Oxycodone is associated with dose-dependent QTc prolongation in patients and low-affinity inhibiting of hERG activity *in vitro*

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WHAT IS ALREADY KNOWN

- During recent years some opioids have been associated with prolonged QT and torsade de pointes (TdP).
- *In vitro* testing has shown that most opioids can block the cardiac potassium channels.
- This indicates that QT prolongation and TdP could be a more general problem associated with the use of these drugs.

WHAT THIS PAPER ADDS

- This study is the first to show that oxycodone dose is associated with QT prolongation and *in vitro* blockade of hERG channels expressed in HEK293.
- Neither morphine nor tramadol doses are associated with the QT interval length.

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AIMS

During recent years some opioids have been associated with prolonged QT interval and torsade de pointes (TdP). *In vitro* patch clamp testing has shown that most opioids can block human ether-a-go-go related gene (hERG) channels that are known to underlie cardiac repolarizing I_{Kr} current. This indicates that QT prolongation and TdP could be a more general problem associated with the use of these drugs. The aims of this study were to evaluate the association between different opioids and the QTc among patients and measure hERG activity under influence by opioids *in vitro*.

METHODS

One hundred chronic nonmalignant pain patients treated with methadone, oxycodone, morphine or tramadol were recruited in a cross-sectional study. The QTc was estimated from a 12-lead ECG. To examine hERG activity in the presence of oxycodone, electrophysiological testing was conducted using *Xenopus laevis* oocytes and HEK293 cells expressing hERG channels.

RESULTS

There were no differences in gender distribution or age between the treatment groups. The known association between methadone dose and QTc was confirmed ($R^2 = 0.09$; $P = 0.02$). Higher oxycodone dose was also associated with longer QTc ($R^2 = 0.21$; $P = 0.02$). A 100 mg higher oxycodone dose was associated with a $10 \text{ ms}^{1/2}$ (95% CI 2–19) longer QTc. Neither morphine nor tramadol dose was associated with the QTc. Electrophysiological testing revealed low-affinity inhibition of the potassium current through hERG channels expressed in HEK293 cells ($IC_{50} = 171 \text{ } \mu\text{M}$ oxycodone).

CONCLUSIONS

Among patients treated with methadone or oxycodone, higher doses were associated with longer QTc. Oxycodone is capable of inhibiting hERG channels *in vitro*.

Introduction

During recent years several cases of torsade de pointes (TdP) associated with use of some opioids have been described. Until now only cases of TdP associated with use of L- α -acetylmethadol or methadone have been reported [1–3]. In epidemiological studies methadone dose has been associated with prolongation of the QT interval on ECG [4–6]. The molecular mechanism has been elucidated through patch clamp test series conducted on cells expressing cardiac ion channels. The fact that many opioids are capable of blocking the human ether-a-go-go related gene (hERG) potassium channels *in vitro* [7] indicates that QT prolongation and TdP could be a more general problem with this particular group of drugs.

During the 1990s there has been a tendency towards more aggressive management of chronic pain and heroin addiction [8]. These new trends in the use of opioids have led to a dramatic increase in opioid sales, possibly reflected by increased doses as well as number of treated patients [9]. In the USA sales of methadone and oxycodone have increased almost 400% from 1997 to 2002, and poisoning death rates seem to be associated with the prescribed doses of opioids regardless of the type of opioid used [10, 11]. European countries also use opioids on a large scale. In Denmark approximately 3% of the adult population use opioids on a regular basis [9, 12].

The mechanism behind poisoning deaths is not simple to investigate in humans, since neither respiratory arrest nor arrhythmia leaves traces that allow for specific identification and diagnosis.

The aims of the present study were: (i) to describe the association between the doses of different opioids and the QT interval, and (ii) to elucidate the mechanism behind QT prolongation through patch clamp test series on hERG channels expressed in *Xenopus laevis* oocytes and mammalian HEK293 cells.

Methods

Patients

One hundred consecutive chronic nonmalignant pain patients from the Multidisciplinary Pain Centre, the National Hospital, Copenhagen, Denmark were included in the cross-sectional study. Inclusion criteria were age >18 years and long-term treatment with oral opioids for chronic pain. The primary analgesic was methadone, oxycodone, morphine or the low-dose opioid analgesic tramadol. All opioid doses except tramadol doses were for comparison converted to equianalgesic doses of oral morphine and relevant equipotency tables were used [13]. Although some reports suggest that the relation between methadone dose and equianalgesic morphine dose is nonlinear, we have used a constant factor to convert

methadone dosages. Tramadol doses were not converted because no suitable conversion formula exists.

Interview

An interview was performed as a standardized questionnaire concerning demographic data, symptoms of syncope, medical history considering heart disease and medical treatment.

Blood sample: Low S-potassium is associated with QT prolongation. S-potassium was measured in all patients willing to give a blood sample.

ECG

A resting ECG of 10 s length was recorded just after the interview. All ECGs were recorded by the same physician using the same 12-lead ECG recorder (Corina; Marquette Hellige, Kettering, UK). The ECGs were printed at paper speed 25 mm s⁻¹ and amplification 10 mm mV⁻¹.

The beginning of the QT interval was measured from the first deflection from the isoelectric line after the P-wave. The ending of the QT interval was defined as the point where the steepest tangent at the descending part of the T-wave crosses the isoelectric line. This is a simplification of the Lagunas method [14]. In the case of a bifid T-wave, the second peak was considered as part of the T-wave only if the amplitude was similar to or higher than the amplitude of the first peak [15]. The longest QT interval measured in lead II, V2 or V5 from each ECG was used in the further analysis of data. The same physician blinded to patient data measured all ECGs twice.

Since adaptation of the QT interval to the heart rate is slow, the correction of the QT interval was done using the mean of all RR intervals in the ECG [16].

The corrected QT interval was calculated using Bazett's formula [17] and a derivative from Fridericia's formula [18]:

$$\text{Bazett: } QTc = \frac{QT}{\sqrt{RR}} \quad \text{Fridericia: } QTc = \frac{QT}{\sqrt[3]{RR}}$$

Nine patients were excluded: three with atrial fibrillation/flutter, two with bundle branch block, one with pacing pacemaker, one patient receiving more than one opioid and one patient because of hypokalaemia (s-K = 2.2 mM); in addition, one patient was excluded due to poor quality of the ECG.

Molecular biology

cDNA encoding hERG1 was introduced into the custom-made vector pXOOM, which is optimized for expression in both *X. laevis* oocytes and mammalian cells [19].

crRNA preparation and capping were performed by *in vitro* transcription using the Ambion T7 m-Message Machine kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. mRNA was purified using the Ambion MEGAclean kit (Ambion) according to the manufacturer's instruction and dissolved in

Tris–ethylenediamine tetraacetic acid buffer to approximate concentrations of $0.1 \mu\text{g } \mu\text{l}^{-1}$. For proof of purity and integrity, mRNA was inspected by gel electrophoresis and concentrations were determined photometrically. mRNA was stored at -80°C until injection.

Expression in *X. laevis* oocytes

Xenopus laevis surgery and oocyte treatment were done as previously described [20]. Oocytes were collected under anaesthesia (Tricain $2 \text{ g } \text{l}^{-1}$; Sigma A-5040; Sigma, St Louis, MO, USA) according to guidelines approved by the Danish National Committee for Animal Studies. Before injection of 50 nl mRNA (approximately 5 ng), oocytes were kept for 24 h at 19°C in Kulori medium consisting of (in mM) 90 NaCl, 1 KCl, 1 MgCl_2 , 1 CaCl_2 , 5 HEPES, pH 7.4 with NaOH. Injection of mRNA was accomplished using a Nanoject microinjector from Drummond (Drummond Scientific, Broomall, PA, USA). Oocytes were kept at 19°C for 1–3 days before measurements were performed.

Electrophysiological recordings in *X. laevis*

Current through expressed hERG channels was monitored using a two-electrode voltage-clamp amplifier (Dagan CA-1B, Minneapolis, MN, USA). Electrodes were pulled from borosilicate glass capillaries on a horizontal patch electrode puller (DMZ universal puller; Zeitz Instruments, München, Germany) and had tip resistance between 0.5 and $2.5 \text{ M}\Omega$ when filled with 2 M KCl. During the experiments, oocytes were placed in a small chamber (volume $200 \mu\text{l}$) connected to a continuous flow system (flow $3 \text{ ml } \text{min}^{-1}$). hERG channels were activated by membrane depolarization and channel activity was measured in Kulori solution consisting of (in mM) 87 NaCl, 4 KCl, 1 MgCl_2 , 1 CaCl_2 , 5 HEPES, pH 7.4 with NaOH. All experiments were performed at room temperature. The condition of each single oocyte was controlled before measurements by recording membrane potentials. Only oocytes with membrane potentials $< -30 \text{ mV}$ were used for current recordings. Oxycodone was dissolved in dimethylsulphoxide (DMSO) and diluted 1000 times in Kulori medium. At this concentration, no effect of the applied vehicle was observed (data not shown).

The hERG channels are activated by voltage ramps. From a resting potential of -80 mV lasting for 2 s, a step protocol from -80 to $+40 \text{ mV}$ in 10-mV increments was applied. Each step lasted for 1 s and was followed by a step to -60 mV for 1.5 s to reveal hERG channel tail current.

Electrophysiological recordings in HEK293 cells

An HEK293 cell line expressing hERG was used for the electrophysiological recordings on a QPatch(tm) automated patch clamp machine. The cells were harvested using detachin and kept on the machine in suspension with a cell density of $3 \times 10^6 \text{ ml}^{-1}$. All experiments were carried out with QPatch, which performs 16 parallel and independent patch clamp recordings on a disposable QPlate™. For

details on QPatch operation and QPlate properties see Wilmsen [21] and Mathes [22].

The QPatch prepares the cells automatically with a built-in centrifuge and pipettes to wash the cells. Positioning of the cells on the measurement site in the QPlate was done by application of negative hydrostatic pressure. Gigaseal and whole-cell configuration were achieved according to the settings specified in the assay. For these experiments suction pulses were used to establish the whole-cell configuration. Once the whole-cell configuration was established, the membrane resistance value (R_{mem}) was continuously checked and the quality of the experiments was assessed post hoc. Cells were held at a holding potential of -85 mV . Every 10 s a voltage step protocol [-50 (20 ms), $+20$ (2 s), -50 (2 s), -85 mV] was executed. This voltage protocol was used for all experiments. Current recordings were done using amplifiers made by Sophion Bioscience (Ballerup, Denmark) and data were filtered at 1 kHz and sampled at 5 kHz. Pseudo leak subtraction was performed off-line using the initial -50-mV step, which is the same potential as the tail current but before the channel population is activated. All experiments were done at room temperature. The solutions were: extracellular (in mM), 145 NaCl, 4 KCl, 2 CaCl_2 , 1 MgCl_2 and 10 HEPES; and intracellular, 120 KCl, 10 KOH/ethyleneglycol tetraacetic acid, 5.374 CaCl_2 , 1.75 MgCl_2 and 10 HEPES. Oxycodone was diluted in extracellular solution from 100 mM stock (DMSO), so the concentration of DMSO was $\leq 0.1\%$ and did not affect the current.

Data were partly analysed using the QPatch assay software (Sophion Bioscience) and partly using Igor (Wavemetrics, Portland, OR, USA).

Methadone was used in a positive control study showing an $\text{IC}_{50} = 2 \mu\text{M}$ (data not shown).

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare demographic group data, and homogeneity of variance was confirmed using Levene's test. All variables are expressed as mean $\pm$ standard error (SE). When data did not have a normal distribution, nonparametric methods (Kruskal–Wallis test) were used and median and percentiles are given. As expected, values in groups were low; Fisher's exact test was used to analyse categorical data.

The association between opioid doses and the QT interval was assessed by linear regression. The parameters in the regression were handled as continuous variables. The reproducibility of the QT measuring was assessed using Bland–Altman's method [23]. The Bland–Altman plot (not shown) showed no relation between the difference and the mean of the two QT measurements. Ninety-five percent of all intervals were measured within $\pm 0.02 \text{ s}$. This corresponds to $\pm 0.5 \text{ mm}$ at the standard ECG.

All statistical calculations were done using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). A P -value < 0.05 was considered to be significant.

Table 1

Demographic data

	Methadone treated	Oxycodone treated	Morphine treated	Tramadol treated	P-value
n	25	27	23	16	–
Female (%)	56.0	66.7	69.6	56.3	0.5711
Age (years)	56.6 (± 2.32)	55.2 (± 2.47)	54.9 (± 2.21)	49.3 (± 2.99)	0.2933
Duration of treatment (years)	8	5	6	2	<0.03
Median (10th and 90th percentiles)	(2–15)	(0.6–13)	(1–10)	(0.2–10)	
Opioid dose (mg)	45	100	120	300	–
Median (10th and 90th percentiles)	(15–120)	(20–360)	(30–300)	(150–600)	
Equianalgesic dose (mg morphine)	170	175	120	–	0.077
Median (10th and 90th percentiles)	(60–480)	(35–630)	(30–300)		
Serotonin reuptake inhibitors (no.)	6 (24%)	5 (18.5%)	2 (8.7%)	5 (31.3%)	0.3176
Tricyclic antidepressant (no.)	2 (8%)	2 (7.4%)	4 (17.4%)	3 (18.8%)	0.5444
Antiepileptic drugs (no.)	7 (28%)	6 (22.2%)	6 (26.1%)	2 (12.5%)	0.7255
Benzodiazepines (no.)	7 (28%)	6 (22.2%)	4 (17.4%)	2 (12.5%)	0.6864
S-potassium (mM)	4.4 (± 0.11)	4.4 (± 0.10)	4.5 (± 0.08)	4.4 (± 0.11)	0.8768
QTc Bazett ($s^{1/2}$)	0.424 (± 0.005)	0.411 (± 0.006)	0.406 (± 0.006)	0.414 (± 0.007)	0.6590
QTc Fridericia ($s^{2/3}$)	0.416 (± 0.005)	0.401 (± 0.007)	0.395 (± 0.005)	0.401 (± 0.007)	0.1759
R-R interval (s)	0.902 (± 0.030)	0.877 (± 0.033)	0.870 (± 0.032)	0.832 (± 0.030)	0.4739

Ethical considerations

The patients were all in long-term treatment with oral opioids and therefore physically dependent on opioid. Every study including participants among such patients has to consider the ethics thoroughly. It is obvious that a patient dependent on a drug may feel forced to participate if the prescribing doctor asks. In this study a physician who was not employed at the Pain Centre informed and examined the participants. The investigation conforms with the principles outlined in the Declaration of Helsinki [24]. All participants gave informed consent and the local ethical review board approved the study [(KF)01-075/04].

Results

Table 1 shows the demographics of the included patients. There were no differences in gender distribution, age or QTc length between the treatment groups. Patients in the methadone, oxycodone and morphine groups were treated with equianalgesic opioid doses.

Table 2 shows the association between opioid doses and QTc using Bazett and Fridericia. The well-known association between methadone doses and QTc was confirmed for both Bazett and Fridericia. A significant association between oxycodone doses and QTc was also found. Higher oxycodone doses were associated with longer QTc (Figure 1). This association was present regardless of the correction formula used (Table 2).

Neither morphine nor tramadol doses were associated with the QT interval length.

Oxycodone has a different effect on potassium current through hERG channels depending on the *in vitro* assay

Table 2

The association between opioid doses and QTc

	R ²	P
Methadone (Bazett)	0.09	0.0165
Methadone (Fridericia)	0.09	0.0155
Oxycodone (Bazett)	0.21	0.0190
Oxycodone (Fridericia)	0.35	0.0016
Morphine (Bazett)	0.02	0.5093
Morphine (Fridericia)	0.03	0.4466
Tramadol (Bazett)	0.05	0.4184
Tramadol (Fridericia)	0.11	0.1994

used. Representative current traces recorded in *Xenopus* oocytes are depicted in Figure 2A for control recordings and in Figure 2B for recordings obtained in the presence of 30 μ M oxycodone. Summarized steady-state currents are shown in Figure 2C and peak tail current as a function of the previous step potential in Figure 2D. The current/voltage relation of the steady-state current was bell-shaped, as expected from the profound inactivation observed in hERG channels. Maximum steady-state amplitude was observed at -10 mV and was 402 ± 54 nA for control and 435 ± 57 nA for oxycodone-treated oocytes. For tail current recordings maximum amplitude recorded at $+40$ mV was 1174 ± 134 nA for controls and 1125 ± 147 nA for oxycodone-treated oocytes. Oxycodone did not inhibit the activity of hERG channels expressed in *X. laevis*.

In HEK293 cells oxycodone was capable of inhibiting the potassium current through hERG channels. Figure 3A shows the traces from a representative cell exposed to oxycodone. The modest current during the depolarizing

Figure 1

The scatter plot shows the corrected QT interval (Bazett) plotted against the oxycodone dose ($n=26$). The solid line is the linear regression line. The slope of the regression line was $0.10 \text{ ms}^{1/2} \text{ mg}^{-1}$ oxycodone (95% CI $0.02\text{--}0.19$)

phase at +20 mV of the voltage protocol (time 0–2 s) is followed by the more pronounced tail current recorded at –50 mV in the remainder of the voltage protocol. In the two highest concentrations of oxycodone a distinct reduction of the current is observed. The blockade of the hERG channels was fully reversible, as illustrated in Figure 3B showing the full current–time relationship from the cell in Figure 3A. The results from 13 individual cells exposed to oxycodone fitted to a Hill-equation gives an $\text{IC}_{50} = 171 \text{ } \mu\text{M}$ (± 14), $n_{\text{Hill}} = 1.1$ (Figure 3C).

Discussion

To our knowledge, this study is the first to report an association between oxycodone and QTc, whereas the association between methadone and QTc is well known and has been described previously [4, 5, 25]. Oxycodone dose was stronger associated to QTcF than to QTcB. In this study this difference is explained by the fact that patients receiving higher doses of oxycodone had lower heart rate. QT prolongation is difficult to use in the clinic, because it can be difficult to define the T-wave and it can occur under several different circumstances. Furthermore, QT is only a surrogate for TdP, and the same QT prolongation induced under two different circumstances or individuals is not necessarily associated with the same risk of TdP [26]. The most frequent mechanism in drug-induced QT prolongation associated with arrhythmia is inhibition of the I_{Kr} current during phase 2–3 of the action potential [27]. This association between oxycodone dose and QTc and the fact that Katchman *et al.* did not test oxycodone inspired us to conduct electrophysiological testing. The molecular background for drug-mediated block of hERG activity has been

revealed in some detail. Each of the four subunits in the ion channel exposes two aromatic amino acid side-chains in the pore region (Tyr652 and Phe656). Hydrophobic interactions between these amino acid side-chains and a charged nitrogen in the drug give possibility for π -interaction or stacking, and thereby blockade of the channel [28]. The binding site for methadone has not yet been demonstrated in mutagenesis studies, but the previously mentioned mechanism seems plausible since methadone has two hydrophobic benzene rings interconnected with single bonds to a carbon atom. Rotation around these single bonds makes the molecule flexible and probably capable of forming hydrophobic interactions with the channels. On the other hand, oxycodone has a more rigid structure, with three interconnected rings and a bridge (13'-C, 17'-N-ethylene bridge) from the nitrogen residue to one of the rings to a carbon atom on the other side of the ring (Figure 4).

We tested the influence of commercially available oxycodone on hERG channels expressed in oocytes from *X. laevis* and HEK293 cells. It was only possible to demonstrate any influence on the potassium current in the HEK293 model. In the HEK293 model we found an $\text{IC}_{50} = 171 \text{ } \mu\text{M}$. Initial experiments were performed in oocytes and the highest oxycodone concentration was $30 \text{ } \mu\text{M}$. As demonstrated in Figure 2, no inhibition of hERG channels was observed at this concentration. This is in contrast to results obtained after heterologous expression in mammalian HEK293 cells, where $30 \text{ } \mu\text{M}$ oxycodone resulted in inhibition of hERG channels of approximately 15%. This discrepancy is probably due to intrinsic differences between oocytes and mammalian cells. From a number of comparisons between the two expression systems it is our general impression that compounds have lower affinities when tested in oocytes compared with mammalian cells. Usually the IC_{50} is compared with maximal plasma concentration (C_{max}), and concern is raised if $\text{IC}_{50}/C_{\text{max}}$ is <30 [29]. The maximal plasma concentration in patients treated with oxycodone for chronic pain is not well investigated. Most available data come from pharmacokinetic studies measuring the plasma concentration in healthy subjects after administration of a small dose. On the other hand, forensic studies often report high serum concentrations, but these subjects are dead and it is not certain whether the drug intake was lethal or at least an intentional overdose. Post mortem blood analysis has shown a concentration of oxycodone as high as $900 \text{ } \mu\text{M}$ in patients who died in accidents [30]. Interpretation of serum concentrations is therefore difficult in patients with chronic use of opioid [31]. Having the very high post-mortem oxycodone concentrations in mind, a possible connection between hERG channel inhibition, QTc prolongation and sudden cardiac death could be suggested. However, it should be emphasized that plasma concentrations of $900 \text{ } \mu\text{M}$ for any given compound might induce unspecific blockade of several ion channels and therefore

Figure 2

hERG channel activity measured in *Xenopus laevis* oocytes in the absence or presence of 30 μM oxycodone are demonstrated in A and B, respectively. The hERG channels were activated with the indicated voltage protocol (insert in B). Currents, normalized and summarized, are given for steady state (squares) in C and peak tail (circles) in D. Controls are in black and oxycodone in grey ($n = 8$). hERG activity is unaltered in the presence of oxycodone

make the interpretation of the molecular effect of the compound difficult.

Whether the hERG blocking properties of oxycodone found in HEK293 cells can explain the ECG findings in this study is disputable. First of all, the ECG study had a cross-sectional design, which cannot provide causality. Second, the QTc of methadone-treated subjects was longer than in the oxycodone-treated group, indicating that the oxycodone-induced hERG blockade is less important. There might be confounding factors that we have not adjusted for. Furthermore, we did not measure oxycodone concentration in patients and we cannot say whether serum concentrations of oxycodone in the present population are comparable to IC₅₀ found *in vitro*.

A prospective study with ECG recordings prior to initiation of oxycodone treatment would be a valuable supplement to this study. Even if the QT prolonging effect of oxycodone is confirmed in a prospective study, it is not certain that it is associated with arrhythmia. To our

knowledge, until now no cases of TdP have been reported in patients treated with oxycodone.

It is not possible to show any causal connections from a cross-sectional study and therefore we are only able to describe the association between opioid doses and QTc.

QTc is only a surrogate measure for arrhythmia, and despite a significant QTc prolongation it is not possible to predict arrhythmia. Drugs associated with equivalent QTc prolongation do not necessarily carry the same risk of TdP [26].

The tramadol treated group is small which might conceal a QT-prolonging effect.

Conclusion

Among chronic nonmalignant pain patients treated with long-term methadone or oxycodone, higher doses of either were associated with longer QTc. Oxycodone does

Figure 3

hERG channel activity expressed in HEK293 cells during exposure to different concentrations of oxycodone. (A) Superimposed traces from a representative cell exposed to oxycodone in concentrations ranging from 0.4 to 100 μM. Filled circles are the peak tail current, open triangles the steady-state current and open circles the leak as measured at −50 mV early in the voltage protocol. In the two highest concentrations of oxycodone a distinct reduction of current is observed. (B) The full current–time relationship from the cell in A. The concentration of oxycodone added to the cell is indicated on bars above. Peak tail current, steady-state current and the leak are indicated with the same symbols as in A. (C) The mean effects from 13 cells exposed to oxycodone. This has been fitted to a Hill-equation to give an $IC_{50} = 171 \mu M$, $n_{Hill} = 1.1$. Error bars are SD

Figure 4

The chemical structure of methadone and oxycodone. Both compounds have a structure with hydrophobic rings. The bonds between the benzene rings of methadone are much more flexible than the three-ring structure of oxycodone

inhibit hERG channels expressed in HEK293 cells, although with very low affinity. Causality between oxycodone inhibition of hERG channels and QTc prolongation in pain patients cannot be addressed in the present study due to cross-sectional design.

At the investigated dose levels neither morphine nor tramadol doses seem to be associated with QTc interval length.

Competing interests

None declared.

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