The pH-Partition Profile of the Anti-Ischemic Drug Trimetazidine May Explain Its Reduction of Intracellular Acidosis

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Received September 21, 1998; accepted February 3, 1999

Purpose. The anti-ischemic drug trimetazidine (TMZ) acts by a combination of molecular mechanisms which begin to be understood. Thus, it acts in the micromolar range to significantly reduce intracellular acidification during ischemia. To search for a possible physicochemical explanation of this phenomenon, we investigated the transfer mechanisms of the various electrical forms of this dibasic drug.

Methods. The transfer characteristics of TMZ were studied by electrochemistry at the water/1,2-dichloroethane interface. Cyclic voltammetry was used to measure the formal potentials of singly and doubly protonated forms of TMZ (noted TH+ and TH2+, respectively) as a function of aqueous pH, and the partition coefficient of neutral TMZ (log P0) was measured by two-phase titration. Results. log P0 was measured to be 1.04 ± 0.06, and the acid-base dissociation constants in water were deduced to be pK0 = 4.54 ± 0.02 and pK2 = 9.14 ± 0.02. The partition coefficients of TH+ and TH2+ were found to be respectively log P0[TH+] = −3.78 ± 0.16 and log P0[TH2+] = −9.84 ± 0.30, which agrees well with the charge being delocalized on two nitrogen atoms in TH+. The pH-pH profile of TMZ was then established in the form of its ionic partition diagram.

INTRODUCTION

Trimetazidine (TMZ) is a dibasic compound (Fig. 1) marketed in a number of countries as a safe cellular anti-ischemic devoid of hemodynamic effects. The molecular mechanisms of its anti-ischemic effects in the myocardium and neurosensory organs are insufficiently understood, but recent studies have revealed a variety of relevant activities (1–4).

Thus, most bioelectric activities in retina and inner ear are strongly modified by ischemia, but these deleterious effects were prevented by TMZ 10−6 to 10−5 M in blood plasma. Myocardial mitochondrial protection was evidenced in many tests, in particular in the efficacy of oral TMZ to maintain high levels of ATP despite hypoxia and calcium overload. This action is not due to an antioxidant effect (which is absent in TMZ), but to its ability to switch cellular metabolism towards preferential glucose utilization. Another noteworthy effect of TMZ 10−6 M is to significantly prevent intracellular acidification during ischemia. An action on Na+/H+ exchange or on Na+, K+-ATPase was excluded, as was a simple buffering effect. To gain insight into the underlying mechanism, we used electrochemical techniques to study the pH- and potential-dependent partitioning behavior of TMZ. Our results indeed suggest a partition mechanism whereby TMZ could facilitate proton transfer from an acidic to a neutral aqueous compartment, across a lipidic compartment.

The interface between two immiscible electrolyte solutions (ITIES) is a simple model of biological membranes, and constitutes a powerful means of simulating drug transfer processes. In this respect, the water/1,2-dichloroethane (1,2-DCE) solvent system is an interesting complement to the n-octanol/water and alkane/water systems (5) because the partitioning between water and 1,2-DCE is mainly governed by hydrophobic interactions and by H-bonding in the water phase (6) The water/1,2-DCE system gives more weight to H-bonding than the n-octanol/water system, as seen also in structure-permeation relationships which consistently demonstrate the importance of H-bonding between permeant and membranes (7,8).

Electrochemical measurements in this system offer new opportunities to measure the pharmacokinetically relevant lipophilicity of ionizable solutes (9–12). Furthermore, cells function by creating a potential difference across membranes (see Mitchell’s chemiosmotic model (13)). Electrochemistry has then the additional advantage of being able to investigate the interfacial movement of ions in an electric field as existing in cells. Even though there is no current/voltage source in the body, concentration differences between cytosol, intercellular space and membranes creates a potential difference of ~70 mV. This value could appear very small at first glance, but it must be remembered that the cellular membrane is only 3.5 nm thick. Thus, the potential gradient across the membrane is ~200,000 V cm−1. In simple biological models such as biphasic systems,

KEY WORDS: trimetazidine; lipophilicity; proton transfer; ionic partition diagram; ITIES.
Moreover, $\Delta_{\phi} \phi_i^0$ is in fact the standard Gibbs energy of transfer ($\Delta_{\phi} G_{\text{trans}}^{0,\text{w-w}}$) expressed in a potential scale, and these two quantities are simply related by:

$$\Delta_{\phi} \phi_i^0 = \frac{\Delta G_{\text{trans}}^{0,\text{w-w}}}{z_i F}$$

(2)

The standard transfer potential can be understood as a Gibbs energy of transfer normalized by the charge, and it accounts for the difference of solvation in the two adjacent phases (14). As discussed later, $\Delta_{\phi} \phi_i^o$ is a more relevant parameter than $\Delta G_{\text{trans}}^{0,\text{w-w}}$ to estimate the lipophilicity of an ionic species.

Upon variation of $\Delta_{\phi} \phi$, the thermodynamic equilibrium is displaced, and a certain time is needed to reach a new equilibrium state. However, for reversible (i.e., kinetically fast) ion transfer controlled by diffusion at the interface, it can be estimated that the equilibrium is instantaneous, so that Eq. 1 is valid whatever the rate of the potential sweep, and it can be rewritten in terms of partition coefficients:

$$\log P_i = \frac{z_i F}{RT \ln 10} (\Delta_{\phi} \phi_i^0 - \Delta_{\phi} \phi_i^o)$$

$$= \frac{z_i F}{RT \ln 10} \Delta_{\phi} \phi_i^o - \frac{\Delta G_{\text{trans}}^{0,\text{w-w}}}{RT \ln 10}$$

(3)

where $P_i$ is the partition coefficient of 1 and $P_i^o$ its formal partition coefficient (i.e., its partition coefficient when $\Delta_{\phi} \phi = 0$).

Contrary to charged species, the partition coefficient of a neutral species, $P_N$, is a unique quantity related to its standard Gibbs energy of transfer $\Delta G_{\text{trans}}^{0,\text{w-w}}$ by:

$$\log P_N = \log \left( \frac{a_i}{a_N^o} \right) = -\frac{\Delta G_{\text{trans}}^{0,\text{w-w}}}{RT \ln 10}$$

(4)

Thus, aqueous pH, $\Delta_{\phi} \phi$ and log $P_N$ influence the nature and the amount of each species in both phases. At the ITIES, no redox reaction occurs upon application of a Galvani potential difference between the two phases, and the current resulting from a potential sweep is due to a flux of ions across the interface. It can easily be shown that the aqueous bulk concentration of the transferring species $c_i^*$ is related to the maximum forward peak current $I_{\text{peak}}^{\text{FWD}}$ by the Randles-Sevcik equation (15):

$$I_{\text{peak}}^{\text{FWD}} = 4.463 z_i F A c_i^* \left( \frac{z_i F}{RT} \right)^{1/2} (vD_i^*)^{1/2}$$

(5)

where $A$ is the area of the interface, $D_i^*$ the diffusion coefficient of 1 in the water phase and $v$ is the rate of the potential sweep.

**MATERIALS AND METHODS**

Trimetazidine (1-(2,3,4-trimethoxybenzyl)piperazine) was kindly donated by the Institut de Recherches Internationales Servier (IRIS, F) and was of pharmaceutical grade. The aqueous phase was deionized water (Milli-Q QSP reagent water system, Millipore) with LiCl (Fluka, CH) as aqueous electrolyte, and the pH was adjusted to the desired value by addition of HNO$_3$ or LiOH (Fluka). The organic phase was 1,2-dichloroethane (1,2-DCE) of the highest available purity (Merck, G) with bis