

Parkinson's disease and CYP1A2 activity

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Aims MPTP, a neurotoxin which induces parkinsonism is partially metabolized by the enzyme CYP1A2. Smoking appears to protect against Parkinson's disease (PD) and cigarette smoke induces CYP1A2 activity. Thus, we investigated the hypothesis that idiopathic PD is associated with lower CYP1A2 activity using caffeine as a probe compound.

Methods CYP1A2 activity was assessed using saliva paraxanthine (PX) to caffeine (CA) ratios. Caffeine half-life was also estimated from salivary concentrations of caffeine at 2 and 5 h post dose. 117 treated and 40 untreated patients with PD and 105 healthy control subjects were studied.

Results PX/CA ratios were 0.57, 0.93 and 0.77 in treated patients, untreated patients and healthy control subjects, respectively, with no significant differences between study groups (95% CI: treated patients *vs* controls -0.24, 0.57; untreated patients *vs* controls -0.75, 0.35). However, patients with PD (treated or untreated) had caffeine half-lives shorter than that in controls (treated patients: 262 min, untreated patients: 244 min, controls: 345 min; 95% CI: controls *vs* treated patients 23, 143 ($P=0.003$); controls *vs* untreated patients 19, 184 ($P=0.011$)). Amongst the patients with PD, caffeine half-life was also inversely related to the age of onset of disease ($P=0.012$); gender and concomitant drugs did not influence this significantly.

Conclusions Based on PX/CA ratio, there was no evidence of decreased CYP1A2 activity in patients compared with control subjects. The observed decrease in the elimination half-life of caffeine in PD may be caused by increased CYP2E1 activity, an enzyme that also contributes to the metabolism of caffeine. The latter warrants further investigation.

Keywords: caffeine metabolism, CYP2E1, Parkinson's disease

Introduction

Susceptibility to idiopathic Parkinson's disease (PD) may be dependent upon the interaction of genetic factors, exposure to environmental neurotoxins and efficiency of neuroprotective mechanisms.

MPTP, a neurotoxin that causes parkinsonism, is metabolized by monoamine oxidase to form toxic metabolites [1]. MPTP is detoxified by the liver enzymes flavin monooxygenase (FMO) and cytochromes (CYPs) P450 2D6, 1A2 and 3A4 [2, 3]. These enzymes may therefore be neuroprotective [1, 4–7]. Smoking, which

induces CYP1A2 activity [7, 8], protects against MPTP-induced depletion of striatal dopamine [9] and enhances CYP1A-catalysed detoxification of neurotoxins which cause parkinsonism [10]. Consistent with these observations, epidemiological studies have reported a negative association between smoking and PD [11–15] suggesting a protective effect. It was suggested initially that the association was an artefact of 'diagnostic displacement' [12], 'selective mortality' [13], or 'PD-associated personality traits' [16, 17]. Nonetheless, further studies confirmed the negative association [11, 13, 15, 18, 19]. Putative protective mechanisms include the chemical properties of cigarette smoke [19–21], enzymatic inhibition of monoamine oxidase [22], or protection against free radical damage [20]. However, induction of CYP1A2 activity

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Received 6 August 1999, accepted 5 July 2000.

may also explain the negative association between smoking and the incidence of PD.

CYP1A2 activity can be assessed by measuring the rate of metabolism of caffeine (CA) to paraxanthine (PX). Caffeine is 1-, 3-, and 7-demethylated to form theobromine (TB), PX and theophylline (TP), respectively (Figure 1) [23]. In healthy humans, the 3-demethylation of caffeine by CYP1A2 to form PX accounts for $84 \pm 5\%$ of its metabolism, whereas 1- and 7-demethylation to TB and TP accounts for $12 \pm 4\%$ and $4 \pm 1\%$ of net metabolism, respectively [24] (Figure 1). The use of plasma PX/CA ratios at 5 h after a dose of caffeine provides a robust estimate of CYP1A2 activity *in vivo* [25, 26].

We tested the hypothesis that patients with PD have decreased activity of CYP1A2 relative to disease-free control subjects. Caffeine and paraxanthine were measured in saliva rather than blood plasma as collection is less invasive and salivary concentrations have been reported to reflect plasma concentrations accurately [26]. In order to validate our findings we also examined caffeine half-life, which has also been reported to be a valid reflection of CYP1A2 activity in normal individuals [25, 26].

Methods

Power calculation

The intra- and inter-individual variability of caffeine elimination is reported to be 21% and 79%, respectively [27]. Based on this, it was estimated that 40 subjects in each group would be required to detect a 30% difference in CYP1A2 activity with a power of 80% at a significance level of $\alpha = 0.05$.

Subjects

The South Sheffield Research Ethics Committee approved the study protocol and participants gave written informed consent. One hundred and seventeen treated patients with PD, 40 untreated patients with PD and 105 healthy control subjects were recruited from the

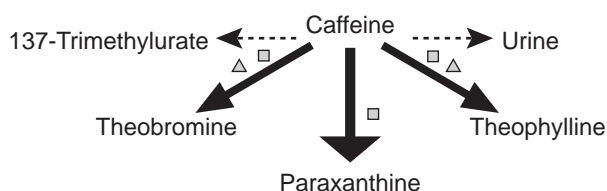


Figure 1 Primary routes of metabolism of caffeine in humans. The thickness of the arrow indicates the relative contribution of each pathway to overall elimination. Major enzymes responsible for each metabolic route are also shown. CYP = cytochrome P450. □ CYP1A2; △ CYP2E1.

Movement Disorder Outpatient Clinic, and the Departmental volunteer database at the Royal Hallamshire Hospital, Sheffield, UK. All subjects were white Caucasians living in the South Yorkshire region and patients fulfilled the diagnostic criteria of the Parkinson's Disease Society (PDS UK): akinesia plus asymmetric rest tremor, rigidity or postural instability and the absence of any other condition that might produce signs of parkinsonism. The demographic details of the three groups are shown in Table 1. To avoid the complications of matching smoking habits between PD and controls, we recruited only subjects who were currently nonsmokers. Subjects receiving medication known to affect CYP1A2 activity were excluded (fluvoxamine, cimetidine, oral contraceptives and quinolone antibacterial agents). Control subjects mainly comprised spouses and friends of patients with PD and were free from neurological disease. It was assumed that the dietary habits of patients and controls, which may influence CYP1A2 activity, are similar in our patient and control groups.

Sample collection

Subjects were asked to abstain from methylxanthine-containing food and drinks for 12 h prior to their participation in and during the study. On the day of the study subjects provided approximately 1.5 ml of saliva in a sterile plastic tube (predose-sample), and then drank a cup of coffee providing approximately 57 mg caffeine (5 g sachet of instant coffee, Nescafé, Nestlé UK Ltd). If the subject had problems producing saliva, a piece of paraffin film (Parafilm[®]) was chewed to aid salivation. Subjects provided two further saliva samples at 2 and 5 h after the

Table 1 Demographic details of patients and controls¹.

	Treated PD ²	Untreated PD	Control subjects
Gender	57M/60F	17M/23F	30M/75F
Age ² (years)			
[range]	64	60	61
	[32-78]	[32-76]	[28-80]
Disease duration (years)	7.0	2.0	
[range]	[0.5-25]	[0.5-7]	

1) The average age of treated patients with PD was significantly higher compared with controls and untreated patients ($P=0.000$ and $P=0.045$, respectively); the distribution of gender among groups was also significantly different ($P=0.009$); Although disease duration was shorter in untreated PD patients ($P=0.0001$), there was no significant difference in age of disease onset between treated and untreated patients. 2) Treatments included: amantadine, apomorphine, benzhexol, benzotropine, bromocriptine, lysuride, co-beneldopa (levodopa with benzerazide), ropinerole, co-careldopa (levodopa with carbidopa), selegiline, pergolide.

caffeine dose. All samples were stored in a freezer. One ml of each sample was pipetted into Eppendorf® microcentrifuge tubes and spun at 13 000 rev min⁻¹ (corresponding to 640 g) in a MSE Microcentaur centrifuge for 10 min. The supernatant was stored at -20°C until assay.

Assay of caffeine and paraxanthine

The method of O'Connell & Zurzola [28] was used to determine concentrations of CA and PX. Protein was removed from saliva samples by precipitation with perchloric acid followed by centrifugation. PX and CA concentrations were measured by h.p.l.c.

The intra-assay reproducibilities for CA and PX were 3% and 7% at lowest concentration (0.05 µg ml⁻¹) and 5% and 9% at highest concentration (1.5 µg ml⁻¹), respectively. Inter-assay variability (over 6 weeks) was consistently less than 15% for both CA and PX.

Data analysis

The activity of CYP1A2 was determined using the ratio of PX to CA concentration in saliva at 5 h after CA dose. In order for this ratio to be a valid reflection of CYP1A2 activity, subjects must abstain from CA-containing food and beverages for a 12–24 h period [29]. This precondition is unlikely to be met consistently in out-patient studies [26, 30–32]. Thus, PX/CA ratio was calculated after CA and PX concentrations were corrected for decline from predose levels by subtracting the value of the following expression:

$$C_{pre} \times e^{\left(-\frac{0.693}{t_{1/2}} \times T\right)}$$

where C_{pre} is the predose saliva concentration of PX or CA and T is the time of the sample (2 or 5 h).

The elimination half lives ($t_{1/2}$ in min) of CA and PX were calculated from:

$$t_{1/2} = \frac{0.693 \times 180}{\ln(C_{(5)}) - \ln(C_{(2)})}$$

where $C_{(5)}$ and $C_{(2)}$ are the saliva concentrations of CA or PX at 5 and 2 h, respectively. The time elapsed between samples was 180 min.

Assuming that the absorption of CA is complete and that it is much faster than the elimination of CA [33, 34], the volume of distribution (V) of CA was estimated from:

$$V = \frac{57}{e^{(\ln(C_{correct(2)}) + (120 \times 0.693/t_{1/2}))}}$$

where $C_{correct(2)}$ is the CA concentration at 2 h after correction for baseline, and the dose of CA is 57 mg.

Hence, CA clearance (CL) was estimated from:

$$C_{pre} \times e^{\left(-\frac{0.693}{t_{1/2}} \times T\right)}$$

In some patients the corrected value of PX or CA concentration was biologically implausible (i.e. negative concentration or half life, or extremely long half life). Such values may result from unusually slow absorption of the caffeine test dose, failure to abstain from methylxanthine-containing foodstuffs during the course of the experiment or failure to adhere to the timings of the experimental protocol. During calculation of CA half-life only a small proportion of subjects showed such nonbiologic values (4 of 117 values in patients with treated PD, 3 of 40 values in patients with untreated PD and 6 of 105 values in control subjects). There was no statistically significant difference in the proportion of such data between subgroups. In calculating PX/CA ratios, data from 25/117 treated patients, 12/40 treated patients and 29/105 control subjects showed nonbiological values. Again, there was no significant difference between subgroups in regard to proportion of these nonbiological values.

Three approaches were followed to overcome the problem of nonbiological values: (1) they were excluded from the analysis (2) they were replaced by the mean from the respective study population, and finally (3) they were left in the data base but nonparametric statistical methods were used to analyse the data.

During the first two statistical analyses, the differences in PX/CA ratios, caffeine half life, volume of distribution and clearance between groups were assessed using repeated measures analysis of variance (MANOVA) followed by Tukey's *post hoc* analysis (SPSS for Windows v7.0). In all subsequent analyses age, gender and concomitant drugs used by PD patients were considered as covariates. Relationships between caffeine half life and covariates were investigated using backwards stepwise multiple linear regression analysis.

A cut-off age for the relationship between the age of onset of PD and caffeine half life (covaried for age) was sought using the following equations:

when age of onset ≤ cut-off age:

$$t_{1/2}(\text{Age Corrected}) = \text{Slope} \times \text{Age of Onset} + \text{Intercept}$$

when age of onset > cut-off age:

$$t_{1/2}(\text{Age Corrected}) = \text{Slope} \times (\text{Cut-Off Age}) + \text{Intercept}$$

where the unknown parameters Slope, Intercept and Cut-Off Age were estimated iteratively by Solver within Excel (v5.0, Microsoft Corporation).

Non-parametric comparisons were made using the Mann-Whitney *U*-test (SPSS for Windows v7.0).

Results

Nearly 90% of subjects were found to have baseline salivary concentrations of CA and/or PX, despite instructions to abstain from CA intake. Implications of this observation are reported elsewhere [29]. The values (and interquartile range) for CA, PX and PX/CA ratios are shown for each subgroup in Table 2. It has been reported that dietary CA can also be used as a probe for CYP1A2 activity [35]. Therefore, predose samples were analysed to calculate values of predose PX/CA ratio.

Non-parametric analysis, including all nonbiological values, revealed the half-life of CA to be significantly shorter in treated or untreated PD patients compared to control subjects ($P=0.001$ and $P=0.008$, respectively; Mann–Whitney U -test). The most common CYP 1A2 activity, PX/CA ratio at 5 h, was not significantly different between groups. Pre-dose PX/CA, however, was significantly lower in both treated and untreated PD patients compared with control subjects ($P=0.000$ and $P=0.012$, respectively; Mann–Whitney U -test).

A summary of the results following parametric analysis of the data (see Methods) is shown in Table 3. Again, there was a significant difference in CA half-life between patients with PD and controls. Both patients with treated PD and those with untreated PD had significantly lower values compared with controls (the gender and age

Table 2 Summary of observed and calculated kinetic parameters values of caffeine (as medians) in control subjects and PD patients¹.

Parameters ²	Control subjects	Untreated PD	Treated PD
CA(0)	587 [254-964]	339 [101-756] ³	162 [41-651] ³
CA(2)	1400 [780-2048]	1025 [671-1891]	993 [858-1546] ³
CA(2) _{cor}	1026 [567-1388]	740 [557-1425]	706 [506-1116]
CA(5)	849 [430-1298]	596 [330-953]	467 [252-869] ³
CA(5) _{cor}	593 [291-841]	425 [284-858]	371 [200-650] ³
CA t _{1/2}	351 [204-446]	197 [141-324] ³	223 [129-341] ³
PX(0)	390 [209-643]	268 [174-442]	242 [79-451] ³
PX(2)	644 [366-879]	427 [281-587] ³	533 [316-743]
PX(2) _{cor}	423 [161-579]	350 [184-517]	322 [152-544]
PX(5)	509 [364-686]	357 [227-518]	376 [217-579]
PX(5) _{cor}	358 [131-519]	300 [151-475]	255 [130-436]
PX t _{1/2}	585 [185-621]	479 [3-1345]	355 [154-835]
PX/CA (0)	0.43 [0.30-0.93]	0.38 [0.12-0.64] ³	0.37 [0.12-0.67] ³
PX/CA (2) _{cor}	0.40 [0.20-0.71]	0.42 [0.21-0.63]	0.44 [0.22-0.74]
PX/CA (5) _{cor}	0.62 [0.30-0.92]	0.63 [0.32-1.00]	0.66 [0.35-1.07]

1- Numbers in bracket show the time of sampling; numbers in square bracket show the values for first and third quartiles; subscript 'cor' shows that the corresponding parameter value is obtained after correction for baseline (see Methods for details). 2- Concentrations values for CA and PX are in ng ml⁻¹; half-life values are in min. 3- Significantly different from control subjects (at $\alpha \leq 0.02$).

Table 3 Summary of differences in mean PX/CA ratio and caffeine half-life between PD patients and control subjects¹.

		Caffeine half-life		
Group		Control subjects	Untreated PD	Treated PD
Control subjects			101 [0.011] (19 to 184)	83 [0.003] (23 to 143)
PX/CA ratio	Untreated PD	-0.20 [0.67] (-0.75 to 0.35)		-18 [0.86] (-99 to 63)
	Treated PD	0.17 [0.60] (-0.24 to 0.57)	0.37 [0.25] (-0.18 to 0.91)	

Values above the diagonal line show the differences in caffeine half-life and those below the line show the differences in PX/CA ratio between study groups; Numbers in square brackets are P values and numbers in italics are 95% CI's for mean differences.

differences between these groups were accounted for using MANOVA).

Multiple regression analysis revealed that caffeine half-life was related to the age of onset of disease and current age of patients in the group with PD ($P=0.041$). Nevertheless, these could explain only 5% of the overall variability. Covariates such as gender and concomitant drugs taken by PD patients did not contribute to the variance of caffeine half-life significantly. Current age was related inversely to caffeine half life in the patient group ($P=0.048$, Figure 2). There is evidence for a different aetiology in early and late onset Parkinson's disease. Despite the existence of analytical tools to define aetiological subgroups (e.g. cluster analysis, modelling), the age cut-off point has often been determined arbitrarily. We sought a cut-off point for the association of age of onset and caffeine half life by means of modelling. The broken line in Figure 2 shows the best model fit when such a cut-off point is considered and determined using an iterative fitting process ($P=0.00005$). The model suggested that beyond 61 years of age, there was no relationship between caffeine half-life and age of onset. This model, although consistent with previous suggestions of different aetiology of early and late parkinsonism in finding a cut-off age, produced only a marginal improvement in fit compared with simple linear regression (dotted line in Figure 2a; $P=0.10$ for the improvement of fit; F -test). No correlation between age and caffeine half-life was observed in the control group or the group of untreated patients with PD when considered separately.

Mean values of estimated volume of distribution and clearance for caffeine (Table 4) were similar to those reported in the literature [27]. However, variability in the values was high, reflecting the indirect method of estimation and limited utility of such parameters. Body weight, a covariate for caffeine volume of distribution [33], did not differ between study groups (mean weight of

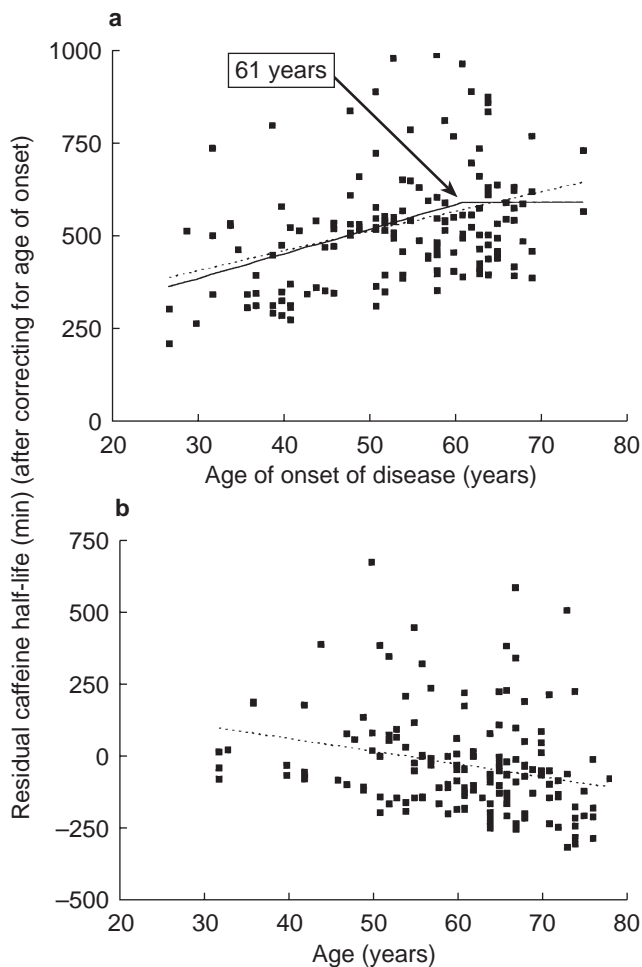


Figure 2 Relationships between caffeine half-life and (a) age of onset and (b) current age of patients with PD after correcting for other covariates.

control subjects \pm s.e.mean = 71 ± 1.4 kg; treated PD = 69 ± 1.5 kg; untreated PD = 70 ± 2.1 kg; $P=0.53$).

Discussion

Since the mean salivary PX/CA ratio 5 h postdose was not different in patients with PD and controls, the original hypothesis that induction of CYP1A2 activity protects against PD by enhancing CYP1A2-mediated detoxification of a neurotoxin was unlikely. Pre-dose PX/CA ratios showed significantly lower values in patients compared to control subjects. This may imply that CYP1A2 activity is lower in patients with PD, but the validity of this measure as a probe for CYP1A2 is doubtful if all subjects were instructed to refrain from caffeine containing food and beverages. The difference between patients and control subjects may alternatively reflect the differences in abstinence from caffeine between control and patient groups [29]. It is established that PX/CA ratio increases with time after caffeine ingestion [25, 36]. Thus, lower

Table 4 Estimated caffeine volume of distribution and clearance values in the study groups¹.

Group	Volume of distribution ² (l)	Clearance ³ (l h ⁻¹)
Treated PD (n = 117)	57 (35)	12 (12)
Untreated PD (n = 40)	50 (27)	10 (7)
Control (n = 105)	58 (47)	9 (7)

Values are means (\pm s.d.) There was no significant differences between groups. Patients with treated PD had significantly higher clearances compared with controls ($P=0.019$).

predose ratios may indicate that the duration of abstinence from caffeine containing beverage had been shorter in the PD group. PX/CA ratio at 5 h (corrected for baseline) is not influenced by such factors. The similarity between control and PD subjects is inconsistent with a true difference in CYP1A2 activity between the groups. Accordingly, the mechanism of the protective effect of smoking in the development of PD remains obscure. It is possible that induction of CYP1A2 in smokers accelerates the removal of a neurotoxin but, as none of our patients were current smokers, we are unable to comment further on this issue.

CYP1A2 activity, as marked by the PX/CA ratio at 5 h post dose, was similar in patients with PD yet the half-life of caffeine was shorter and its clearance faster. This suggests an alternative hypothesis for the role of xenobiotic metabolism in the aetiology of PD. Normally, CYP2E1 is responsible for only a small fraction (about 3%) [25] of the metabolism of caffeine by contributing to 1- and 7-demethylation (Figure 1). However, CYP2E1 is an inducible enzyme [37], and significant up-regulation may account for the enhanced elimination of caffeine in patients with PD. Accordingly, induction of the formation of a neurotoxin from a precursor by CYP2E1 may be a

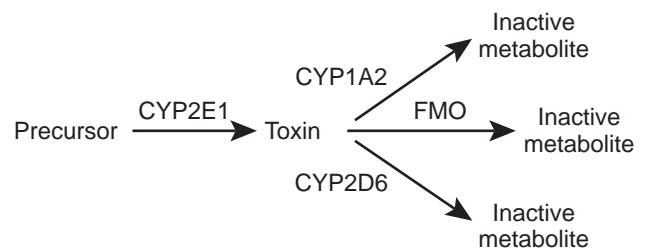


Figure 3 Scheme for the proposed activation and detoxification of a putative neurotoxin causing PD. CYP = cytochrome P450; FMO = flavin monooxygenase.

predisposing factor to PD. Thus, the balance of the activities of different CYPs may determine the rate of production of the putative neurotoxin, CYP2E1 producing the toxin and CYP1A2 and CYP2D6 inactivating it (Figure 3). This scheme is consistent with a recent report that the neuroprotective effect of smoking is reduced by heavy alcohol intake [38].

CYP2E1 is known to be localized in rat substantia nigra dopamine cells [39]. Bandmann *et al.* [40] reported no significant genotypic differences in CYP2E1 between patients with PD and control subjects. However, the genotypes were defined with respect to the coding region of the CYP2E1 gene; regulatory genes may be more important to consider.

It is known that gastric emptying is slower in PD patients [41]. This could have an effect on the estimate of caffeine half-life as measured from 2 and 5 h samples. However, assuming such an effect, it should bias estimates of half-life to longer values compared to controls. This is in contrast to our observation.

Our patients with an early onset of disease exhibited faster metabolism of caffeine compared to those with late-onset disease, this suggests that the enzyme alteration associated with accelerated caffeine metabolism (other than change in CYP1A2) could be a significant risk factor for both the development and progression of PD. An inverse relationship between age and caffeine half-life was observed in the treated patients with PD but not in untreated patients or control subjects. This may reflect an effect of treatment duration or the effect of disease duration on metabolism. Differentiation between such effects would be possible only by comparing treated and untreated patients with similar disease duration, which is problematic.

In conclusion we have demonstrated that a lower PX/CA ratio 5 h after caffeine intake, as a marker of low CYP1A2 activity, is not associated with PD. Faster elimination of caffeine (i.e. shorter half-life) was found in patients with PD, and may indicate a possibility for higher activity of CYP2E1, an enzyme that contributes to caffeine metabolism. The possibility of a relationship between age of onset and short caffeine half-life warrants further studies since this relationship implies that modulation of the activity of the enzymes involved in caffeine metabolism could enhance or retard the rate of progression of PD.

We thank the Special Trustees of the Central Sheffield University Hospitals Trust for financial support for this research.

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