Effects of Surfactants on Amiodarone Intestinal Absorption. I. Sodium Laurylsulfate

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Amiodarone is a widely used antiarrhythmic agent with high variability in therapeutic effects, which appears to be related, at least in part, to its pharmacokinetics, and in particular, gastrointestinal absorption. The drug exhibits physico-chemical properties highly suitable for diffusion across lipophilic absorbing membranes but its low aqueous solubility can act as the rate limiting step for absorption, making it erratic and variable. In studying the intestinal absorption mechanism of amiodarone, a series of experiments using a rat gut *in situ* preparation was performed in the presence of a synthetic anionic surfactant, as a drug solubilizer, i.e., sodium laurylsulfate, at variable supramicellar concentrations (from 2.6 to 104 mM). Absorption rate constants of amiodarone decreased as surfactant concentration increased, the absorption being unusually fast at lower surfactant concentrations. Equations were developed to evaluate the relationship between absorption rate constant and surfactant concentration in the intestinal luminal fluid.

KEY WORDS: amiodarone; intestinal absorption; sodium laurylsulfate; surfactant.

Introduction

Amiodarone hydrochloride is an iodinated benzofurane-derivative antiarrhythmic agent, often used when other antiarrhythmics are inefficient. The therapeutic responses are variable, and side effects frequently occur (1,2) after administration by the oral route. These features may be related to the pharmacokinetics of the drug, and particularly, to its absorption.

The absolute bioavailability of commercially available amiodarone tablets averages approximately 50%, but varies considerably, ranging from 22 to 86% (2–4). The sometimes low and often variable bioavailability of amiodarone may result from N-dealkylation or other metabolism in the intestinal lumen and/or gastrointestinal mucosa, from first-pass metabolism in the liver, and/or from poor dissolution characteristics of the drug. Amiodarone shows physico-chemical properties suitable for diffusion across lipophilic membranes, but its low aqueous solubility could represent the rate limiting step for absorption (5,6).

Drugs with physicochemical characteristics similar to those of amiodarone have shown improved dissolution and absorbability in the presence of surfactants (7–14). The effects of surfactants on passive absorption of xenobiotics have also been reported (15–20). Absorption of water-soluble drugs can be improved through an increase in the permeability of the absorbing membrane directly promoted by the surfactants, whereas absorption of lipophilic, poorly soluble drugs can be improved *via* the wetting action of the surfactants, and by the disarrangement of the aqueous diffusion layer adjacent to the membrane which acts as the limiting factor for absorption of such compounds in the absence of synthetic surfactants. Whereas these effects seem to be exerted by the surfactant at its critical micelle concentration (CMC) or lower, at supramicellar concentrations (SMC) such effects could be masked by micellar solubilization of the solutes. This process reduces the free drug fraction directly available for absorption; however, through further dilution in the gastrointestinal fluid, it could lead to the release of the drug from micelles in an hyperabsorptive state.

This study characterizes intestinal absorption of amiodarone in the presence of increasing surfactant concentrations to determine the importance of each surfactant action on the absorption process of the drug, and to establish conditions leading to optimal amiodarone absorbability.

Materials and Methods

Drug and chemicals. Six sodium laurylsulfate (reagent grade, Merck) solutions (2.6, 5.2, 10.4, 26, 52 and 104 mM in NaCl 0.78%) containing Amiodarone hydrochloride (75 μg/ml) were prepared; the final osmolarity was 300 ± 10 mOsm. The pH of the solution to be perfused was adjusted to 7.0 using NaOH or HCl 0.1 N.

Analytical procedure. Intestinal amiodarone samples were assayed for drug content by HPLC using a procedure developed in our laboratory, which provided an excellent separation and quantitation. The instrumentation consisted of a Perkin Elmer programmable binary pump, series 250, a liquid chromatograph equipped with a Waters C_{18} column (μBondapak 10 μm, 30 × 0.39 cm), and a variable volume injector (Rheodyne P/N 7525047). A Perkin Elmer spectrophotometer, model LC 90 BIO, set at 242 nm, was used to monitor the column effluent. Integration of peak areas was performed using a Perkin Elmer LCI 100 integrator.

For amiodarone evaluations, a mobile phase consisting of a volumetric mixture of methanol and 0.01 M phosphoric acid at pH 3.0 (85:15) was used, at a flow rate of 2 ml/min. Intestinal samples were centrifuged at 3000 r.p.m. for 15 minutes, and 10 μl of the supernatant were injected into the chromatograph. Calibration curves covering the entire range of concentrations in biological samples were obtained. Excellent linear plots relating peak area and concentrations were found; intercept did not significantly differ from zero. Because of the simplicity of the procedure, no internal standard was needed. Variation coefficients ranging from 0.4% to 5.50% were found.

Absorption studies. Male Wistar rats weighing 200–300 g, fasted for 20 h and anesthetized 1 h before the experience by intraperitoneal injection of ethyl-urethane (25 % w/V) were used. The *in situ* rat gut technique was performed using the whole small intestine, adapted as previously described (21–23).

The absorption experiments were developed using ami-

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odarone solutions in the presence of different surfactant concentrations. All solutions were supramicellar. Ten ml of an isotonized amiodarone solutions, in the presence of sodium laurylsulfate and buffered to pH 7, were perfused at 37°C. The remaining concentrations of the drug were measured every 5 min, for a total time of 30 min, using 0.25 ml samples of the perfused solutions. The remaining amounts in the samples were determined using the following equation:

$$A_e = C_e \cdot V_t \quad (1)$$

where A_e is the remaining amount in the sample, C_e the experimental concentrations and V_t the aqueous volume at the sampling times.

Water reabsorption was evaluated separately for each solution according to a previously reported procedure (23).

Water reabsorption process. Reabsorption was identified as an apparent zero-order process (23) accordingly to equation 2.

$$V_t = V_o - k_o \cdot t \quad (2)$$

where V_t is the volume remaining in the luminal content at the sampling times, t, V_o the calculated intercept value at zero time, and k_o the zero order water reabsorption rate constant.

To evaluate its extent and characteristics, the time course of this process was analyzed by linear regression using the data obtained from each perfused solution. The amiodarone remaining in the intestinal lumen at each time unity was then calculated, only when the slope was significantly different from zero value, according to ANOVA test.

Amiodarone absorption rate measurements. Amiodarone absorption was quantified by means of its apparent first order rate constant, through the expression:

$$-\frac{dA}{dt} = k_a \cdot A \quad (3)$$

or its integrated form:

$$A = A_o \cdot e^{-k_a} \cdot t \quad (4)$$

where A values are the remaining amounts in the luminal contents at the sampling times, t, k_a is the absorption rate constant and A_o is the initial drug amount, which is always lower than the actual amount perfused due to membrane uptake and other factors (22,23). Therefore, only the samples obtained between 5 and 30 minutes were used for calculations, i.e., the zero time sample was not used for regression. Both parameters (A_o and k_a) were then calculated according to non-linear regression least squares, and the average values found for each test series were statistically compared through a one-way ANOVA test.

Fitting of models to data. In order to express the absorption rate constant as a function of the sodium laurylsulfate supramicellar concentrations in the perfusion fluid, a previously reported equation was used (16), expressed here as equation 5, as well as some modifications of it expressed as equation 6. The origin of these expressions is given in the Appendix.

Table I. Water reabsorption kinetics; k_o is the reabsorption rate constant, V_o is the calculated intercept value at zero time, and P values mean the significance of the regression.

Sodium laurylsulfate (mM)	$V_o \pm$ s.d. (ml)	$k_o \pm$ s.d. (ml/min)	P
2.6	10.46 ± 0.50	−0.09 ± 0.02	0.0070
5.2	10.76 ± 0.47	−0.09 ± 0.02	0.0018
10.4	10.24 ± 0.31	−0.05 ± 0.01	0.0070
26	10.95 ± 0.56	−0.04 ± 0.03	0.1846
52	10.40 ± 0.53	−0.02 ± 0.02	0.4819
104	10.08 ± 0.50	0.03 ± 0.02	0.2750

Model 1

$$k_a = \frac{k_{af}}{1 + \frac{V_m}{V_a} \cdot P_i} \quad (5)$$

Model 2

$$k_a = \frac{k_{ao} \cdot 10^{\left(\frac{V_m}{V_a} \cdot a\right)}}{1 + \frac{V_m}{V_a} \cdot P_i} \quad (6)$$

where k_{af} represents the absorption rate constant for the free amount of drug; k_{ao} is the absorption rate constant which performs in the presence of the surfactant at its critical micelle concentration (CMC, i.e. the absorption rate constant of the drug in the absence of surfactant micelles); P_i represents the *in vivo* partition coefficient of the amiodarone between micelles and aqueous phase; a, in equation 6, is a constant which depends on the experimental technique used; V_m is the micellar volume and V_a the aqueous volume, so that the ratio V_m/V_a can be expressed as the concentration of the surfactant in the perfusion fluid. Although some variation in V_a exists because water reabsorption, this variation is in practice irrelevant within the 30 minutes interval, so that k_a values truly represent the influence of each initial surfactant concentration on absorption.

The fitting process was performed by means of nonlinear weighted least squares regression, using the Marquardt algorithm, in a IBM-PC computer. In order to check the goodness of the fits, the correlation coefficients between estimated and experimental values, and the Akaike information criterion (AIC) were used. As a complementary criterion the weighted sum of squares found for each fit was also calculated.

Table II. Average absorption rate constants and initial amounts of amiodarone, (k_a and $A_o \pm$ s.d.) found at different starting sodium laurylsulfate concentrations.

Sodium laurylsulfate concentration (mM)	Absorption rate constant $k_a \pm$ s.d. (h^{-1})	Amiodarone amount at intercept $A_o \pm$ s.d. (mg)
2.6	2.86 ± 0.26	0.61 ± 0.04
5.2	2.22 ± 0.22	0.68 ± 0.05
10.4	1.40 ± 0.09	0.61 ± 0.05
26	0.98 ± 0.11	0.60 ± 0.05
52	0.84 ± 0.10	0.58 ± 0.06
104	0.91 ± 0.13	0.62 ± 0.04

Table III. ANOVA test for absorption rate constants found in the presence of surfactant at different starting surfactant concentrations.

ANOVA test					
Variability	D.f.	Sum of squares	Mean squares	F	P
Sets	5	27.4695	5.4939	202.7365	0.00001
Error	41	1.1110	0.0271		
Total	46	28.5806			

Results

Water reabsorption tests. Parameter values and statistical figures for water reabsorption kinetics for the six sets of data are shown in Table I. According to these results, amiodarone remaining amounts were adequately corrected for reabsorption (23).

Absorption kinetic parameters. Absorption rate constants, k_a, and concentrations of amiodarone at the intercept, A_o, calculated according to equation 4 (mean ± s.d. of 8 animals per set), are shown in Table II.

Significant differences between k_a values were found when the ANOVA test was applied, as shown in Table III. No significant differences between A_o values were found.

Correlations between absorption rate constant and surfactant concentration. In Table IV, parameter values, r, weighted sums of squares and AIC values found by fitting equations 5 and 6 to the data are given. In Figure 1, graphs are plotted according to the fitting equations.

Discussion

Water reabsorption studies. Water reabsorption in the rat small intestine was shown to be significant only for tests developed with amiodarone solutions in the presence of the surfactant at the three lower concentrations used, i.e. 2.6, 5.2 and 10.4 mM. In these series of experiments, the water reabsorption constant was significantly different from zero, whereas for amiodarone solutions with 26, 52, and 104 mM of sodium laurylsulfate, the water reabsorption process was not significant (Table I). It seems evident that the extent of water reabsorption becomes irrelevant when a given surfactant concentration is attained in the perfusion solution. It should be pointed out that all the perfusion solutions handled were isotonic; therefore, the opinion of the authors is that this effect might have been induced by the surfactant itself.

Table IV. Parameter values found by fitting the selected equations to data. Statistical figures found for each fit are also shown.

Fitting equation	Parameters	Parameter values ± SD	AIC	WSS	r
5	k_{af} (h^{-1})	2.1 ± 0.6	−1.833	0.378	0.864
	P_i (l/mM)	0.028 ± 0.017			
6	k_{ao} (h^{-1})	4.2 ± 0.6	−20.94	0.016	0.996
	P_i (l/mM)	0.22 ± 0.05			
	a (1/mM)	0.0069 ± 0.0006			

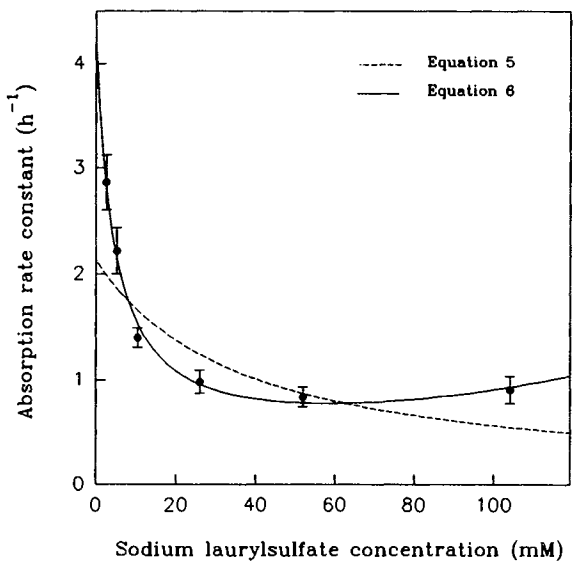


Fig. 1. Graphical plot representing the fits of equations 5 and 6 to data (see Table IV).

Amiodarone absorption in the presence of surfactant. The usual dose of amiodarone in patients, given orally, is 200 mg per day. This dose was used as a reference to calculate the allometric amount of amiodarone to be administered to the experimental animals, according to the ratio (24):

$$\frac{Human\ dose}{Human\ weight} = \frac{Animal\ dose}{Animal\ weight} \qquad (7)$$

If a mean weight of 75 kg for humans is assumed and since the mean weight of the animals was about 250 g, the working dose would be 0.783 mg, which was rounded to 0.75 mg. This amount of drug was dissolved in 10 ml of the perfusion fluid with the aid of variable amounts of the surfactant; the minimal concentration of surfactant suitable for amiodarone solubilization in the isotonic vehicle was 2.6 mM.

The amiodarone absorption process was apparently first-order kinetics and was characterized as a passive diffusion across the intestinal lipoidal membrane. Since amiodarone has a high molecular weight (681.8 daltons), diffusion through the aqueous pores does not have to be taken into account (25,26).

Table II shows that the apparent absorption rate constants of amiodarone decrease as surfactant concentration increases; the differences were statistically significant on the ANOVA test (Table III). This is probably no more than a consequence of the multiple effects of the surfactant on absorption, with an outstanding predominance of micellar solubilization (16). Correlation between apparent absorption rate constants of amiodarone and surfactant concentration in perfusion fluids could aid in quantifying each of these effects by means of equations 5 and 6.

Fitting biophysical absorption models to data. **Model 1.** Equation 5, representative of this model, accounts only for micellar solubilization. The absorption rate constant of amiodarone in the presence of surfactant micelles decreases as the amount of these latter (i.e. surfactant concentration in

the perfusion liquid) increases. Thus, k_a values range from a rather high value (which would be found in the presence of surfactant at CMC, when V_m/V_a equals to zero) to a negligible value (when surfactant concentration is very high, that is, when V_m/V_a tends to infinity). Fitting equation 5 to the data, however, demonstrates that some other factor, apart from micellar solubilization, is participating (Fig. 1); correlation coefficients between experimental and calculated values as well as the AIC figures are rather poor.

Model 2. This model not only accounts for micellar solubilization but also for membrane polarity changes elicited by the surfactant. The fitting of equation 6, representative of this model, to data, is much better than in the case of equation 5 (Fig. 1; Table IV). This was confirmed by the Snedecor F-test, with the aid of which significant differences clearly favouring model 2 were found ($F = 67.87$, $P < 0.0037$). This seems to indicate that the increase in membrane polarity by the surfactant is a continuous function of the concentration of the latter, even when CMC has been surpassed. In other words, the number of molecules (or ions) of surfactant interacting with the membrane increases along the entire range of concentrations and, consequently, the partition system membrane/luminal fluid continuously changes.

The effect of surfactant on the stagnant aqueous diffusion layer adjacent to the membrane (16) could not be determined here since no absorption tests for amiodaone in the absence of surfactant were available. The insolubility of the drug in the vehicle thus prevents quantitation of the drug in luminal samples. It seems reasonable to select Model 2 as the best in explaining the amiodarone absorption profiles found in our experimental conditions.

The membrane polarity change has been attributed to a partial and reversible solubilization of some membrane lipids or proteins (27,28) and can give rise to serious membrane damage when surfactant concentration is abnormally high.

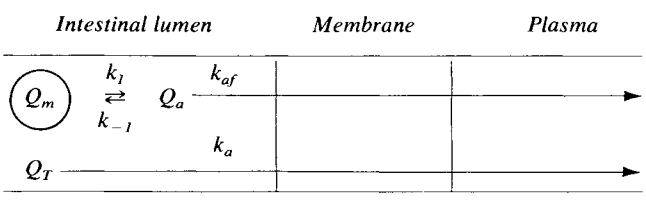
Practical implications. The high absorption rate constant found for amiodarone in the presence of 2.6 mM sodium laurylsulfate (2.86 h^{-1}), or an absorption half-life of about 15 min, means that only two hours would be necessary for complete absorption of the drug, if the proper surfactant concentration was present in the small intestine. However, the intestinal or hepatic first-pass effects, would not be bypassed.

The results presented here could lead to designing more reliable oral dosage forms of amiodarone containing a suitable dose of surfactant, as a solid dispersion or similar preparation (8,13,29).

Appendix

Model 1

This model can be represented as:



The model was developed by Plá-Delfina *et al.* (16) on the following bases:

(1) At supramicellar concentrations of surfactant (SMC), the drug is distributed between micelles and aqueous luminal fluid according to its *internal* partition coefficient, P_i, between these two phases.

(2) Only the free fraction of drug (i.e. drug dissolved in the aqueous media) is available for membrane diffusion.

(3) The surfactant increases membrane polarity until CMC is attained.

(4) The synthetic surfactants remove the limiting step for absorption which is performed by the aqueous diffusion layer adjacent to the membrane at its luminal side.

From these assumptions, equation 5 was derived (16), which predicts that the absorption rate constant of the drug in the presence of surfactant at SMC tends to decrease as surfactant concentration increases, from a maximum value (which is found at CMC) to a minimum value, zero, when $P_i \cdot V_m/V_a$ tends to infinity.

Model 2

A fundamental difference between this model and model 1 was that assumption 3 was modified as follows:

(3) The surfactant increases membrane permeability as much as its concentration in luminal fluid increases, as a result of its tendency to be preferentially arranged along the interfaces without disturbing the equilibrium with the molecules (or ions) in the solution.

As a result, the partition system continuously changes, even above CMC, since more and more surfactant molecules are present in the membrane as surfactant concentration increases, leading to a progressive increase in membrane polarity. Since it has been shown with surfactant at CMC (16), as a consequence of assumption 4, that:

$$k_{af} = B \cdot P_v^f \tag{1A}$$

where P_v represents the partition coefficient of the drug between membrane and luminal fluid, and B and f are constants, when CMC is surpassed, it can be reasonably assumed that P_v will change as a consequence of the increasing membrane polarity. This degree of change can be approached with the aid of some reversed-phase chromatographic partition principles (30) which we believe to be completely applicable to our particular case:

$$K' = 10^{(a \cdot \varphi + b)} \tag{2A}$$

where K' represents the capacity factor (or any other true partition constant, such as P), φ represents the fraction of organic solvent in the aqueous chromatographic eluent, and a and b are constants. In our case, a whole homology between K' and P_v, and between V_m/V_a and φ, respectively was assumed, so that:

$$P_v = 10^{\left(c \cdot \frac{V_m}{V_a} + d\right)} \tag{3A}$$

That is, P_v is a function of micellar volume. By substituting P_v for its value in equation 1A, and rearranging, we have:

$$k_a = \frac{B \cdot 10^{f \cdot d} \cdot 10^{f \cdot \left(c \cdot \frac{V_m}{V_a}\right)}}{1 + \frac{V_m}{V_a} \cdot P_i} \qquad (4A)$$

Renaming the parameters of equation 4A, equation 6 is obtained, which is the expression of a complex curve, the shape of which depends on the characteristics of the interactions drug/micelles and drug/membrane, these latter devoid of its aqueous diffusion layer. The intercept of the curve (when $V_m = 0$) is k_{ao}. This value represents k_{af} in Model 1, because from equation 10A when $V_m = 0$, k_a is k_{af} (i.e. at CMC the absorption rate constant, k_a, is k_{af} from Model 1 and k_{ao} from Model 2).

The remaining points of the curve will depend on two factors: (1) The lipophilicity of the drug (which governs its *internal* partition coefficient, P_i, and, therefore, its solubilization into the micelles), and (2) the modification in membrane permeability caused by the surfactant, $10^{\left(\frac{V_m}{V_a} \cdot a\right)}$, (which governs the passage of the free fraction of the drug across the membrane).

These two factors appear in equation 6 as the denominator and the numerator, respectively.

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