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Molecular determinants of loperamide and *N*-desmethyl loperamide binding in the hERG cardiac K^+ channel

Roy J. Vaz [a,*], Jiesheng Kang [b], Yongyi Luo [c], David Rampe [d,*]

[a] Integrated Drug Discovery, Sanofi US, 153-2nd Ave., Waltham, MA 02451, USA
[b] Prelinical Safety, Sanofi US, 153-2nd Ave., Waltham, MA 02451, USA
[c] Drug Metabolism & PK, Sanofi US, 153-2nd Ave., Waltham, MA 02451, USA
[d] Preclinical Safety, Sanofi US, 55 Corporate Blvd, Bridgewater, NJ 08807, USA

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ABSTRACT

Abuse of the common anti-diarrheal loperamide is associated with QT interval prolongation as well as development of the potentially fatal arrhythmia torsades de pointes. The mechanism underlying this cardiotoxicity is high affinity inhibition of the human ether-a-go-go-related gene (hERG) cardiac K+ channel. *N*-Desmethyl loperamide is the major metabolite of loperamide and is a close structural relative of the parent molecule. To date no information is available regarding the affinity of *N*-desmethyl loperamide for human cardiac ion channels. The effects of *N*-desmethyl loperamide on various cloned human cardiac ion channels including hERG, KvLQT1/mink and $Na_v1.5$ were studied and compared to that of the parent. *N*-Desmethyl loperamide was a much weaker (7.5-fold) inhibitor of hERG compared to loperamide. However, given the higher plasma levels of the metabolite relative to the parent, it is likely that *N*-desmethyl loperamide can contribute, at least secondarily, to the cardiotoxicity observed with loperamide abuse. We used the recently solved cryo-EM structure of the hERG channel together with previously published inhibitors, to understand the basis of the interactions as well as the difference that a single methyl plays in the hERG channel blocking affinities of these two compounds.

Introduction

Loperamide is a common anti-diarrheal medication that is available without a prescription. The drug exerts its anti-diarrheal effect via activation of peripheral μ-opioid receptors. Despite this mechanism of action, abuse of loperamide has historically been limited by its poor oral bioavailability coupled with limited brain penetration. In recent years this situation has changed with opioid addicts consuming large doses of loperamide in an attempt to produce euphoria or to prevent opioid withdrawal symptoms.[1] Loperamide abuse leads to the generation of cardiac conduction disturbances characterized by QRS and QT prolongation on the electrocardiogram and the development of the potentially lethal torsades de pointes arrhythmia.[2,3] This has led to a warning from the U.S. Food and Drug Administration about the causal relationship of loperamide overdose to serious ventricular arrhythmias.[4] We and others have previously shown that loperamide is a potent inhibitor of the human ether-a-go-go-related gene K^+ channel (hERG) and this activity likely underlies the QT prolongation and torsades de pointes associated with loperamide abuse.[5,6] Loperamide is also an effective inhibitor of the human cardiac sodium channel which may contribute to QRS prolongation.[5] Following ingestion, loperamide is *N*-demethylated in the liver to form a major metabolite, *N*-desmethyl loperamide[3] which is a close structural relative to loperamide (Fig. 1). The purpose of the present study was to examine the effects of *N*-desmethyl loperamide on cardiac ion channels and compare them with the parent molecule. Furthermore, the binding of loperamide, *N*-desmethyl loperamide and other well-known torsadogenic drugs were modeled using the newly available cryo-EM structure of the hERG channel[7] in an attempt to better define the molecular determinants underlying these drug/hERG interactions.

* Corresponding authors.
E-mail address: roy.vaz@sanofi.com (R.J. Vaz).

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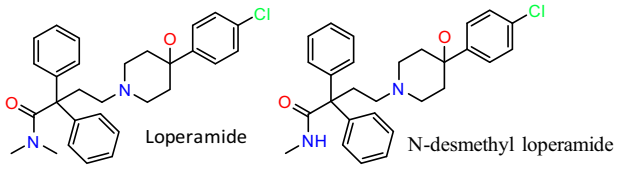

Fig. 1. Structure of loperamide and *N*-desmethyl loperamide.

Table 1
Effects of Loperamide and *N*-desmethyl loperamide on the cardiac channels - KvLQT1/minK, $Na_v1.5$ and hERG.

Channel	*N*-Desmethyl loperamide	Loperamide[a]
KvLQT1/minK	4 and 23% inhibition @1 and 10 μM, respectively	17 and 65% inhibition @1 and 10 μM, respectively
$Na_v1.5$	IC_{50} 483 nM (326–718 nM 95% CL)	IC_{50} 239 nM (206–277 nM, 95% CL)
hERG	IC_{50} 245 nM (220–272 nM, 95% CL)	IC_{50} 33 nM (25–44 nM, 95% CL)

[a] Loperamide data taken from Ref. 5.

Fig. 2 shows the effects of *N*-desmethyl loperamide on various human cardiac ion channels. Ion channel currents were recorded using the whole-cell configuration of the patch clamp technique as we have previously described for loperamide.[5] KvLQT1/minK is the channel that carries the slow component of the delayed rectifier current in the human heart (I_{Ks}). KvLQT1/minK channel currents were inhibited by 4 ± 1% (n = 4) and 23 ± 2% (n = 5) following exposure to 1 and 10 μM *N*-desmethyl loperamide, respectively (Fig. 2A, D). *N*-desmethyl loperamide blocked human cardiac sodium channel ($Na_v1.5$) currents with an IC_{50} of value of 483 nM (326–718 nM, 95% confidence limits, n = 5, Fig 2B, 2D). Finally, *N*-desmethyl loperamide inhibited hERG channel currents with an IC_{50} value of 245 nM (220–272 nM, 95% confidence limits, n = 5–7, Fig. 2C, D). The threshold for inhibition of hERG was 30 nM where inhibition measured 5.9 ± 2% (p = 0.029, paired *t*-test, n = 7).

Following oral administration of therapeutic doses of loperamide to healthy volunteers, peak plasma levels of *N*-desmethyl loperamide are approximately 2-fold higher than the parent compound.[8,9] Although data is scarce, in cases of high dose loperamide abuse, plasma levels of the metabolite may achieve somewhat higher multiples. A recent report of 2 patients presenting with cardiac arrhythmias secondary to loperamide abuse revealed loperamide plasma levels of 120 and 76 ng/ml and *N*-desmethyl loperamide of 560 and 630 ng/ml.[10]

Since it was not available in the literature, we conducted protein binding of *N*-desmethyl loperamide in fresh human plasma using rapid equilibrium dialysis.[11] Protein binding was tested at concentrations of 30, 300, and 1000 ng/mL and averaged 95.7 + 0.4% which is similar to the 95–97% protein binding reported for loperamide.[4,12] Assuming 96% protein binding for each compound, free plasma levels of loperamide measure 10 and 6.3 nM while for *N*-desmethyl loperamide they measure 48 and 54 nM for these two patients. With these parameters in mind,

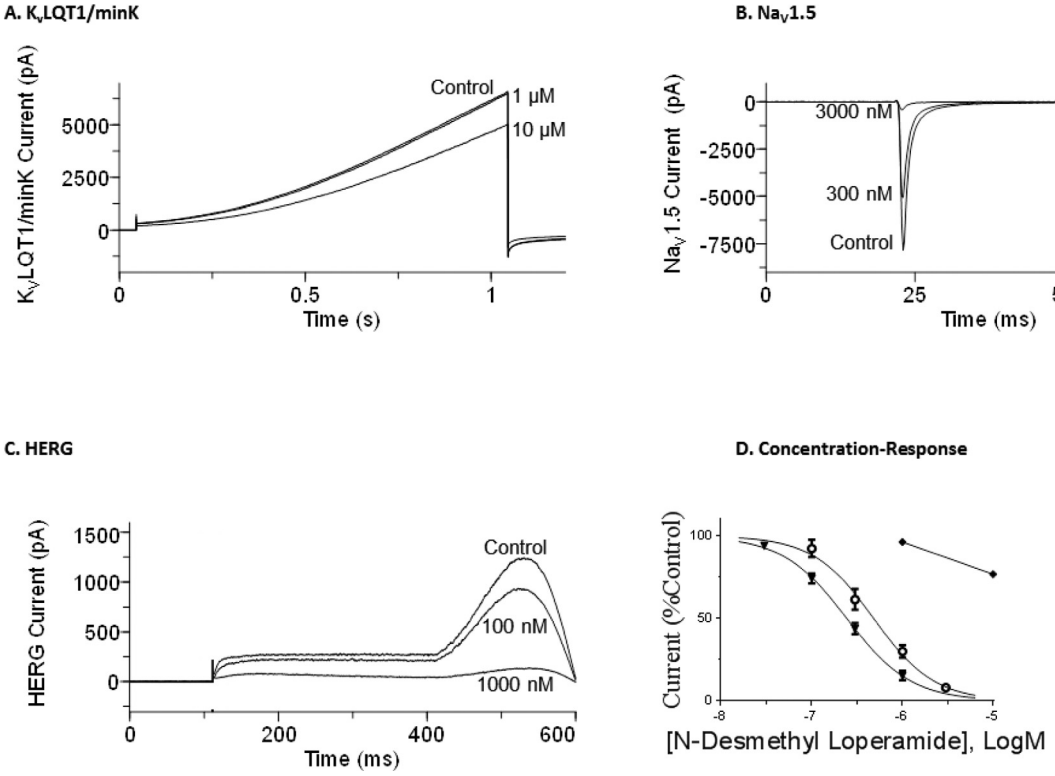

Fig. 2. Effects of *N*-desmethyl loperamide on cardiac ion channel currents. A. *N*-desmethyl loperamide inhibition of KvLQT1/minK channel currents. Cells were held at −80 mV and depolarized for 1 s to +20 mV at a frequency of 0.2 Hz at room temperature. B. Inhibition of Nav1.5 channel currents by *N*-desmethyl loperamide. Cells were held at −70 mV and depolarized to −20 mV at a frequency of 1 Hz. C. *N*-desmethyl loperamide inhibition of hERG. Cells were held at −80 mV and depolarized for 300 ms to +20 mV followed by a −0.5 V/s ramp back to −80 mV at 37 °C. D. Concentration-response relationships for *N*-desmethyl loperamide inhibition of hERG (filled triangles), Nav1.5 (open circles) and KvLQT1/minK (filled diamonds). Error bars denote standard error of the mean (n = 4–7). Where no error bars are visible they are contained within the symbol.