Olanzapine-mediated cardiotoxicity is associated with altered energy metabolism in isolated rat hearts

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Olanzapine is an antipsychotic drug routinely used for the treatment of schizophrenia. Although the olanzapine treatment is associated with disturbed electrical heart activity, the exact mechanism underlying this severe adverse effect remains unclear. Recently, olanzapine administration was demonstrated to be associated with elevation of blood glucose and lower levels of free fatty acids. Therefore, we investigated the effect of acute olanzapine administration on pathways regulating the cardiac energy metabolism in an isolated heart. Electrical activity and contractile parameters were recorded in isolated, spontaneously beating, adult male rat hearts, perfused with either olanzapine (100 nmol/l) or the vehicle for 10 min. Regulation of key signalling molecules was evaluated by immunoblotting and ATP levels were measured spectrophotometrically. Olanzapine prolonged the QTc intervals and induced a higher number of premature ventricular beats. Furthermore, olanzapine significantly decreased the coronary flow, the rate-pressure product and the contractility (+dP/dt and –dP/dt). These changes were associated with an increased acetyl-CoA carboxylase phosphorylation and tissue ATP levels. We also found a trend for lower phosphorylation levels of Akt and its downstream products AS160, a key regulator of GLUT4 trafficking and glycogen synthase kinase-3β in olanzapine-treated hearts when compared to vehicle-treated controls. These data should contribute to the elucidation of mechanisms that underlie the adverse cardiac effects of olanzapine.

Key words: olanzapine, cardiotoxicity, ventricular arrhythmias, ATP, acetyl-CoA carboxylase

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INTRODUCTION

Olanzapine belongs to the second generation of antipsychotics and is routinely used in the clinic for the treatment of schizophrenia. It is well known that schizophrenic patients suffer from cardiometabolic disorders (Jones et al., 2013; Pasternak et al., 2014; Wang et al., 2014), and administration of antipsychotic drugs is followed by higher morbidity and mortality (Jones et al., 2013). Several adverse effects, particularly prolonged QT intervals, higher rates of arrhythmias (Dineen et al., 2003; Drici et al., 1998; Gurovich et al., 2003), elevated risk of hyperlipidemia (Dudek et al., 2016; Horska et al., 2016; Takeuchi et al., 2015), increased blood glucose and lower circulating levels of free fatty acids (Albaugh et al., 2012; Klingerman et al., 2014) have been reported after olanzapine administration. Despite the fact that the incidence of sudden cardiac or sudden unexpected death in olanzapine-treated patients is twice as high as in nonusers (Salvo et al., 2016), the mechanism underlying the severe, adverse cardiac effects remains poorly understood.

Large amounts of energy must be generated in order to support the mechanical functions of the heart and disturbances in energy metabolism may be associated with cardiac dysfunction (Fillmore et al., 2014). Fatty acids and glucose are the two main sources of ATP in the heart. In addition to the substrate availability and end-product inhibition, two main signalling pathways regulate the cardiac energy metabolism: the Akt and 5’AMP-activated protein kinase (AMPK) signalling pathways. Under physiological conditions, Akt is activated by insulin or growth factors, and promotes glucose metabolism by stimulating translocation of the glucose transporters, primarily GLUT-4 in the heart, to the plasma membrane. Under conditions of metabolic stress, the AMPK pathway is activated and promotes production of energy via fatty acid oxidation and phosphorylation of the acetyl-CoA carboxylase (ACC) (Fillmore et al., 2014; Kang et al., 2012). Importantly, increased fatty acid oxidation in the heart inhibits glucose utilization and is associated with a decreased oxygen efficiency and reduced contractile function under certain conditions (Stanley et al., 1997). Furthermore, dysregulated fatty acid metabolism may lead to lipotoxicity, which refers to the accumulation of toxic lipid metabolic intermediates that may lead to cellular stress and dysfunction, and potentially cell death (D’Souza et al., 2016).
Maintenance of energy homeostasis is crucial for preserving cellular physiology (Cieslak et al., 2016; Goralska et al., 2017). However, the involvement of the energy metabolism regulation in adverse cardiac effects of antipsychotics has not yet been investigated. Therefore, we investigated the effects of olanzapine on both, cardiac function and activation of key signalling molecules that regulate the energy metabolism in the isolated heart.

**METHODS**

**Animals.** Experiments were carried out according to Directive 2010/63/EU and were approved by Local Bioethical Committees.

**Experimental design and isolated heart perfusions.** Hearts were randomly divided into either olanzapine- or vehicle- treated hearts. Isolated heart preparations were established as described below. Isolated hearts, after a short stabilization period (~ 15 min), were perfused in the unloaded (Langendorff) mode for five minutes, to collect baseline measurements, and then continuously perfused with either the vehicle (aqua pro injectione) or 100 nmol/l olanzapine (Zyprexa, Eli Lilly; Utrecht, NLD) via the aortic cannula for an additional 10 minutes (Fig. 1, upper panel). Two series of perfusions were performed: in the first (n=5), the contractile function, coronary flow and ECGs were monitored; in the second (n=3–4), the ventricular tissue was snap-frozen in liquid nitrogen at the end of the perfusion period and stored at −80°C for biochemical analyses.

Male, Wistar rats (230–270 g, 4–5 months) were maintained on a standard laboratory chow and tap water *ad libitum*. Rats were anesthetized by i.p. injection of 100 mg/kg ketamine and 10 mg/kg xylazine, and, in the first series, anticoagulated with heparin (500 IU i.p.). Hearts were explanted and perfused *via* the aorta at 80 mm Hg with a modified Krebs-Henseleit solution (Farine et al., 2016; Knezl et al., 2017) and gassed with 95% O₂ and 5% CO₂.

**Functional measurements.** For contractile measurements, a latex balloon was inserted into the left ventricle and pressure measurements were recorded using a LabChart 7 Pro version 7.3.7 (AD Instruments; Spechbach, Germany). Coronary effluent was harvested manually to determine the coronary flow 10 minutes after vehicle or olanzapine administration. For electrocardiogram (ECG) recordings, needle electrodes (AD Instruments; Spechbach, Germany) were inserted into the left ventricle. Electrode signals were

![Figure 1. Experimental protocol and heart function.](image-url)

**Upper panel:** Experimental protocol. **Lower panels:** Changes in hemodynamics after 10 min of either the vehicle (n=5, grey box) or 100 nmol/l olanzapine (n=5, white box) administration. (A) Left ventricular systolic pressure (SP; mmHg), **p<0.01. (B) Rate pressure product (RPP; mmHg* BPM), *p<0.05. (C) Coronary flow (CF; ml/min), *p<0.05. (D) Maximal rates of contraction (+dP/dt; mmHg/s), *p<0.05. (E) Maximal rates of relaxation (−dP/dt; mmHg/s), *p<0.05.
amplified with the FE136 Animal Bio Amplifier (AD Instruments; Spechbach, Germany) and recorded with a PowerLab 8/30 (AD Instruments; Spechbach, Germany). ECGs were recorded for the entire perfusion period.

Heart rate was calculated from the ECG recordings. Peak systolic pressure and their product (rate-pressure product; RPP), +dP/dt and –dP/dt were calculated from the ventricular pressure measurements.

To evaluate ECGs, QT intervals were analyzed using LabChart 7 Pro version 7.3.7 (AD Instruments; Spechbach, Germany). Corrected QT (QTc) was analyzed according to the following formula: QTc=QT/(RR/1.2), where t is the normalization factor according to the basal RR interval duration in rats and equals 150 ms (Kmeova & Klimas, 2010). To examine the effect on QT interval, averages of 10 consecutive beats from the 10th minute after vehicle or olanzapine administration were compared. Development of ventricular arrhythmias was defined according to the Lambeth Convention (Curtis et al., 2013). The sum of ventricular premature beats (VPBs) during 10 min of vehicle or olanzapine infusion was compared.

Isolation of ventricular cardiomyocytes. Ventricular cardiomyocytes were isolated as previously described (Ségalen et al., 2008). Briefly, isolated hearts were perfused via the aortic cannula with calcium-free Joklik’s medium for 10 min. Next, the heart was perfused with 0.09% collagenase and 0.7% BSA in Joklik’s medium for 15 min. After the atria removal, ventricles were opened and incubated in an incubation buffer (10 ml, 4°C) for 10 min at 35°C. The heart was subsequently dissected and the suspension of cells was incubated at 37°C for 5 min, whereas the Ca²⁺ concentration was increased every minute in steps of 200 μmol/l to 1 mmol/l. Next, the cell suspension was filtered through 180 μm filter mesh and centrifuged at 17 000 ×g for 1 min. Cells were allowed to adhere to the laminin-coated plates in the HEPES-modified medium 199 with 10% fetal calf serum for 1 h. Finally, cardiomyocytes were treated with either the vehicle (0.001 % DMSO) or 100 nmol/l olanzapine (Sigma Aldrich, St. Louis, USA) for 10 min.

Immunoblotting. Frozen ventricular tissue or ventricular cardiomyocytes were homogenized in a lysis buffer containing Tris-HCl (20 mmol/l), phenylmethylsulfonyl fluoride (1 mmol/l), NaCl (137 mmol/l), EGTA (1 mmol/l), EDTA (10 mmol/l), NaF (20 mmol/l), sodium pyrophosphate (1 mmol/l), sodium orthovanadate (1 mmol/l), glycerol (10%), NP-40 (1%), aprotinin (4 μg /ml), leupeptin (4 μg /ml), and pepstatin (4 μg /ml). Lysates were centrifuged (12000 rpm, 20 min, 4°C) and supernatants were stored at −80°C until use. Total protein concentration of the supernatant was determined with the BCA protein assay kit (Pierce Biotechnology; Waltham, USA) according to the manufacturer’s instructions. Lysates (75 μg protein) were separated on 7.5% SDS-polyacrylamide gels (BioRad; Hercules, USA), and transferred to nitrocellulose membranes (Li-COR Biosciences; Lincoln, USA). The membranes were blocked for 1 h with the Odyssey Blocking Buffer (Li-COR Biosciences; Lincoln, USA) in PBS and then incubated overnight at 4°C with anti-Akt (#9272, 1:1000), anti-AS160 (#2670, 1:1000), anti-GSK-3β (#9271, 1:1000), anti-IRβ (#9271, 1:1000), anti-phosphoSer473-Akt (#473, 1:1000), anti-phosphoThr476-AS160 (#4288, 1:1000), anti-phosphoSer9-GSK3β (#9323, 1:1000), anti-phosphoSer79-ACC (#11818, 1:1000). Anti-GSK-3β and anti-IRβ were purchased from Santa Cruz Bio-technology (Dallas, USA). All other antibodies were purchased from Cell Signaling Technology (Danvers, USA). The following day, membranes were incubated with anti-mouse (Li-COR Biosciences; Lincoln, USA) or anti-rabbit (Invitrogen; Carlsbad, USA) secondary antibodies (1:10000) for 1 h at room temperature. Bands were detected with the Odyssey NIR imaging system (Li-COR Biosciences; Lincoln, USA).

ATP levels. Tissue ATP levels were determined spectrophotometrically as previously described (Niederberger et al., 2017). Briefly, frozen ventricular tissue was powdered, resuspended in perchloric acid (7%), homogenized in a Dounce homogenizer and neutralized with KOH/KHCO₃ (1 mol/l). 200μl of sample was then added to 800μl of the assay buffer containing Tris-HCl (60 mmol/l, pH 7.5), MgSO₄₄ (40 mmol/l, glucose (2 mmol/l), nicotinamide adenine dinucleotide phosphate (524 μmol/l) and glucose-6 phosphate dehydrogenase (2 U/ml). The ATP assay was initiated by adding hexokinase (2 U/ml) and absorbance was measured at 340 nm. ATP levels were calculated after linear extrapolation from standard curves prepared with known quantities of ATP.

Isolation and function of the heart mitochondria. Heart mitochondria were isolated as previously described (Misak et al., 2013). Briefly, the heart was disrupted with scissors and a Potter–Elvehjem homogenizer with 20 strokes. Homogenate was centrifuged at 950×g for 5 min at 4°C. The remaining supernatant was decanted to a separate tube and centrifuged at 8500×g for 10 min at 4°C. The pellet was resuspended in an isolation buffer (65 mmol/l sucrose, 225 mmol/l mannitol, 0.25 mmol/l EDTA, 10 mmol/l HEPES, pH 7.4) and centrifuged at 8500×g for 10 min at 4°C. Finally, the pellet was resuspended in a 400 μl volume of isolation buffer. Total protein (mg/ml) was determined using the Modified Lowry Protein Assay Kit (Thermo Fisher, Waltham, USA). Mitochondrial preparations were used within 2 h. Functional parameters of isolated mitochondria were estimated by the Mitocell MT-200A system (Strathkelvin Instruments, Scotland). Mitochondria were resuspended in a respiration buffer (120 mmol/l KCl, 0.5 mmol/l EGTA, 3 mmol/l KH,PO₄, 2% dextran, 12.5mmol/l HEPES, pH 7.4) at 30°C in the presence of substrates (10 mmol/l glutamate+5 mmol/l malate) and treated with either the vehicle (0.001% DMSO) or 100 nmol/l olanzapine (Sigma Aldrich, St. Louis, USA) for 10 min. Next, 125 μmol/l ADP was added in order to induce state 3 (phosphorylating respiration) and 100 mmol/l carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) was added to induce state 3α (maximum uncoupler-stimulated respiration). Respiration rate before and after addition of the vehicle, olanzapine, ADP or FCCP was calculated as the negative slope of oxygen concentration. The ATP/O ratio (the relationship between ATP production and oxygen consumption) was calculated as the ratio between ADP added and the amount of oxygen consumed during state 3.

Data analysis and statistics. The results are presented as mean values ± S.E.M. Differences between groups were evaluated with t-tests. A value of p<0.05 was considered as significant. Analyses were performed with the GraphPad Prism (GraphPad Software, Inc.; San Diego, USA). The Shapiro-Wilk test was used to test distribution normality in the groups.
RESULTS

Effects of olanzapine on cardiac function

We evaluated the effect of acute olanzapine treatment (100 nmol/l, 10 min) on contractile function and electrical activity of isolated rat hearts. The contractile function in the olanzapine-treated hearts was depressed when compared to the vehicle-treated hearts, with significant reductions in the peak systolic pressure ($p=0.007$, Fig. 1A), rate pressure product ($p=0.02$, Fig. 1B), coronary flow ($p=0.01$, Fig. 1C), $\frac{dP}{dt}$ ($p=0.03$, Fig. 1D) and $-\frac{dP}{dt}$ ($p=0.03$, Fig. 1E). Next, we analysed the effect of olanzapine on electrical activity of the hearts. The olanzapine treatment resulted in a significant prolongation of the QT interval in comparison to the vehicle-treated isolated hearts ($p<0.0001$, Fig. 2A). There were no significant changes between the groups in the heart rate (0.23, Fig. 2B). The olanzapine administration significantly increased the QTc interval duration ($p=0.046$, Fig. 3A). There were no significant changes between the groups in the heart rate (0.23, Fig. 2B). The olanzapine administration significantly increased the QTc interval duration ($p=0.046$, Fig. 3A). There were no significant changes between the groups in the heart rate (0.23, Fig. 2B). The olanzapine administration significantly increased the QTc interval duration ($p=0.046$, Fig. 3A).

Effects of olanzapine on cardiac energy state and pathways regulating the energy metabolism

To determine if olanzapine-mediated cardiotoxicity is associated with changes in regulation of the energy metabolism of the hearts, we examined the effect of acute olanzapine treatment (100 nmol/l, 10 min) on the cardiac ATP content. Significantly higher ATP levels were associated with the depressed cardiac function, in olanzapine-treated hearts ($p=0.046$, Fig. 3A).

In order to investigate the mechanism by which olanzapine affects the energy metabolism specifically in the...
myocardium, we used isolated rat ventricular mitochondria and cardiomyocytes. We found a trend for higher ATP/O ratio after olanzapine incubation as compared to the vehicle treated isolated mitochondria ($p=0.16$, Fig. 4A), however, these changes were not statistically significant. Olanzapine did not alter the rates of basal respiration and the rates of maximal respiration (data not shown). Similarly to the whole heart, incubation with olanzapine (100 nmol/l, 10 min) significantly increased the level of phosphorylated ACC as compared to the vehicle treated isolated cardiomyocytes ($p=0.03$, Fig. 4B).

We also measured the effects of olanzapine on phosphorylation of key proteins in the glucose metabolism: Akt kinase (Ser$^{473}$), AS160 protein (Thr$^{642}$), regulator of GLUT4 trafficking), and GSK3β (Ser$^{9}$, regulator of glycogen synthesis). Acute administration of olanzapine appeared to decrease phosphorylation of Akt ($p=0.07$, Fig. 5A) and its downstream targets AS160 ($p=0.11$, Fig. 5B) and GSK3β ($p=0.16$, Fig. 5C), however, these changes were not statistically significant.

**DISCUSSION**

Although olanzapine is one of the most prescribed antipsychotics for the treatment of schizophrenia, little is known about the molecular mechanisms of its adverse cardiac effects. We observed that acute olanzapine treatment was associated with a higher incidence of ventricular arrhythmias, as well as depressed contractile function and coronary flow in isolated rat hearts. In addition, we were able to demonstrate that olanzapine increases phosphorylation levels of ACC and tissue ATP levels in the heart, suggesting increased fatty acid oxidation. Furthermore, we observed a lower trend for phosphorylation of Akt, AS160 and GSK3β in olanzapine-treated hearts when compared to the vehicle-treated ones. These data indicate that acute exposure to olanzapine impairs cardiac rhythm and function in association with dysregulation of fatty acid metabolism in the isolated rat heart.

A higher incidence of sudden cardiac death has been linked to administration of antipsychotic drugs (Salvo et al., 2016), and QT prolongation and higher rates of arrhythmias have been reported in patients treated with...
olanzapine (Dincen et al., 2003; Drici et al., 1998; Guo et al., 2009; Gurovich et al., 2003). Consistent with previous studies, we observed a higher number of premature ventricular beats and an increase in the QTc interval after acute administration of 100 nmol/l olanzapine. The selected dose was based on steady-state plasma concentration of olanzapine, reaching ~ 30 ng/ml (96 nmol/l) with the use of 15 mg/day (Attarbaschi et al., 2007; Fellows et al., 2003; Mauri et al., 2005; Weigmann et al., 2001). Blockage of hERG potassium channels results in increased action potential duration and represents one of the most common causes of QT prolongation (Recanatini et al., 2005). Furthermore, inhibition of hERG channels has been proposed as an underlying mechanism of olanzapine-induced impairment of the cardiac electrical activity (Lee et al., 2015), and response to treatment in schizophrenic patients is modulated by alterations in expression of the hERG isoforms in the brain (Apud et al., 2012). In addition, Morissette et al. reported that olanzapine blocks the rapid component of the delayed rectifier potassium current in guinea pig ventricular myocytes (Morissette et al., 2006). Thus, it is tempting to speculate that inhibition of specific potassium channels may be the mechanism by which olanzapine induces the prolonged QTc intervals following acute administration in isolated rat hearts.

Importantly, the observed disturbances in electrical activity of the heart after olanzapine administration were associated with depressed contractile function and reduced coronary flow. As the heart rate remained unchanged, decrease in the rate-pressure product resulted from a significant reduction in the peak systolic pressure. These findings are consistent with those of Leung and colleagues, who reported that a single olanzapine injection significantly decreased blood pressure and left ventricular contraction, but did not affect the heart rate in rats (Leung et al., 2014). In contrast, cumulative dose of olanzapine induced reduction in heart rate associated with a decrease in the developed pressure and coronary perfusion pressure in an isolated guinea pig heart (Guo et al., 2009). The decrease in coronary perfusion pressure could be responsible for the reduced coronary flow and contractility observed in our study. Under these conditions and according to our study in the isolated heart, we may conclude that olanzapine mediates changes in the cardiac functions via local, cardiac action(s), although the precise underlying mechanisms remain to be determined.

Under physiological conditions, the heart primarily uses fatty acids in order to meet its high energy demands. However, dysregulation of fatty acid metabolism may lead to accumulation of toxic lipid species, impaired myocardial contractility and arrhythmias (D’Souza et al., 2016; Schulze et al., 2016). ACC plays a crucial role in regulation of fatty acid oxidation. Under conditions of metabolic stress, ACC is phosphorylated and deactivated by 5’AMP-activated protein kinase (AMPK), thereby reducing production of the malonyl CoA and relieving its inhibition of carnitine palmitoyltransferase I (CPT-I), a key regulator of fatty acid cytosolic-mitochondrial trafficking. Excessive fatty acid oxidation may overwhelm capacity of the tricarboxylic acid cycle, thereby resulting
in accumulation of toxic acylcarnitines (D’Souza et al., 2016). In the present study, olanzapine treatment was associated with a significantly higher phosphorylation of ACC and elevated ATP levels in the hearts, suggesting increased rates of mitochondrial fatty acid oxidation. The observed increase in phosphorylated ACC levels was also present in isolated rat ventricular cardiomyocytes, suggesting that olanzapine may alter the energy metabolism and thereby contractile function of cardiomyocytes. In contrast to our study, Takami et al. showed that apiprazol, but not olanzapine or other antipsychotics, increased phosphorylation of ACC, AMPK and intracellular ATP levels in PC12 cells (Takami et al., 2010). Reasons for these discrepant findings are unclear, but may result from the different models employed.

Several reports confirm that olanzapine mediated cytotoxicity is associated with increased production of reactive oxygen species (ROS) (Eftekhari et al., 2016; Heiser et al., 2010; Stanley et al., 2010; Martins et al., 2010). We observed a trend for higher mitochondrial efficiency (Yang et al., 2016). Several studies suggest that glycolytically derived ATP is crucial for maintenance of the cellular ion homeostasis (Van Emous et al., 2001; Weiss & Maslow, 2004; Xu et al., 1995). Since cytosolic malonyl-CoA levels reciprocally regulate fatty acid and pyruvate oxidation (Stanley et al., 1997), we may speculate that the lower activity of ACC may be associated with lower activity of pyruvate dehydrogenase (PDH) and a related electrical dysfunction (Rozanski & Xu, 2002; Runnman et al., 1990; Stanley et al., 1997). In addition, accumulation of toxic lipid species has been linked with inhibition of Akt signalling, as well as disturbed AMPK activity (Schulze et al., 2016). In our study, acute administration of olanzapine appeared to lower phosphorylation of Akt and its downstream target AS160, a key regulator of GLUT4 trafficking. Similarly, phosphorylation of GSK3β, regulating the glycolysis synthesis, appeared to be decreased in olanzapine-treated hearts, which is in line with depressed Akt/GSK3β phosphorylation reported after olanzapine administration in the rat skeletal muscle and liver (Engl et al., 2005; Mondelli et al., 2013). Moreover, a recent report provided a direct link between AS160 phosphorylation levels and impaired electrical activity of the heart (Quan et al., 2015).

Cardiac energy requirements are extremely high in order to meet demands for contractile function, as well as maintenance of the ion gradients. It is important to note that cardiac ATP levels depend on both, the rates of production and utilization. Since we observed higher ATP levels in association with depressed cardiac function, we may speculate that lower utilization of ATP was responsible for this effect of olanzapine. Along different lines, both inhibition and augmentation of K_{ATP} channel activity have been reported after treatment with antipsychotics (Kinosita et al., 2006; Yang et al., 2004). These channels are closed under physiological conditions and tend to open with metabolic stress in order to save energy. Previous studies demonstrated that blockage of K_{ATP} channels was mediated by serotonin and histamine agonists (Boney & Nelson, 1996; Kleppisch & Nelson, 1995; Yang et al., 2004). Given that olanzapine is a serotonin and histamine antagonist, it is possible that olanzapine promoted K_{ATP} channel opening. In addition, intracellular accumulation of fatty acids triggers the opening of K_{ATP} channels (Liu et al., 2001), however K_{ATP} activators fail to shorten the prolonged QT interval mediated by the hERG channel blockers (Testai et al., 2010). Based on these points, we may speculate that opening of the K_{ATP} channels may contribute to the observed elevation of cardiac ATP content.

In conclusion, acute olanzapine administration led to impaired electrical activity, and reduced contractile function and coronary flow in the isolated rat heart. Our data support the concept that olanzapine stimulates fatty acid oxidation via phosphorylation and inactivation of ACC. Although the contractile function was reduced in olanzapine-treated hearts, cardiac ATP content was elevated. The antagonistic action of olanzapine on H_{1} receptors is one mechanism that may contribute to its metabolic and contractile effects in the heart. It should, however, be highlighted that further studies, particularly chronic and in vivo experiments, are needed to confirm this hypothesis.

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Conflict of Interest

None.

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