Correlation Between Serum Entecavir Concentration and Virological Response in Patients with Chronic Type B Hepatitis

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Data Collection **B**
Statistical Analysis **C**
Data Interpretation **D**
Manuscript Preparation **E**
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Background: This study was conducted to investigate the relationship between trough concentrations of serum entecavir and the virological response of patients with chronic type B hepatitis (CHB).

Material/Methods: A total of 59 CHB patients who had been receiving antiviral therapy with entecavir for >3 months were included in this study. Serum entecavir concentrations, HBV DNA levels, and other biochemical indicators were determined after drug treatments.

Results: The serum entecavir concentrations in the good response and poor response groups were 0.58±0.38 and 0.43±0.15 ng/mL, respectively. The antiviral efficacy was 52.38%, 65.63%, and 100% in low, middle, and high entecavir groups, respectively. The baseline HBV DNA level among the patients with poor response was significantly higher than in the group with good response. Among the 14 patients with a high viral load, 5 patients showed a good response and had a higher entecavir concentration than the other 9 patients with poor response. Entecavir in patients with cirrhosis was higher than in those without cirrhosis (0.63±0.45 ng/mL *vs.* 0.46±0.16 ng/mL), and the virological response rate in patients with cirrhosis was higher than in those without cirrhosis (83.33 *vs.* 51.43%). Cirrhosis progression was reversed in 3 patients with high serum entecavir concentration.

Conclusions: Serum entecavir concentrations vary among individuals, and higher serum entecavir concentration is correlated with more efficient viral clearance. Therefore, for patients with poor response, high doses may be beneficial for viral clearance.

MeSH Keywords: **Antiviral Agents • Hepatitis, Chronic • Virology**

Abbreviations: **CHB** – chronic type B hepatitis; **HBV** – hepatitis B virus; **TDM** – therapeutic drug monitoring; **HDV** – hepatitis D virus; **HAV** – hepatitis A virus; **HCV** – hepatitis C virus; **HEV** – hepatitis E virus; **HIV** – human immunodeficiency virus; **PCR** – polymerase chain reaction; **ALT** – alanine aminotransferase; **AST** – aspartate aminotransferase

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Background

Chronic hepatitis B (CHB) is a global public health problem; more than 100 million people in China are infected with HBV [1] and are at increased risk for cirrhosis, liver failure, and hepatocellular carcinoma. The guanosine analogue entecavir is an antiviral drug commonly used in EASL in clinical practice [2]. Entecavir can be tri-phosphorylated and compete with the natural substrate used for DNA synthesis (guanosine triphosphate). Furthermore, entecavir displays a high degree of specificity for HBV [3,4]. In a phase III clinical trial of Chinese patients with chronic type B hepatitis (CHB), the ratio of HBV clearance (HBV DNA level decreased to below the lower limit of detection) was 76%, 79%, and 89%, after 48, 96, and 144 weeks, respectively, of treatment [5–8].

Entecavir triphosphate functionally inhibits all 3 activities of the HBV reverse transcriptase: (1) base priming, (2) reverse transcription of the negative strand from the pregenomic messenger RNA, and (3) synthesis of the positive strand of HBV DNA. Clinical pharmacokinetics studies showed that entecavir is rapidly absorbed, and reaches its peak concentration at 0.5–1.5 h, with a bioavailability of >70%, widely distributing throughout the body [9]. The half-life of entecavir phosphate (the active form) is approximately 15 h. Entecavir is primarily excreted by the kidneys, and renal insufficiency decreases drug clearance [7,9,10]. Therefore, entecavir may be used for drug monitoring (TDM). According to the drug instructions and clinical expert consensus, entecavir should be taken on an empty stomach and should not be taken within 2 h before or after a meal. Therefore, it is recommended that patients take the drug before going to bed, which makes it extremely difficult to collect steady-state trough concentration data for a TDM. A previous study on entecavir pharmacokinetics (PK) in CHB patients suggested that serum entecavir concentrations remain basically the same for 24 h after administration [11]. We measured the serum entecavir concentration the following morning as an estimate of the trough concentration for studying the correlation between serum entecavir concentrations and the virological response of CHB patients. While physicians usually prescribe a fixed dose of entecavir (0.5 mg/d) for all patients according to the label or their own personal experience, virus clearance is achieved in most patients. However, a few patients fail to achieve an effective virological response or become resistant to entecavir, and the virus cannot be cleared in these patients, even after long-term antiviral treatment. The reasons for treatment failure may be related to high viral load, low entecavir concentration, or poor medication compliance. Viral resistance or breakthrough may be associated with low drug concentrations and dependent on adherence to medication [12,13].

This study investigated the correlation between serum entecavir concentrations and the virological response of hepatitis B patients, for the purpose of providing a new perspective on optimizing the treatment of those patients.

Material and Methods

Subjects

A total of 59 patients diagnosed with chronic type B hepatitis (CHB) at our hospital from January 2017 to December 2017 were included in this study. The diagnosis was based on EASL 2012 Clinical Practice Guidelines on the management of hepatitis B virus infection [14]. The inclusion criteria were: male or female patients with CHB (HBsAg-positive or -negative) and compensated hepatitis B cirrhosis and aged between 18 and 65 years. We excluded patients with hepatitis B combined with HAV, HCV, HDV, HEV, or HIV infection, decompensated cirrhosis or liver cancer, creatinine clearance of <50 mL/min, hepatitis B combined with severe diseases of the circulatory system, and nervous system or immune system. Patients with mental illness and pregnant or lactating women were also excluded.

Informed consent was obtained from all patients and the study protocols were approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University.

Treatment

The entecavir antiviral treatment regimen was 1 entecavir tablet (0.5 mg/tablet) taken before bedtime (9–11 pm) on an empty stomach every day for >3 months, and patients who did not adhere to the regimen were excluded. HBV DNA levels and markers of HBV infection, liver functions, and renal functions were measured before and after medication, and the serum entecavir concentration was measured after at least 1 week of continuous medication.

Blood samples and measurement of indicators

We collected 5 mL peripheral venous blood from each patient 8–10 h after drug administration (8 a.m. the next day), and serum was separated and stored at -80°C until use. Entecavir concentrations were measured with a Waters Acquity UPLC-TQD ultra performance liquid chromatography tandem mass spectrometer (Waters, Stamford, CT, USA). A quality control standard used for entecavir was purchased from the China National Institutes for Food and Drug Control.

Table 1. Demographics and baseline characteristics of 59 subjects (mean ±SD).

	Good response group	Poor response group	*p* Value
Number of cases	38	21	>0.05
Age (years)	47.00±10.63	39.83±10.27	>0.05
Sex (Male/Female)	26/12	18/3	>0.05
BMI (kg/m²)	22.90±2.58	22.93±2.36	>0.05
eAg (+/−)	16/20	11/10	>0.05
ALT (IU/L)	31.19±22.86	41.84±30.76	>0.05
GFR (ml/min/1.73 m²)	97.34±20.71	100.77±23.04	>0.05
Total cholesterol (mmol/L)	4.13±0.97	4.27±0.85	>0.05
AST (IU/L)	30.11±12.46	36.58±22.56	>0.05
Triglycerides (mmol/L)	1.07±0.55	1.09±0.36	>0.05
ALB (g/L)	46.21±3.51	46.91±4.43	>0.05
Treatment time (months)	18.89±21.87	16.24±26.30	>0.05
Cases with high viral load	5	9	>0.05

Laboratory assays

Serum HBV DNA was quantified by real-time fluorescence quantitative PCR using a SLAN 96P System, and the HBV nucleic acid quantitative assay kit produced by Zhijiang Biotechnology, Shanghai (cat. no. 20170902). HBsAb, HBsAg, HBeAb, HBeAg, and HBcAg were detected by chemiluminescence microparticle immunoassay using an ARCHITECTi2000 automatic immunoassay analyzer and kits (Abbott, USA). Plasma ALT, AST, bilirubin, albumin, and triglycerides were analyzed using automatic biochemical analyzers at the First Affiliated Hospital of Zhejiang University Clinical Laboratories.

Statistical analysis

Data are expressed as means ±SD. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21, (IBM Corp., Armonk, NY, USA). Quantitative variables are expressed as mean values, categorical variables are expressed as counts and percentages, and HBV DNA levels were log-transformed before analysis. Comparisons between groups of quantitative and qualitative variables were analyzed using the *t* test and chi-square test (or Fisher's exact test), respectively. P value of less than 0.05 (two-tailed) indicated a significant difference.

Results

Patient baseline data

Three months after entecavir treatment, 38 of the 59 patients (64.41%) showed a good response and their HBV DNA level decreased to below the detection limit (30 copies/mL), and the other 21 showed a relatively poor response and their HBV DNA level was decreased by at least 2 orders of magnitude, but was still over the detectable limit.

The good response and poor response groups did not significantly differ in age, sex, body mass index (BMI), baseline liver functions, serum virus level, or treatment time (Table 1). All patients displayed good tolerance to the antiviral therapy, and no patient complained of severe discomfort.

High serum entecavir concentrations gave rise to better virological response

The serum entecavir concentration in the good response group was higher than in the poor response groups (0.58±0.38 *vs.* 0.43±0.15 ng/mL), but the difference was not statistically significant (*p*>0.05, Figure 1). As a control, serum entecavir concentration was also determined in 10 CHB patients who did not receive entecavir antiviral therapy. The value in each of those patents was 0 ng/mL.

The baseline HBV DNA level in all patients was (5.95±1.87) $\log_{10}$ copies/mL. The baseline HBV DNA in the poor response group was 7.00±1.73 $\log_{10}$ copies/mL, which was significantly higher than that in the good response group (5.21±1.64 $\log_{10}$ copies/mL, *p*<0.05) before treatment. After treatment, the HBV DNA levels in the good response group decreased to undetectable levels, while the levels in the poor response group decreased by 4.00±2.07 $\log_{10}$ copies/mL (*p*=0.0248, Figure 2).
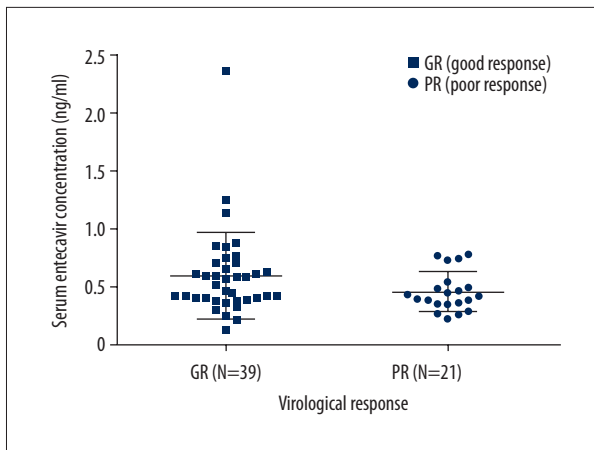
Figure 1. Serum entecavir concentration distribution in good response group (GR, filed square,) and poor response group (PR, open cycle), horizontal lines indicate Mean ±SD. The serum entecavir concentration is 0.58±0.38 ng/mL(range from 0.12 to 2.36) in the good response group, while 0.43±0.15 ng/mL (range from 0.21 to 0.78) in the poor response group, P>0.05.

Figure 2. HBV DNA levels in good response group (GR) and poor response group (PR) before (filled column) and after (open column) treatment. * Denotes significant difference between the columns under the bar.

According to the distribution range of entecavir serum concentration, the 25th percentile of entecavir was 0.3722 ng/ml and the 75th percentile was 0.7844 ng/ml. Therefore, the patients were divided into low, middle, and high entecavir groups (entecavir <0.4 ng/mL, 0.4–0.8 ng/mL, and >0.8 ng/mL). The rate of virological response was 52.83% (11/21), 65.63% (21/32) and 100% (6/6) in these groups, respectively.

Since in each entecavir serum concentration group the virological response was different, we further evaluated the antivirus response for patients with a high ($>10^7$ copies/mL) and in low ($<10^7$ copies/mL) viral load. The results showed that patients in with a serum entecavir concentration of <0.8 ng/mL, a high viral load was correlated with a lower virological response rate (Fisher's exact test probability=0.003), while all patients with a serum entecavir concentration of >0.8 ng/mL had good responses (Table 2).
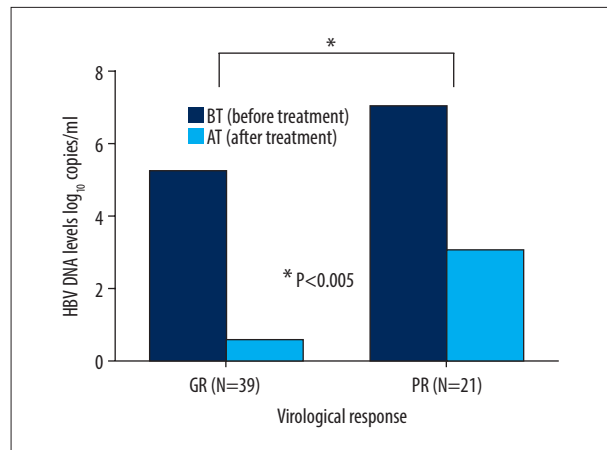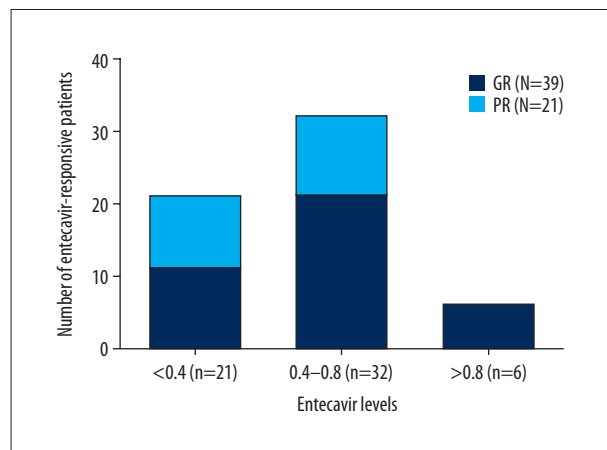

Figure 3. Numbers of virological response patients with different entecavir concentration groups.

Among the 14 patients with a high viral load, the serum entecavir concentration in 5 patients with a good response was 0.68±0.36 ng/mL, which was significantly higher than that in the poor response group (0.44±0.21 ng/mL, *p*=0.0110, Figure 3).

Table 2. Virological response of patients with different viral loads in 3 serum entecavir concentration groups.

Patient's response	Serum entecavir concentration (ng/mL)					
	<0.4		0.4–0.8		>0.8	
	Viral load (copies/mL)					
	$>10^7$	$>10^7$	$>10^7$	$>10^7$	$>10^7$	$>10^7$
Good	1	10	2	19	2	4
Poor	2	8	7	4	0	0
p value	>0.05		<0.05			

Table 3. Demographics and clinic characteristics of cirrhosis and non-cirrhosis patients.

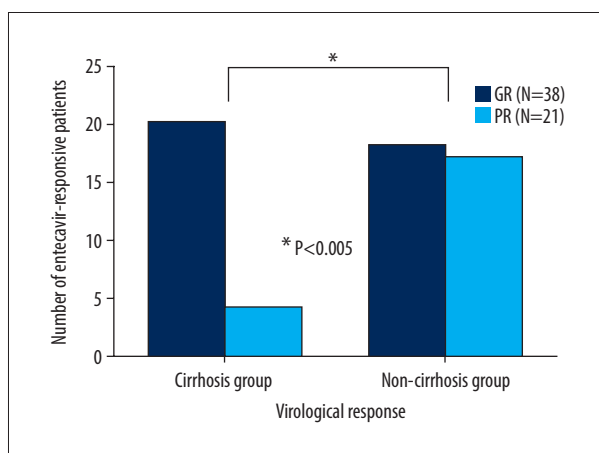|	Cirrhosis group (n=24)	Non-cirrhosis (n=35)	*p* Value
Serum entecavir concentration (ng/ml)	0.63±0.45	0.46±0.16	<0.05
Age (years)	46.88±11.89	41.51±10.36	>0.05
Sex (Male/Female)	18/6	26/9	>0.05
BMI (kg/m²)	23.86±2.13	22.26±2.54	<0.05
Baseline viral load (log₁₀ copies/ml)	5.19±1.56	6.39±1.95	<0.05
ALT	35.33±27.68	34.59±25.47	>0.05
AST (U/L)	26.74±11.17	40.34±31.68	<0.05
ALB	45.88±3.92	46.81±3.78	>0.05
GFR (ml/min/1.73 m²)	93.23±20.97	100.78±20.99	>0.05
Total cholesterol (mmol/L)	1.15±0.75	1.12±0.55	>0.05
Triglycerides (mmol/L)	3.73±0.79	4.28±0.84	<0.05

Figure 4. Numbers of virological response patients in cirrhosis group and non-cirrhosis group.

Cirrhosis patients had better response than non-cirrhosis patients

Based on clinical diagnosis, the 59 patients were divided into cirrhosis (n=24) and non-cirrhosis (n=35 cases) groups (Table 3). Analysis showed that the serum entecavir concentrations in the cirrhosis group were significantly higher than those in the non-cirrhosis group (0.63±0.45 ng/mL *vs.* 0.46±0.16 ng/mL, *p*=0.033), and they also differed significantly in body weight index (*p*=0.0292), aspartate aminotransferase level (*p*=0.0304), and total cholesterol (*p*=0.0104). Furthermore, the baseline serum entecavir concentration in the non-cirrhosis group were significantly higher than in the cirrhosis group (6.39±1.94 *vs.* 5.19±1.56 log₁₀ copies/mL, *p*=0.0471).

The rate of virological response in the cirrhosis group was significantly higher than that in the non-cirrhosis group (83.33% *vs.* 51.43%, *p*<0.05, Figure 4), and was also higher than in all patients (83.33% *vs.* 64.41%, *p*<0.05).

Three of the 59 patients had a serum entecavir concentration of >1 ng/mL; they were diagnosed with cirrhosis upon the initiation of entecavir treatment and responded well to the treatment (Table 4). Two of the 3 patients had been receiving entecavir for >5 years, and current liver elasticity imaging showed they had moderate fibrosis (F2). One of the 3 patients had been taking entecavir for 7 years (since 27 years old) for treatment of decompensated liver cirrhosis and hepatic encephalopathy. That patient had normal liver function, but that liver elasticity imaging suggested moderate fibrosis. The other patient had a serum entecavir concentration of 2.36 ng/mL and had been receiving

Table 4. Response of 3 cirrhosis patients with >1 ng/ml serum entecavir concentration to entecavir treatment.

Patient code	Sex	Age	HBV DNA level (log10 copies/mL)		Serum entecavir concentration	Months of antiviral treatment
			Baseline	After treatment		
414	Male	50	4.93*10E5	0	2.3624	>45
484	Male	36	3.93*10E7	0	1.2421	84
210	Male	60	2.31*10E9	0	1.1275	Not available

entecavir treatment for 5 years. That patient also had normal liver function with moderate fibrosis and a recent liver biopsy at G1S1.

Discussion

We found significant differences in the serum entecavir concentrations among the study subjects.

In our study, all patients took entecavir at a dose of 0.5 mg/d, and showed an average serum entecavir concentration of 0.53±0.32 ng/mL with a p5-95 range of 0.220–1.11275 ng/ml. These results are similar to results in subjects ≥18 years old [11].

Several studies have shown that entecavir is very effective against HBV. The ETV-023 study detected a significant decrease (5.07 logs on average) in HBV DNA levels after the patients had been treated with entecavir for 12 weeks [5,7,10,15]. In our study, the results after the same treatment period were similar to those in the earlier studies. However, the reduction in the poor response group was less remarkable (4.00±2.07 logs).

The rates of anti-virus response in the 3 entecavir concentration groups were 52.38%, 65.63%, and 100%, respectively. The high concentration group (>0.8 ng/mL) showed a 100% virological response rate, suggesting that higher serum entecavir concentrations is beneficial for viral clearance and a better efficacy. This result contradicts the idea proposed by Boglione et al. [16], who believed that serum entecavir concentrations are negatively correlated with decreases in HBV.

Baseline viral loads are also correlated with the rate of virological response. Patients that showed a poor response had a significantly higher baseline viral load than those who showed a good response. The term "high viral load" is defined in various ways and varied from 10^7 to 10^9 copies/mL, and the responsive rate of "high viral load" patients was only 76.5% or 85.71%, while the "non-high viral load "patients were close to 100% [17–19]. In this study, we defined a "high viral load" as HBV DNA >10^7 copies/mL, and found that patients with different viral loads also showed different virological responses. Furthermore, among the 14 patients with high viral loads, 5 patients with good responsive had higher entecavir serum concentrations than the remaining 9 patients with poor responsive. In previous studies, patients' initial viral load was barely taken into consideration when investigating the correlation between serum entecavir concentration and viral clearance. The patients were treated for a long period of time, but some of the "high viral load" patients still responded poorly, suggesting that a higher viral load makes it more difficult to achieve viral clearance. Prescribing a fixed recommended dose of entecavir for all patients without referring to their initial viral load may not achieve sufficient efficacy, while increasing entecavir serum concentration may achieve

a higher virological response rate. On the other hand, medication nonadherence is an important cause of viral resistance or breakthrough [20–22], and at least 90% long-term adherence is required in most long-term therapy [22]. Compliance is reflected in entecavir concentration, and TDM is necessary.

Cirrhosis patients had higher entecavir concentrations and higher virological response rate compared with non-cirrhosis patients, which may be due to lower initial viral loads in the patients with cirrhosis. The main cause of CHB-induced cirrhosis is that chronic inflammation of the liver and long-term inflammatory damage lead to liver fibrosis, which eventually progresses to cirrhosis [23]. Entecavir inhibits DNA replication of HBV, reduces the incidences of liver cancer and cirrhosis [24,25], and helps alleviate liver fibrosis [26]. The higher serum entecavir concentrations in patients with cirrhosis may be due to the pathophysiological mechanism of hyperdynamic circulation [25]. Cirrhosis of the liver may cause peripheral vasodilation, reduced circulating blood, activation of the renin-angiotensin system, renal vasoconstriction, decreased glomerular filtration, sodium retention, and reduced drug clearance [27]. Meanwhile, the apparent volume of drug distribution may also be reduced [28], contributing to an increase in the serum entecavir concentration. On the other hand, microstructural changes in the livers of patients with cirrhosis may reduce the transport of the drug into cells [27]. However, the EC50 of entecavir is only 3.75 nmol/L [29], thus entecavir is effective even at very low concentrations. The higher virological response rates among patients with cirrhosis may be related to higher serum entecavir concentrations. However, this hypothesis requires further verification.

All 3 patients in our study with a serum entecavir concentration of >1 ng/mL showed improvement after long-term entecavir treatment. Similar results reported that after long-term ETV treatment (mean=6 years), 10 patients with severe hepatic fibrosis/cirrhosis showed improvement when compared with their baseline status (Ishak fibrosis score decreased by >1 point) [30]. Furthermore, some of the patients improved from cirrhosis at baseline to moderate liver fibrosis. Kuo et al. assessed the changes of liver stiffness measurement using transient elastography for CHB patients undergoing more than 5 years of entecavir therapy, showing that the mean value of liver stiffness after treatment was 10.1 kPa, while the initial level was 12.5 kPa [31]. Our results are in line with these studies that entecavir delayed the progression of or partially reversed liver cirrhosis. However, whether this is due to high serum entecavir concentrations requires further investigation.

Conclusions

We have shown that there are significant variations in the serum entecavir concentration, and high serum entecavir

concentration is correlated with a good prognosis. Patients with a poor entecavir response and a low serum entecavir concentration may benefit from a high entecavir dosing that generates higher serum entecavir concentration, especially in high viral load patients who need more drug exposure to clear the virus and prevent virological breakthrough. Low entecavir serum concentration may be caused by poor adherence in long-term therapy and lead to poor virological response. Needless to say, further studies are needed to define the optimal serum entecavir concentrations in CHB patients. The serum concentration of entecavir determined the morning after taking the drug may be a stable, reliable, clinically-relevant, and practical indicator for use in clinical studies and the routine TDM of entecavir. Further studies with larger sample sizes are required to confirm whether a higher dose of entecavir would generate

higher concentration and improve the drug's efficacy in patients with a high viral load and a poor response. Such studies are also needed to further elucidate the role of entecavir in patients with cirrhosis and the prognosis for those patients.

Declaration

Ethics approval and consent to participate: The study was approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University, and written consent was received from every participant.

Conflict of interests

None.

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