Pergamon

Biochemical Pharmacology, Vol. 48, No. 5, pp. 851–857, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)E0116-3

COMMENTARY

IS THERE A LINK BETWEEN THE PHOTOTOXIC OR ANTIOXIDANT PROPERTIES OF AMIODARONE, AN ANTIARRHYTHMIC DRUG, AND ITS LIPOPHILIC CHARACTER?

NICOLE PAILLOUS* and SUZANNE FERY-FORGUES

Laboratoire des Interactions Moléculaires et Réactivité Chimique et Photochimique, URA-CNRS 470, Université Paul Sabatier, 31062 Toulouse Cedex, France

Key words: amiodarone; cordarone; phototoxicity; lipid peroxidation; antioxidant; lipophilicity; membrane; photosensitivity

Amiodarone is a benzofuranic derivative originally introduced in Europe in 1964 as an antianginal drug because of its vasodilatator properties. Some years later, it was also shown to be highly effective in the treatment of intractable cardiac arrhythmias, for which it is now prescribed frequently [1, 2]. The drug exhibits antiarrhythmic effects by prolonging the duration of the action potential and is opposed to cardiac hyperexcitability. It displays a bradycardic effect and tends to slow down auriculoventricular conduction. In addition to its coronary vasodilator action, amiodarone reduces systemic resistance and myocardial contractability, and it contributes to restoring the balance of oxygen supply and demand [3, 4].

However, amiodarone therapy is associated with severe side-effects on the lungs, liver, nervous system, thyroid gland, cornea and skin [2, 5]. The drug induces adverse phototoxic effects in patients after exposure to sunlight [6]. A common feature of all these side-effects is the accumulation of the drug in a great variety of tissues where lysosomal inclusions may be detected [7]. Long-term administration perturbs phospholipid metabolism, leading to cellular phospholipidosis [8].

Amiodarone hydrochloride, 2-butyl-3-(3′,5′-diiodo - 4′ - β - *N* - diethyl - aminoethoxybenzoyl) benzofuran hydrochloride (cordarone R), belongs to a class of cationic amphiphilic drugs whose relatively highly lipophilic moiety can strongly interact with membranes (Scheme 1).

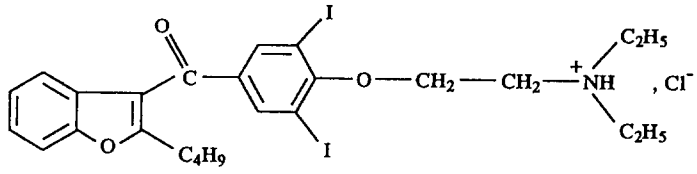
Previous observations have suggested that part of both the therapeutic activity of the drug and its side-effects may be related to its lipophilicity. Here, we will examine the data given in the literature concerning the incorporation of amiodarone into membranes and the possible consequences on its phototoxic and antioxidant properties. More particularly, its capacity to induce or inhibit lipid peroxidation will be considered with regard to its location in the membranes. The chemical aspects of the problem will be emphasized, whereas the biological and pharmacological aspects, which are largely beyond the scope of this report, will be discussed only briefly. Our discussion will be focused on amiodarone. The case of its main metabolite, desethylamiodarone, will not be considered, as both compounds have neighbouring structures and frequently similar effects.

* Corresponding author: Dr. Nicole Paillous, Laboratoire des Interactions Moléculaires et Réactivité Chimique et Photochimique, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse Cedex, France. Tel. (33) 61 55 62 74; FAX (33) 61 25 17 33.

Interactions of amiodarone with membranes

Amiodarone displays a low water-solubility and a strong lipophilic character, which could explain most of its behaviour. *In vitro*, these hydrophobic properties are reflected by a high partition coefficient value, which, apart from discrepancies, should be above 16,500 [9, 10] and a Σf value up to 9.46 [11], micelle formation [12], and spreading at the air–water interface [13]. *In vivo*, amiodarone is taken up efficiently by the cells [14, 15], although more slowly than other cationic amphiphilic drugs. The effect of amiodarone on the physical state of membrane phospholipids has been investigated in several models, ranging from multilamellar vesicles [10, 16–19] to natural membranes [9, 20, 21]. In pure lipid membrane models, a striking feature of amiodarone behaviour is that this compound has a fluidizing effect below the phase transition temperature (gel state) of phospholipids and a rigidizing effect above this temperature (liquid crystalline phase), as has been shown by Chatelain *et al.* [17] and by Sautereau *et al.* [19] from fluorescence polarization experiments. Chatelain *et al.* also established that this effect on fluidity depends on amiodarone concentration and on the length of the lipid acyl chain.

NMR studies [16] have shown that amiodarone is present in the outer monolayer of the liposome, as found by Eriksson [22] for other amphiphilic drugs. In fact, the location of the probe in the lipid matrix is the subject of much controversy; some authors have found the drug deeply buried in the hydrocarbon core of the liposomes [17] while others believe that the drug is located near the hydrocarbon/water interface [16, 19]. A deeper insight into the con-



Scheme 1.

troversy shows that contradictions between the different authors are mostly superficial. In our opinion, it is just to consider four distinct cases: the drug being found in either its ionized or neutral form and interacting with lipids in either the gel phase or the liquid crystalline phase. Several pK_a values have been reported for amiodarone: 5.6 [23], 6.56 [24], and 7.4 [25] in aqueous medium and 9.12* in a mixture of water and organic solvent. This reflects the difficulty encountered with classical potentiometric or spectroscopic methods in the determination of the aqueous dissociation constants of a poorly water-soluble product. Surface potential measurements, which are not affected by the lack of solubility of amiodarone, allowed the determination of the ionization constant at $pK_a = 8.7 \pm 0.5$. This value, which seemed more correct for a tertiary amine, proved to be in line with calculations performed later [18]. This means that at physiolological pH, amiodarone is mainly in its protonated form.

As long as the liquid crystalline phase is considered, amiodarone has the same influence as cholesterol at equal concentration on the bilayer structure. A comparison between the two compounds was made by Chatelain *et al.*, due to similarities in behaviour and structure [17] (a bulky hydrophobic ring with a short polar tail). Hence, these authors postulated an identical location for amiodarone and cholesterol in the phospholipid phase. The decrease of fluidity due to amiodarone was found to be independent of pH, hence of the ionization state of the drug [17]. A comparison was made with cholesterol and a cholesterol analogue bearing a doubly charged polar tail, which had a negligible influence upon micro-viscosity variations of the lipid bilayer [26]. However, we think that the cholesterol analogue may be located at a different depth in the membrane than the parent compound, because the ionized tail could bring it nearer to the hydrophilic part of the bilayer. Actually, variations of the macroscopic view of the lipid fluidity, as determined by fluorescence polarization, may reflect only the presence of a drug along the acyl chain (and until there, the comparison with cholesterol seems to be relevant to us), but may not be able to give more precise information about the depth of the drug location.

Using multinuclear NMR, Jendrasiak *et al.* [16] have shown that amiodarone does not order the hydrocarbon interior of the bilayer to the extent that cholesterol does, and that the effect obtained is closely dependent on pH. At acidic pH, amiodarone alters the signal arising from the phosphatidylcholine trimethylammonium group in a way quite different from that found with cholesterol, but the difference gets smaller and smaller as the pH turns basic. Addition of thiocyanate ions into the medium leads to significant variations of the proton and phosphorus NMR spectra in acidic medium, whereas the spectum remains unchanged when amiodarone is in its neutral form. Moreover, Ferreira *et al.* [18] demonstrated, by differential scanning calorimetry, that only the ionized form of amiodarone has the capability of decreasing the transition temperature and the enthalpy of melting, the variation being linearly correlated with the number of charged amiodarone moles. From these observations, we assume a different location of amiodarone in the liquid crystalline phase according to its ionization state. In its ionized form, amiodarone would be located near the interfacial region of the bilayer, the tertiary amine group of amiodarone neighbouring the head-group region of the lipid matrix. This is in agreement with Sautereau *et al.* [19], who observed, by UV spectroscopy, an interaction between amiodarone and the polar head of negatively charged lipids. On the contrary, neutral amiodarone would be more deeply embedded in the bilayer.

If we now consider lipids in the gel phase, the main difference is that the effect of the drug upon lipid fluidity is now related to pH. The neutral form of amiodarone leads to a far weaker decrease of ordering than the ionized form does. This has been attributed to the formation of clusters between neutral amiodarone molecules, thus minimizing the interaction with lipids and consequently the destabilization [18]. On the contrary, the repulsion due to the charge would favour the distribution of ionized drug molecules into the bilayer, as suggested by Ferreira *et al.* [18]. A change in solubility was already noticed in the liquid crystal phase since the liposomes became turbid and milky when the pH was increased [16]. In the X-ray diffraction study reported by Trumbore *et al.* [10], the liposomes were in the gel phase (dipalmitylphosphatidylcholine at 4°), and it can be assumed that amiodarone was poorly ionized due to the low degree of hydration used. In these conditions, the drug was located in the bilayer core. The orientation of the drug in the membrane has not been experimentally achieved yet, although it has been suggested that the high electron density observed in the acyl chain region of the bilayer would be better explained by the long axis of the drug lying parallel to the acyl chain. Since

* Gachon R, *Sanofi-Research. Internal Scientific and Technical Report*, 1981.

surface pressure measurements [13] showed that the area occupied by an amiodarone molecule was the same whatever the pH, it may be supposed that the conformation of the drug remains unchanged, whereas its penetration in the bilayer should be mediated by the charge borne by the polar head. Under physiological conditions, membrane lipids are in their liquid crystalline phase so that the mobility of the acyl chain should allow amiodarone to get closer to the unsaturated double bond. The drug should then be able to play either its photosensitizing role or, in the absence of light, its protective role towards oxidative stress.

The specific interaction between amiodarone and a particular lipid species is still controversial. Changes of calcium binding by monolayer shows no difference in the interaction of amiodarone with phosphatidylserine, phosphatidylinositol and phosphatidylethanolamine [27]. In the same way, Chatelain and Laruel [9] found that the drug incorporates similarly in the different lipid systems investigated, although electrostatic interactions could be masked by high partition coefficients. On the contrary, Sautereau *et al.* [19] attributed the difference found in the behaviour of amiodarone incorporated in phosphatidylcholine or phosphatidylglycerol to electrostatic interaction with the negatively charged lipids. This drug–lipid interaction could take into account another side-effect frequently encountered in patients, namely the formation of drug–lipid aggregates, especially on the cornea.

Lipophilicity and phototoxicity

Phototoxicity appears to be one of the properties of amiodarone that may be connected with its membrane affinity. Cutaneous amiodarone-induced photosensitivity is well known [6]. After long-term administration of the drug, the skin of patients exhibits an increased sensitivity to sunlight, characterized by intense burning, emergence of erythema, and swelling on the exposed area followed sometimes by a slate-grey pigmentation [5, 28]. This pigmentation is attributed to the granular accumulation of lipofuscin-type pigments located within superficial dermal macrophages [29]. These manifestations meet the criteria of phototoxic reactions [30]: they appear in most patients after exposure to sunlight (from 2 weeks to 2 months after the start of therapy) and disappear when the treatment is stopped, although some symptoms persist for weeks [31, 32]. In fact, amiodarone is not only phototoxic but may also cause photoallergic reactions. Photoallergy is generally hidden by phototoxicity and can be observed only when amiodarone is eliminated completely from the tissues. In addition, ocular problems are pointed out frequently, but they are reversible after withdrawal of the drug [28, 33].

The first requirement for a drug to be phototoxic is that sufficient drug levels are present in the skin during exposure to the sun. Actually, it is known that amiodarone is eliminated slowly, since the mean elimination half-life is more than 40 days and it accumulates in tissues 100–1000 times more than in plasma [34–36]. The incorporation of amiodarone within a lipidic matrix allows the excited drug to induce photochemical reactions at this level [37].

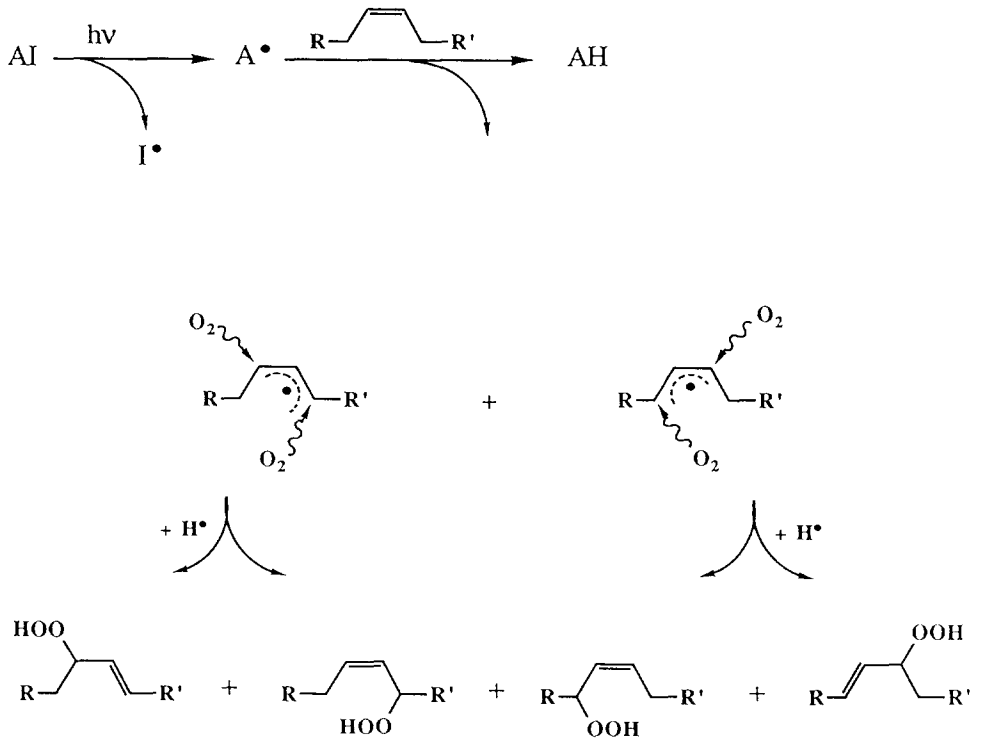
Most of the studies on drug-induced photosensitization indicate that immediate phototoxicity and related inflammatory effects may result from a photoinduced oxidative stress on membranes, leading to lipid peroxidation. In the case of amiodarone, the involvement of such reactions has been studied *in vitro*. Experiments carried out by Hassan *et al.* [37] have shown that amiodarone photosensitizes the hemolysis of red blood cells and is phototoxic to lymphocytes. The membrane damage observed on red blood cells results from both oxidative and non-oxidative processes. The influence of specific scavengers, such as sodium azide, mannitol, superoxide dismutase and catalase, on the photohemolysis rate suggests that free radical species, $O_2^{\cdot -}$, H_2O_2 and perhaps singlet oxygen are involved in the photodamage. The effects of the quenchers were lower than those usually observed. This is consistent with the fact that these oxidative species generated by amiodarone in the core of the membrane may react preferentially with membrane components instead of diffusing into water to encounter the quencher.

At a molecular level, Paillous and Verrier [38] demonstrated that irradiation of an alcoholic solution of amiodarone leads to a photodehalogenation of the drug. This loss of iodine atoms observed *in vitro* was in agreement with the iodine traces detected in macrophages coming from the skin of patients [39]. The photodehalogenation occurs via a radical mechanism established from ESR measurements [38, 40] and generating a carbon-centered radical:

$$AI \rightarrow A^{\cdot} + I^{\cdot}$$

In an aerated medium, irradiation of amiodarone also promotes the formation of active oxygen species. With a high intersystem crossing quantum yield, this drug generates $^1O_2$, as shown by trapping experiments [38]. It also undergoes a photoionization [40] that may explain the subsequent formation of superoxide anion detected by ESR.

These various radical species may attack the unsaturated phospholipids. This was confirmed by Li and Chignell [40], who detected the formation of the dienyl radical derived from linoleic acid during the photolysis of amiodarone in the presence of unsaturated fatty linoleic acid. This process may be at the origin of lipid peroxidation. A new series of experiments carried out by incorporating the drug into liposomes used as membrane model systems supports this assumption [19]. Under these conditions, it was shown that amiodarone photosensitizes the oxidation of unsaturated phospholipids while saturated phospholipids remain unchanged. From the whole set of these data it could be postulated that the radical formed by photolysis of amiodarone removes an allylic hydrogen atom from the unsaturated phospholipid, thus initiating lipid peroxidation (Scheme 2). This requires amiodarone to be close to the double bond. It may be assumed that this condition is more easily fulfilled when the lipid matrix is in its liquid crystalline phase as is the case *in vivo*, due to the relative mobility of the acyl chain. It would be interesting to know whether the drug maintains its efficacy when embedded in a rigid gel matrix, which could provide further evidence for



Scheme 2.

the location hypothesis. The incorporation of amiodarone in the hydrophobic core of membranes may govern the whole photoreactive process. In this respect, lipophilicity must be considered as a key factor of the phototoxic properties of the drug.

The assumption that amiodarone photosensitizes lipid peroxidation *in vivo* is supported by the presence of lipofuscin deposits detected in the skin of patients during amiodarone therapy. In fact, the formation of lipofuscin-type pigments is attributed to a reaction of lipid peroxidation by-products on free amino groups of biological compounds [41].

The importance of lipophilicity in the emergence of phototoxic properties is supported by the study of the behaviour of benziodarone. This drug has a chemical structure very similar to that of amiodarone, and it has the same photochemical reactivity. It undergoes a photodehalogenation reaction with the same quantum yield and generates the same amount of singlet oxygen. However, benziodarone, which exhibits weaker lipophilicity, does not appear to be phototoxic.*

Lipophilicity and antioxidant properties

Unexpectedly, amiodarone, which is phototoxic, has also been reported to elicit antioxidant properties [11]. A similar problem was pointed out by Kochevar in the case of non-steroidal anti-inflammatory drugs [42]. She underlined the "incongruity" that many of these agents induce phototoxic reactions related to inflammatory phenomena, which are at the opposite of their therapeutic indications. She wondered whether it was due to a coincidence or to a specific mechanism.

Recently, Rekka *et al.* [11] demonstrated that amiodarone could inhibit non-enzymatic lipid peroxidation. The peroxidation was induced by Fe^{2+}/ascorbic acid and tested on inactivated rat hepatic microsomal fraction. From the nineteen antiarrhythmic drugs tested simultaneously, amiodarone appeared to be one of the most potent antioxidants. This antioxidant activity of the drugs, quantified by the percent of inhibition of lipid peroxidation, was related by a parabolic relationship to their lipophilicity, expressed by their R_M or Σf values. Hence, it may be assumed that the insertion of amiodarone into the membrane facilitates a protective action against a radical oxidation in the same way that it favours a lipid oxidative process initiated by amiodarone, as previously shown. Nevertheless, the existence of an antioxidant activity implies that the lipid peroxidation reaction induced by amiodarone photosensitization is of some importance in order not to be inhibited completely by the antioxidant effect. From a chemical point of view, the reaction mechanism of the drug towards hydroxyl radicals has not been elucidated. It may be imagined that the latter react on the aromatic ring part of the molecule. It should be noted that this property is consistent with the fact that partial oxidation of amiodarone is observed during photosensitization experiments.

Rekka *et al.* suggested that the antioxidant activity of some antiarrhythmic drugs, including amiodarone,

* Paillous N, unpublished results.

may be part of their mode of action. It is more and more obvious that ischemia and reperfusion produce an oxidative stress that may cause arrhythmia [43–47]. Lipid peroxidation and free radical production can be stimulated by disturbances in calcium homeostasis [48]. The increase in calcium concentrations is able to cause myocardial after-contractions, to activate phospholipases and, therefore, to enhance free radical formation from acid metabolism [49]. It seems that a correlation can be established between the membrane damage resulting from the oxidative stress and rhythm disturbances.

An oxidative stress produced by polynuclear neutrophils also participates in the generation of reperfusion arrhythmias and myocardial damage. It has been demonstrated that amiodarone inhibits this stimulated superoxide anion production [50, 51]. This drug effect on the oxidative stress constitutes an additional mechanism of its activity. Its antioxidant power bestows a cardioprotective function upon amiodarone in addition to its antiarrhythmic activity and appears as a beneficial feature for the treatment of cardiac failure.

Relationship with the antiarrhythmic activity of the drug

The biological action of the drug is partly associated with its capacity to inhibit phospholipases and Na^+/K^+ ATPase, and possibly α and β adrenoreceptors, by a non-competitive mechanism.

After long-term treatment, amiodarone, like other cationic amphiphiles, induces a generalized phospholipidosis by modulating the activity of Ca^{2+}-dependent membrane phospholipases and Ca^{2+}-independent acid phospholipases [52–54]. It is noteworthy that the relative proportions of the lipids accumulating in the cell vary according to the nature of the cell considered [21, 46, 53, 55]. The inhibitory effect on phospholipases, as well as the increase in the lipid pool, may be part of the protective effect of amiodarone in the case of acute ischemia [55], which induces a dramatic lipid hydrolysis. As the inhibition of catabolism yields a modification of the membrane composition, an important consequence is the alteration of fluidity [36, 56].

Both the effect on lipid metabolism observed after chronic administration of the drug and the physicochemical effect due to the insertion of amiodarone into the membrane lead to a change in lipid mobility.

The direct effect of the drug upon membrane fluidity is far more complex on biological membranes than on membrane models. The incorporation of amiodarone into natural membranes may induce a dose-dependent decrease in fluidity as is the case with erythrocyte [21] and synaptic membranes [20], the same effect being observed before and after the transition temperature [20]. This effect is modulated according to the nature of the cells.

It is well-known that the variation of the bilayer fluidity can modify the affinity of receptors and the activity of membrane enzymatic systems. This could partly explain the effect of amiodarone on β-adrenergic receptors [36] and on the receptors involved in calcium regulation [57, 58], as well as the decrease induced in the activity of enzymes such as Na^+/K^+ ATPase [20], acetylcholine esterase, NADH dehydrogenase, and Ca^{2+}-dependent membrane phospholipase C.

A direct action of amiodarone upon membrane proteins must also be taken into account since hydrophobicity is expressed here by a partition coefficient equally high for lipids and for proteins [9]. Amiodarone could induce a possible change in protein conformation or distribution, thus leading to functional alterations. Additionally, as the protein content of membranes influences lipid microviscosity, the effect on fluidity could therefore be explained for drug doses that do not induce a change in lipid composition [21]. Hydrophobicity, which is responsible for this wide range of effects, could then be one of the key determinants that contribute to the antiarrhythmic properties of the drug.

In conclusion, the now well-established strong affinity of amiodarone for membranes appears to play a role in both the antiarrhythmic properties and some of the side-effects of the drug. In particular, its action in generating or inhibiting radicals may be favoured by its presence in the cell membranes. From a general point of view, the lipophilicity of the drug that is sometimes searched for to enhance the therapeutic properties may be a double-edged weapon.

REFERENCES

1. Rosenbaum MB, Chiale PA, Halpern MS, Nau GJ, Przybylzky J, Levi RJ, Lazzari JO and Elizari MV, Clinical efficacy of amiodarone as an antiarrhythmic agent. *Am J Cardiol* **38**: 934–944, 1976.
2. Gill J, Rennie HC and Fitton A, Amiodarone. An overview of its pharmacological properties, and review of its therapeutic use in cardiac arrhythmias. *Drugs* **43**: 69–110, 1992.
3. Zipes DP, Prystowsky EN and Heger JJ, Amiodarone: Electrophysiologic actions, pharmacokinetics and clinical effects. *J Am Coll Cardiol* **3**: 1059–1071, 1984.
4. Singh BN, Jewitt DE, Downey JM, Kirk ES and Sonnenblick EH, Effects of amiodarone and L8040, novel antianginal and antiarrhythmic drugs, on cardiac and coronary haemodynamics and on cardiac intracellular potentials. *Clin Exp Pharmacol Physiol* **3**: 427–442, 1976.
5. Harris L, McKenna WJ, Rowland E and Krikler DM, Side effects and possible contraindications of amiodarone use. *Am Heart J* **106**: 916–923, 1983.
6. Rappersberger K, Honnigsmann H, Ortel B, Tanew A, Konrad K and Wolff K, Photosensitivity and hyperpigmentation in amiodarone-treated patients: Incidence, time course and recovery. *J Invest Dermatol* **93**: 201–209, 1989.
7. Hruban Z, Pulmonary and generalized lysosomal storage induced by amphiphilic drugs. *Environ Health Perspect* **55**: 53–76, 1984.
8. Adams PC, Holt DW, Storey GCA, Morley AR, Callaghan J and Campbell RWF, Amiodarone and its desethyl metabolite: Tissue distribution and morphologic changes during long-term therapy. *Circulation* **72**: 1064–1075, 1985.
9. Chatelain P and Laruel R, Amiodarone partitioning with phospholipid bilayers and erythrocyte membranes. *J Pharm Sci* **74**: 783–784, 1985.
10. Trumbore M, Chester DW, Moring J, Rhodes D and Herbette LG, Structure and location of amiodarone in a membrane bilayer as determined by molecular mechanics and quantitative X-ray diffraction. *Biophys J* **54**: 535–543, 1988.

11. Rekka E, Mannhold RM, Bast A and Timmerman H, Molecular pharmacological aspects of antiarrhythmic activity I. Class I and class III compounds and lipid peroxidation. *Biochem Pharmacol* **39**: 95–100, 1990.
12. Ravin LJ, Shami EG and Rattie ES, Micelle formation and its relationship to solubility behavior of 2-butyl-3 - benzofuranyl - 4 - [2 - (diethylamino)ethoxy] - 3,5 - diiodophenyl ketone hydrochloride. *J Pharm Sci* **64**: 1830–1834, 1975.
13. Ferreira J, Brasseur R, Chatelain P and Ruysschaert JM, Properties of amiodarone monolayer spread at the air–water interface. *J Pharm Pharmacol* **38**: 561–566, 1986.
14. Riva E, Gerna M, Neyroz R, Urso R, Bartosek I and Guaitani A, Pharmacokinetics of amiodarone in rats. *J Cardiovasc Pharmacol* **4**: 270–275, 1982.
15. Debbas MMG, Du Cailor C, Sassine A, Derancourt J, Demaille J and Puech P, Determination of cardiac and plasma drug levels during long-term amiodarone therapy. *Eur J Clin Invest* **13**: 123–127, 1983.
16. Jendrasiak GL, McIntosh TJ, Ribeiro A and Porter RS, Amiodarone–liposome interaction: A multinuclear NMR and X-ray diffraction study. *Biochim Biophys Acta* **1024**: 19–31, 1990.
17. Chatelain P, Ferreira J, Laruel R and Ruysschaert JM, Amiodarone induced modifications of the phospholipid physical state. A fluorescence polarization study. *Biochem Pharmacol* **35**: 3007–3013, 1986.
18. Ferreira J, Chatelain P, Caspers J and Ruysschaert JM, Ionization state of amiodarone mediates its mode of interaction with lipid bilayers. *Biochem Pharmacol* **36**: 4245–4250, 1987.
19. Sautereau AM, Tournaire C, Suarès M, Tocanne JF and Paillous N, Interactions of amiodarone with model membranes and amiodarone-photoinduced peroxidation of lipids. *Biochem Pharmacol* **43**: 2559–2566, 1992.
20. Chatelain P, Laruel R and Gillard M, Effect of amiodarone on membrane fluidity and Na^+/K^+ ATPase activity in rat-brain synaptic membranes. *Biochem Biophys Res Commun* **129**: 148–154, 1985.
21. Chatelain P, Brotelle R and Laruel R, Decrease in lipid mobility in rat erythrocyte membrane after amiodarone chronic treatment. *Biochem Pharmacol* **36**: 1564–1565, 1987.
22. Eriksson LEG, Interaction of charged amphiphilic drugs with phosphatidylcholine vesicles studied by NMR. *Biophys Chem* **26**: 9–18, 1987.
23. Andreasen F, Agerbaek H, Bjerregaard P and Gøtzsche H, Pharmacokinetics of amiodarone after intravenous and oral administration. *Eur J Clin Pharmacol* **19**: 293–299, 1981.
24. Bonati M, Gaspari F, D'Aranno V, Benfenati E, Neyroz P, Galletti F and Tognoni G, Physicochemical and analytical characteristics of amiodarone. *J Pharm Sci* **73**: 829–831, 1984.
25. Canada AT, Lesko LJ and Haffajee CI, Disposition of amiodarone in patients with tachyarrhythmias. *Curr Ther Res* **30**: 968–974, 1981.
26. Lyte M and Shinitzky M, Cholesteryl-phosphoryl-choline in lipid bilayers. *Chem Phys Lipids* **24**: 45–55, 1979.
27. Luellmann H, Ploesch H and Ziegler A, Calcium replacement by cationic amphiphilic drugs from lipid monolayers. *Biochem Pharmacol* **29**: 2969–2974, 1980.
28. Chalmers RJG, Muston HL, Srinivas V and Bennett DH, High incidence of amiodarone-induced photosensitivity in Northwest England. *Br Med J* **285**: 341, 1982.
29. Zachary CB, Slater DN, Holt DW, Storey GCA and MacDonald DM, The pathogenesis of amiodarone-induced pigmentation and photosensitivity. *Br J Dermatol* **110**: 451–456, 1984.
30. Ferguson J, Addo HA, Jones S, Johnson BE and Frain-Bell W, A study of cutaneous sensitivity induced by amiodarone. *Br J Dermatol* **113**: 537–549, 1985.
31. Marcus FI, Fontaine GH, Frank R and Grosgogeat Y, Clinical pharmacology and therapeutic application of the antiarrhythmic agent, amiodarone. *Am Heart J* **101**: 480–493, 1981.
32. Walter JF, Bradner H and Curtis GP, Amiodarone photosensitivity. *Arch Dermatol* **120**: 1591–1594, 1984.
33. Texier L, Verin P, Gendre P, Gauthier Y, Mme M, Coville P and Gauthier O, Les syndromes oculo-cutanés iatrogènes. *Bordeaux Méd* **10**: 1545–1552, 1974.
34. Holt DW, Tucker GT, Jackson PR and Storey GCA, Amiodarone pharmacokinetics. *Am Heart J* **106**: 840–847, 1983.
35. Singh BN, Vekatesh N, Nademanee K, Josephson MA and Kannan R, The historical development, cellular electrophysiology and pharmacology of amiodarone. *Prog Cardiovasc Dis* **31**: 249–280, 1989.
36. Honegger UE, Zuehlke RD, Scuntaro I, Schaefer MHA, Toplak H and Wiesmann UN, Cellular accumulation of amiodarone and desethylamiodarone in cultured human cells. Consequences of drug accumulation on cellular lipid metabolism and plasma membrane properties of chronically exposed cells. *Biochem Pharmacol* **45**: 349–356, 1993.
37. Hassan T, Kochevar IE and Abdulah D, Amiodarone phototoxicity to human erythrocytes and lymphocytes. *Photochem Photobiol* **40**: 715–719, 1984.
38. Paillous N and Verrier M, Photolysis of amiodarone, an antiarrhythmic drug. *Photochem Photobiol* **47**: 337–343, 1988.
39. Trimble JW, Mendelson DS, Fetter BF, Ingram P, Gallagher JJ and Shelburne JD, Cutaneous pigmentation secondary to amiodarone therapy. *Arch Dermatol* **119**: 914–918, 1983.
40. Li ASW and Chignell CF, Spectroscopic studies of cutaneous photosensitizing agents—IX. A spin trapping study of the photolysis of amiodarone and desethyl-amiodarone. *Photochem Photobiol* **45**: 191–197, 1987.
41. Dillard CJ and Tappel AL, Fluorescent damage products of lipid peroxidation. In: *Methods in Enzymology* (Eds. Colowick SP and Kaplan NO), Vol. 105, pp. 338–341. Academic Press, New York, 1984.
42. Kochevar IE, Phototoxicity of nonsteroidal inflammatory drugs. Coincidence or specific mechanism? *Arch Dermatol* **125**: 824–826, 1989.
43. Stewart JR, Blackwell WH, Crute SL, Loughlin V, Greenfield LJ and Hess ML, Inhibition of surgically induced ischemia reperfusion injury by oxygen free-radical scavengers. *J Thorac Cardiovasc Surg* **86**: 262–272, 1983.
44. Gauduel Y and Duvelleroy MA, Role of oxygen radicals in cardiac injury due to reoxygenation. *J Mol Cell Cardiol* **16**: 459–470, 1984.
45. Peterson DA, Asinger RW, Elsperger KJ, Homans DC and Eaton JW, Reactive oxygen species may cause myocardial reperfusion injury. *Biochem Biophys Res Commun* **127**: 87–93, 1985.
46. Zweier JL, Flaherty JT and Weisfeldt ML, Direct measurement of free radical generation following reperfusion of ischemic myocardium. *Proc Natl Acad Sci USA* **84**: 1404–1407, 1987.
47. Garlick PB, Davies MJ, Hearse DJ and Slater TF, Direct detection of free radicals in the reperfused rat heart using electron spin resonance spectroscopy. *Circ Res* **61**: 757–760, 1987.
48. Julicher RHM, Sterrenberg L, Koomen JM, Bast A and Noordhoek J, Evidence for lipid peroxidation during the calcium paradox in vitamin E-deficient rat heart. *Naunyn Schmiedebergs Arch Pharmacol* **326**: 87–89, 1984.

49. Saxon ME, Stabilizing effect on antioxidants and inhibitors of prostaglandin synthesis on after-contractions in Ca^{2+}-overloaded myocardium. *Basic Res Cardiol* **80**: 345–352, 1985.
50. Wysocka E, Wysocki H, Siminiak T and Szczepanik A, Effect of selected antiarrhythmic drugs on the superoxide anion production by polymorphonuclear neutrophils *in vitro*. *Cardiology* **76**: 264–269, 1989.
51. Siminiak T, Wysocki H, Veit A and Maurer HR, The effect of selected antiarrhythmic drugs on neutrophil free oxygen radicals production measured by chemiluminescence. *Basic Res Cardiol* **86**: 355–362, 1991.
52. Shaikh NA, Downar E and Butany J, Amiodarone—an inhibitor of phospholipase activity: A comparative study of the inhibitory effects of amiodarone, chloroquine and chlorpromazine. *Mol Cell Biochem* **76**: 163–172, 1987.
53. Duane PG, Rice KL, Charboneau DE and Niewoehner DE, Amiodarone-induced endothelial injury is associated with phospholipase C-mediated hydrolysis of membrane phospholipids. *J Lab Clin Med* **120**: 955–963, 1992.
54. Wilson BD, Clarkson CE and Lippmann ML, Amiodarone causes decreased cell-mediated immune responses and inhibits the phospholipase C signaling pathway. *Lung* **171**: 137–148, 1993.
55. Shaikh NA, Effect of amiodarone therapy on the time course of myocardial phospholipid hydrolysis during *in vitro* total ischemia in cat hearts. *J Mol Cell Cardiol* **24**: 507–521, 1992.
56. Padmavathy B, Devraj NS and Devraj H, Hematological and erythrocyte membrane changes induced by amiodarone, in rats. *Indian J Physiol Pharmacol* **36**: 276–278, 1992.
57. Kodavanti PRS, Pentyala SN, Yallapragada PR and Desaiah D, Amiodarone and desethylamiodarone increase intrasynaptosomal free calcium through receptor mediated channel. *Naunyn Schmiedebergs Arch Pharmacol* **345**: 213–221, 1992.
58. Kachel DL, Moyer TP and Martin WJ II, Amiodarone-induced injury of human pulmonary artery endothelial cells: Protection by α-tocopherol. *J Pharmacol Exp Ther* **254**: 1107–1112, 1990.