA-activated human neutrophils (data not shown). Hence, we presume that the same iminoquinone intermediate is formed by activated neutrophils. The positions to which NAC and GSH bind to the reactive metabolite of 7-hydroxyfluperlapine are the same as when they bind to the reactive metabolite of clozapine and its other analogs (Liu and Uetrecht, 1995). Also analogous to the reactive metabolite of clozapine, the iminoquinone was shown to be unreactive toward the “harder” nucleophile, N-acetyl-L-lysine. Therefore, it is reasonable to speculate that the iminoquinone of 7-hydroxyfluperlapine will modify sulfhydryl-containing proteins in a manner similar to that of the nitrenium ion generated from clozapine and olanzapine. These results also provide the justification for the assumption that the anticlozapine antibodies for the detection of 7-hydroxyfluperlapine-modified proteins.

Using the clozapine-NAC-KLH adduct as an immunogen, a polyclonal antiserum had been generated previously. This antiserum also recognized fluperlapine and its 7-hydroxyl metabolite, with high affinity. With fewer structural similarities to clozapine, mianserin and procarbazine had lower affinities for the antiserum (Fig. 8). It seems that the presence and orientation of the methylpiperazine ring is important for high-affinity binding of the antibodies. Therefore, we were able to use this antiserum to investigate covalent binding of the reactive intermediate generated by 7-hydroxyfluperlapine to human neutrophil proteins.

At various concentrations (2–20 μM), 7-hydroxyfluperlapine covalently bound to PMA-activated human neutrophils. Western blotting shows that both clozapine and 7-hydroxyfluperlapine modify a neutrophil polypeptide with the molecular mass of 58 kDa, which we suspect is MPO. However, when compared with clozapine, 7-hydroxyfluperlapine tends to modify neutrophil polypeptides with higher molecular masses. When fluperlapine was incubated with PMA-activated neutrophils, no fluperlapine-modified protein was detected even when higher concentrations were used. Fluperlapine is inert toward oxidation chemically and biochemically, whereas its 7-hydroxyl metabolite is bioactivated to the reactive iminoquinone, which forms covalent adducts with sulfhydryl-containing nucleophiles. Hence, it is probable that the iminoquinone generated by
activated neutrophils is also responsible for the covalent binding of 7-hydroxyfluperlapine to neutrophil proteins.

In the presence of GSH or NAC (5 mM), the neutrophil protein modification by the iminoquinone intermediate of 7-hydroxyfluperlapine was effectively inhibited (Fig. 11B). In these experiments, the 7-hydroxyfluperlapine-GSH or 7-hydroxyfluperlapine-NAC adducts were subsequently detected in the incubation supernatant by LC/MS in SIM mode. These results indicate that most of the reactive iminoquinone species formed by activated neutrophils is generated extracellularly and it is trapped by added GSH or NAC before it can bind to cellular proteins. This observation is in accord with the previous studies on neutrophil activation, which show the release of MPO enzyme by neutrophils on external stimulation (Bentwood and Henson, 1980; Chatham et al., 1994). Hence, much of the binding may occur to surface proteins; however, this does not exclude the possibility that some of the extracellularly-formed reactive species may bind to intracellular proteins. N-Acetyl-L-lysine did not inhibit the binding of neutrophil proteins by the iminoquinone and there was no N-acetyl-L-lysine adduct detected in the supernatant. This implies that the iminoquinone intermediate selectively reacts with sulfhydryl nucleophiles and only binds to sulfhydryl-containing proteins. This is similar to the reactivity of the nitrenium ion of clozapine.

The covalent binding of the iminoquinone to MPO was demonstrated using purified human MPO in presence of H$_2$O$_2$ and Cl$^-$. With 7-hydroxyfluperlapine at a concentration as low as 2 μM, the MPO was modified by the bioactivated reactive intermediate. The modified MPO polypeptide has a molecular mass of 58 kDa, which is the heavy subunit of the enzyme (Andrews and Krinski, 1981). The MPO is presumably bound by the iminoquinone intermediate via its cysteine residues. A proposed bioactivation pathway for fluperlapine is summarized in Fig. 12.

It has been widely believed that the bioactivated reactive intermediates are probably responsible for most drug-induced idiosyncratic reactions. Iminoquinones are reactive Michael acceptors, and their formation from drugs has been associated with several idiosyncratic drug reactions. For example, the iminoquinone intermediates of acetaminophen and amiodarone generated by liver cytochrome P-450 enzymes have been implicated in the idiosyncratic hepatitits caused by these drugs (Jewell et al., 1995; Parkinson, 1996; Chen et al., 1998). The same iminoquinone metabolite of amiodarone is also formed by activated human neutrophils and may be responsible for the drug-induced agranulocytosis (Winstanley et al., 1990; Tingle et al., 1995). It has also been shown that many other drugs associated with high incidence of agranulocytosis can be oxidized to iminoquinone-type intermediates (Uetrecht, 1992a; Uetrecht et al., 1994; Ju and Uetrecht, 1999). Even though nominally the reactive metabolite of fluperlapine is different from that of clozapine, i.e., imиноquinone rather than nitrogen ion, the reactivity, orientation of binding, and physical structure of the two are very similar. We propose that the subsequent covalent binding and/or redox cycling of this reactive intermediate is responsible for fluperlapine-induced agranulocytosis and subsequent steps in the mechanism may be very similar to those involved in clozapine-induced agranulocytosis. The current study also suggests that during studies of the mechanism of an idiosyncratic drug reaction, in addition to testing the ability of the drug to form a reactive metabolite, the additional metabolism of the major stable metabolites should also be investigated.

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**References**


