Oral type II collagen in the treatment of rheumatoid arthritis. A six-month double blind placebo-controlled study

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Abstract

Objective

To evaluate the efficacy of oral chicken type II collagen (CII) in the treatment of rheumatoid arthritis (RA).

Methods

Sixty patients with clinically active RA of long duration (mean 7.2 ± 5.5 years) were treated for 6 months with oral chicken CII at 0.25 mg/day (n = 31) or with placebo (n = 29) in a double-blind randomized study.

Results

The response rate to treatment of the collagen-treated group, based on the ACR 20% criteria, was higher than that of the control group but this difference was not statistically significant at any time. Intention-to-treat (ITT) analysis did not show statistically significant improvement in any of the several secondary outcome measures over the 6 months of the study in the collagen-treated patients in comparison with the placebo-treated group. However, in 2 collagen-treated patients we observed a clinical remission according to the criteria of the American Rheumatism Association.

Conclusion

Our study seems to show that the oral treatment of RA patients with chicken CII is ineffective and results in only small and inconsistent benefits. Furthermore, our results raise the possibility that in a sub-group of patients oral collagen administration, usually considered devoid of harmful effects, may actually induce disease flares.

Key words

Rheumatoid arthritis, oral tolerance, type II collagen.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by the migration, activation, and proliferation of immunocompetent cells within synovial membranes. While the etiology of the disease remains unknown, evidence that a subset of primed lymphocytes intervenes in the perpetuation, and possibly in the induction, of the synovial inflammatory process has prompted researchers to investigate therapies aimed at selectively modifying cellular reactivity (1). Oral tolerance is defined as the suppression of immune system reactivity toward an antigen by means of the oral administration of the antigen itself (2). Oral administration of self-antigens has been shown to effectively modulate the immune response in several animal models, including autoimmune encephalomyelitis (3), uveitis (4), and type I diabetes (5). Several lines of evidence suggest that type II collagen (CII), which is preponderant among the structural proteins of articular cartilage, could behave as an autoantigen in RA:

1) In rats, immunization with CII is followed by the development of a polyarthritis morphologically similar to RA (6-8).
2) In RA patients, antibodies to CII have been found in 15-30% of cases in the peripheral blood, and in up to 50% of cases in the synovial fluid (9-20).
3) Oral feeding with CII has been shown to ameliorate polyarthritis not only in rats immunized with CII but also in rats immunized with Freund adjuvant (21).

The suppression by oral CII of Freund adjuvant-induced arthritis, an experimental model where CII should not act as a self-antigen, can be explained by the phenomenon of "bystander suppression", by which tolerance is induced not only toward the administered antigen, but toward other antigens of the same tissue as well. This mechanism represents the theoretical basis of the application of the oral tolerance principle to autoimmune diseases where the causative antigen is unknown, or where multiple causative antigens are involved (22).

The induction of peripheral tolerance to a specific antigen is related to the dosage of the administered antigen, active suppression being induced by low doses, and anergy being induced by high doses (23). The oral administration of antigens in a low dosage is supposed to induce, in the lymphatic tissue of gut, the activation of regulatory T lymphocytes (Th2) that subsequently migrate to affected organs (in the case of RA, synovial membranes). At this level, a second contact with the involved antigen should stimulate Th2 lymphocytes to produce anti-inflammatory cytokines such as interleukin-4 (IL4), IL10, and tumor growth factor β (TGFβ), thus explaining the observed clinical results (24).

The efficacy of the oral administration of CII extracted from chicken or bovine cartilage has recently been evaluated in the treatment of adult (25-28) and juvenile (29) RA. The aim of the present study was to assess the long-term efficacy of oral CII administration in the treatment of adult RA of long duration.

Patients and methods

Sixty patients, 53 females (88.3%) and 7 males (11.6%), with clinically active RA, were recruited at our Rheumatology Unit. Eligibility criteria were: (1) RA diagnosed according to the 1987 ARA criteria (30); (2) age more than 18 years; (3) disease duration of at least 12 months; (4) clinically active disease, i.e.: at least 4 painful joints, 4 swollen joints, morning stiffness 45 min, erythrocyte sedimentation rate (ESR) 28 mm/1 hr or CRP > 1.05 mg/dl; (5) concurrent steroid dosage stabilized at 10 mg prednisone equivalents/day; and (6) written informed consent.

Patients were required to complete a disease-modifying antirheumatic drug (DMARD) washout period that lasted at least one month before starting the study CII treatment; no DMARDS were permitted during the study.

Exclusion criteria were: (1) liver, kidney, cardiovascular and neoplastic disease, and (2) multiple end-stage articular deformities not modifiable by drug treatment.

Study design

The study, lasting 6 months, was performed according to a double-blind, parallel groups, randomized design. It was approved by the local ethical committee.

Concomitant treatment
Patients were left on their steroid treatment, and the dose of the drug was left unchanged for the 2 weeks preceding enrollment and throughout the entire study. Similarly, the dosage of nonsteroidal anti-inflammatory drugs (NSAIDs) was kept constant over the study period, and paracetamol was prescribed for pain control. A baseline evaluation was performed after at least 4 weeks of washout from second-line treatments.

Clinical evaluation
All patients were evaluated at baseline and after 1, 2, 3, 4, and 6 months of treatment. At each visit the following variables were evaluated: Ritchie's articular index (31), the number of joints painful on movement (32), the number of swollen joints (32), the physician's and patient's assessment of improvement on a semi-quantitative scale (0 = nil, 1 = poor, 2 = fair, 3 = good, 4 = excellent), the duration of morning stiffness in minutes, the intensity of morning stiffness evaluated on a 100 mm Visual Analogue Scale (VAS), left and right hand grip strength in mmHg (33), Arthritis Impact Measurement Scale (AIMS) shortened version (34-35), and pain intensity evaluated on a VAS.

Assessment of efficacy
Responders were defined according to ACR 20% criteria for improvement in RA clinical trials (36): reduction ≥ 20% from baseline in both tender and swollen joints, plus an improvement ≥ 20% in at least three of the following items: (1) patient pain assessment (VAS); (2) ESR or CRP; (3) AIMS; (4) physician's global assessment evaluated on a 5-point Likert scale; (5) patient's global assessment evaluated on a 100 mm VAS.
The cumulative rate of ACR20 responders over the 6 months was chosen as the primary outcome measure.

Laboratory variables
The following determinations were performed at baseline, and at the 3rd and 6th month: ESR (mm/1st hr), C-reactive protein (CRP), blood glucose, creatinine, aspartate aminotransferase (AST), sodium, potassium, blood cell count (BCC), urinalysis, and antibodies to CII. The ESR and CRP were also determined at the 1st, 2nd and 4th month. CRP (normal value < 1.05 mg/dl), and rheumatoid factor (n.v. < 40 I.U./ml) were determined by nephelometric methods.
Sera obtained at baseline and at the 3rd and 6th months were stored at -80°C. IgG antibodies to native collagen II were determined by an immunoenzymatic assay. All samples from the individual patients were tested on the same plate, with 10 specimens from blood donors as controls. The determination was repeated 3 times. Antibodies to CII were considered to be present when the optical density (OD) of the patient's specimen exceeded the mean value + 3 standard deviations of the donors' controls. Positive results were expressed as the OD ratio of patient serum to control sera (17).

Collagen preparation
Type II native collagen was extracted from chicken sternal cartilage according to the method described by Trentham (37), and dissolved in 0.1 M acetic acid to a final concentration of 0.5 mg/ml. Active drug and placebo were prepared in indistinguishable sterile dropper bottles containing either 7.5 mg of CII in 15 ml of solution or 15 ml of 0.1 M acetic acid, and stored at -20°C. The drug was dispensed to the patients in thermal containers by a biologist not involved in the clinical follow-up. Patients were instructed to store the drug in their refrigerators at 4-6°C, and to take the daily dose (0.25 mg of CII) in the morning before breakfast, diluted in unsweetened orange or grapefruit juice.

Statistical analysis
Statistical evaluation was performed by standard procedures (38). Baseline characteristics were evaluated by the two-tailed Fisher's exact test, the t-test and the Wilcoxon test, as appropriate. The rate of responders at different time points was evaluated by life table analysis, which allows the analysis of censored data, and the differences between the collagen group and the placebo group were evaluated by the log-rank test. Further efficacy analysis was performed on patients classified in 2 categories: a) the intention to treat population (ITT) comprising all enrolled patients; and b) the per protocol population which included only those patients who completed the entire 6-month study period.
Endpoint changes from baseline values in efficacy variables were analyzed for the ITT group and the PP group by analysis of variance on values corrected for the initial values, steroid treatment and disease duration (ANCOVA). The physician's and patient's assessment of improvement versus baseline was analyzed by the Wilcoxon rank sum test. The rate of responders in the 2 groups at single timepoints was compared by the two-tailed Fisher's exact test. A logarithmic regression model including the disease duration, steroid dosage, and baseline AIMS and ESR was adopted to evaluate the impact of the baseline presence of antibodies to CII on response to treatment in the collagen group. Endpoint changes from baseline values for selected variables (AIMS score, ESR, and CRP) in the collagen group according to baseline anticorpal status (positive or negative) was evaluated by Kruskal-Wallis ANOVA.

Results

Patients characteristics and study withdrawals
Sixty patients were enrolled and randomly assigned to receive either oral collagen (n=31) or placebo (n = 29). The characteristics of the patients (Table I) and the efficacy variables (Table II) at entry were similar in the two groups. Eleven patients (18.3%) did not complete the study, 5 in the collagen group (16.1%), and 6 in the placebo group (20.6%). One patient in the placebo group died from cerebral hemorrhage due to rupture of an angioma at the 4th month. Four patients - 2 from the collagen group and 2 from the placebo group - withdrew from the study for personal reasons unrelated to the disease or the efficacy of treatment. Three patients in the placebo group were withdrawn due to lack of efficacy, and 3 patients in the collagen group were withdrawn due to worsening of disease (2 at the second and 1 at the fourth month). Only mild side effects were recorded in the 2 groups. Laboratory values were unchanged over the 6 months of the study (data not shown).

Table I. Characteristic of the patients by treatment group.

	Intent to treatment			Per protocol analysis		
	Placebo n = 29	Collagen n = 31	P	Placebo n = 23	Collagen n = 26	P
Sex (women/men)	24/5	29/2	NS	18/5	25/1	NS
Age mean ± SD (range)	55.3 ± 11.9	56.3 ± 13.9	NS	52.9 ± 10.7	55.9 ± 13.2	NS
Disease duration in months (mean ± SD)	89.8 ± 79.6	84.5 ± 56.0	NS	89.8 ± 81.7	89.6 ± 56.8	NS
RF positive N(%)	15 (51.7)	19 (61.2)	NS	12 (52.1)	16 (61.5)	NS
Radiological lesions	24 (82.7)	25 (80.6)	NS	20 (86.97)	25 (96.1)	NS
N positive (%)	3 (10.3)	5 (16.1)	NS	2 (8.6)	5 419.27	NS

RF = rheumatoid factor.

Table II. Baseline characteristics (mean ± SD).

	Intention to treat analysis (last recorded value)			Per protocol analysis (sixth month follow-up)		
	Placebo n = 29	Collagen n = 31	P	Placebo n = 23	Collagen n = 26	P
Right grip strength (mmHg)	142.24 ± 70.88	122.58 ± 73.11	0.295	130.65 ± 73.60	114.23 ± 61.35	0.399
Left grip strength (mmHg)	135.69 ± 74.02	114.19 ± 82.25	0.293	124.13 ± 77.88	101.54 ± 72.32	0.298
Severity of pain (VAS mm)	58.96 ± 18.68	56.13 ± 20.96	0.583	60.43 ± 18.34	54.61 ± 20.65	0.305
Duration of morning stiffness (min)	102.07 ± 84.54	90.81 ± 86.21	0.612	116.09 ± 87.80	99.42 ± 89.05	0.514
Ritchie's index	10.59 ± 8.07	8.61 ± 6.05	0.286	10.91 ± 8.34	7.96 ± 5.59	0.148
Swollen joints (number)	9.55 ± 5.59	10.06 ± 6.06	0.735	9.69 ± 5.13	10.15 ± 6.10	0.779
Tender joints (number)	13.76 ± 8.97	13.00 ± 8.74	0.741	14.96 ± 9.55	12.15 ± 7.87	0.366
AIMS	21.41 ± 11.13	22.32 ± 10.92	0.751	22.83 ± 11.46	22.07 ± 10.76	0.815
Morning stiffness (VAS min)	52.76 ± 26.08	49.84 ± 27.74	0.676	55.22 ± 25.61	53.27 ± 25.81	0.792
ESR (mm/1 hr)	49.59 ± 24.32	49.00 ± 27.84	0.347	41.26 ± 25.92	53.12 ± 27.06	0.125
CRP (mg/dl)	1.77 ± 2.60	2.74 ± 2.54	0.071	2.04 ± 2.83	3.04 ± 2.60	0.072

VAS: Visual analogue scale; ESR: Erythrocyte sedimentation rate; CRP C reactive protein; AIMS: Arthritis measurement scales.

Table III. Mean differences versus baseline.

	Intention to treat analysis (last recorded value)			Per protocol analysis (sixth month follow-up)		
	Placebo n = 29	Collagen n = 31	P	Placebo n = 23	Collagen n = 26	P
Right grip strength (mmHg)	5.57 ± 11.17	22.20 ± 10.81	0.305	10.33 ± 12.54	36.25 ± 11.80	0.157
Left grip strength (mmHg)	7.27 ± 11.69	21.91 ± 11.31	0.386	9.36 ± 13.70	33.07 ± 12.89	0.234
Severity of pain (VAS mm)	-8.52 ± 4.31	-10.74 ± 4.17	0.721	-13.07 ± 3.89	-14.98 ± 3.67	0.741
Duration of morning stiffness (min)	-6.36 ± 17.66	-10.66 ± 17.08	0.866	-12.46 ± 15.32	-40.13 ± 14.41	0.217
Ritchie's index	0.32 ± 1.20	-10.43 ± 1.16	0.665	1.29 ± 1.28	-2.60 ± 1.20	0.138
Swollen joints (number)	1.28 ± 0.97	-0.62 ± 0.94	0.177	1.22 ± 0.01	-1.81 ± 0.95	0.043
Tender joints (number)	-1.25 ± 1.48	-3.51 ± 1.44	0.291	-0.77 ± 1.51	-6.09 ± 1.42	0.020
AIMS	-5.37 ± 1.92	-5.05 ± 1.86	0.908	-5.82 ± 1.76	-7.66 ± 1.76	0.498
Morning stiffness (VAS min)	-8.51 ± 5.19	-13.97 ± 5.02	0.464	-9.69 ± 5.09	-22.20 ± 4.79	0.094
Patient assessment (VAS mm)	24.44 ± 6.14	32.62 ± 5.94	0.355	28.19 ± 7.11	37.76 ± 6.69	0.353
ESR (mm/1 hr)	-0.84 ± 3.86	2.21 ± 3.74	0.584	-5.73 ± 4.24	-1.03 ± 3.99	0.053
CRP (mg/dl)	0.55 ± 0.49	0.84 ± 0.48	0.692	0.67 ± 0.57	0.35 ± 0.55	0.699

VAS: Visual analogue scale; ESR: Erythrocyte sedimentation rate; CRP C reactive protein; AIMS: Arthritis measurement scales.

Table IV. Responders at each interval monitored, by treatment group [ACR criteria (36)], expressed as no. and percentages (between parentheses).

Month	1st		2nd		3rd		4th		6th	
	Placebo N = 29	Collagen N = 31	Placebo N = 29	Collagen N = 29	Placebo N = 29	Collagen N = 28	Placebo N = 27	Collagen N = 27	Placebo N = 23	Collagen N = 26
Responders	1(3.4)	4 (12.9)	3 (10.3)	6 (20.67	3 (10.3)	8 (28.4)	3 (11.1)	11 (40.7)	5 (21.7)	10 (38.4)
Non-responders	28 (96.5)	27 (87.0)	26 (89.6)	26 (79.3)	26 (89.6)	20 (71.4)	24 (88.8)	16 (59.2)	18 (78.2)	16 (61.5)
p*	N.S.		N.S.		N.S.		0.02		N.S.	

* Fisher's tailed exact test

Table V. Evaluation of treatment efficacy by the physicians and patients at the last follow-up visit.

	Physician				Patients			
	ITT (N = 60)		PP (N = 49)		ITT (N = 60)		PP (N = 49)	
	Placebo (N = 29)	Collagen (N = 31)	Placebo (N = 23)	Collagen (N = 26)	Placebo (N = 29)	Collagen (N = 31)	Placebo (N = 23)	Collagen (N = 49)
Nil	18 (62.1%)	12 (38.7%)	13 (56.5%)	8 (30.8%)	17 (58.7%)	13 (42.0%)	12 (52.1%)	10 (38.5%)
Poor	6 (20.7%)	8 (25.8%)	6 (26.1%)	7(26.9%)	1 (3.4%)	2 (6.4%)	1 (4.4%)	1 (3.8%)
Fair	2 (6.9%)	2 (6.4%)	1 (4.4%)	2 (7.7%)	6 (20.7%)	6 (19.3%)	6 (26.1%)	5 (19.3%)
Good	3 (10.3%)	8 (25.8%)	3 (13.0%)	8 (30.8%)	4 (13.8%)	9 (29.1%)	3 (13.0%)	9 (34.6%)
Excellent	0 (0.0%)	1 (3.2%)	0 (0.0%)	1 (3.8%)	1 (3.4%)	1 (3.2%)	1 (4.4%)	1 (3.8%)
p	0.045		0.036		0.177		0.197	

ITT: Intent to treat; PP: Per protocol.

Table VI. Baseline clinical and laboratory characteristics of responders versus non-responders among the collagen-treated patients after 6 months of treatment.

Characteristics	Responders (N = 10)	Non-responders (N = 16)	P
RF positive [no. (%)]			
Sex (women/men)	9/1	16/0	N.S.
Radiological lesions [no. (%)]	9 (90)	16 (100)	N.S.
Collagen II antibody [no. positive (%)]	1 (10)	4 (25)	N.S.
ESR mm/1st hr (mean ± SD)	51.6 ± 24.7	58.6 ± 22.2	N.S.*
Desease duration in months (mean ± SD)	90.8 ± 70.5	88.7 ± 48.9	N.S.*

Fisher's tailed exact test; * Student's unpaired T-test

Efficacy variables

The results of the analyses on the efficacy variables are summarized in Table III for both the ITT and PP groups. In the ITT population, no statistically significant differences were recorded between the collagen- and placebo-treated patients. In the PP population the number of swollen and tender joints at the end of the study was significantly decreased compared to the placebo group. The only two clinical remissions [according to ARA criteria (39)] observed were from the collagen group, one patient after 2 months and one after 3 months of treatment. In one patient clinical remission still persists 6 months after treatment discontinuation, while in the second patient the disease slowly relapsed. Oral collagen was significantly more efficacious than placebo in the physician's assessment for both the ITT and PP populations ($p = 0.045$ and $p = 0.036$ respectively by the Wilcoxon rank sum test), but not in the patient's assessment (Table V). Finally, in the collagen group no significant differences in the clinical and laboratory variables were found between the responders and the non-responders (Table VI).

*Correlation of antibodies to native collagen with clinical and laboratory value*s

At baseline 8 patients (13.3%) tested positive for antibodies to CII, 5 in the collagen group (16.1%) and 3 in the placebo group (10.3%). No significant differences between antibody-positive and antibody-negative patients were found for the mean disease duration (84.71± 71.85 vs 99.0±42.91 mos), steroid dosage (4.52 ±3.17 vs 5.31±4.32 mg/day), AIMS score (21.21 ± 11.01 vs 26.25 ± 9.91), ESR (45.48 ± 25.96 vs 48.00 ± 29.35 mm/1st hr), or CRP (2.20±2.57 vs 3.16±2.79 mg/dl). The rate of responders in the collagen group was not significantly related to the presence of antibodies to CII at baseline either by Fisher's exact test ($P = 0.368$) or after correction for potentially confounding variables (disease duration, steroid dosage, baseline AIMS score, ESR, CRP) in a logistic regression model ($p = 0.347$ by the Wald test). Furthermore, endpoint changes from baseline values were not significantly different between the 2 groups (AIMS: -7.00±6.6 vs -5.00±11.8; VES: +3.20±8.8 vs +1.15 ±17.9; CRP: +1.21±1.8 vs +0.60±2.1).

Conclusions

The potential role of oral type II collagen administration in the treatment of rheumatoid arthritis is still unknown. While collagen-treated patients showed a tendency toward improvement in three out of the four double blind, placebo controlled studies published thus far (25-28), the efficacy of this treatment has not been conclusively established (40). Uncertainty still exists concerning the optimal dosage of CII and the patient characteristics associated with a better clinical response.

In this study, a single dosage of CII was tested in order to limit data dispersion. Dosage selection was based on recent clinical data (27) supporting experimental evidence (23) that a lower dosage of oral antigen might be more efficacious in the induction of oral tolerance. The selected dosage (250 mg/day) is similar to the lower dosage tested in the studies by Trentham (25) and Barnett (27). Furthermore, chicken collagen was prepared by the same technique adopted in these studies.

Overall, the results of our study are in good agreement with the published data. A higher response rate by the ACR criteria was observed in the collagen group compared to the placebo group, without reaching statistical significance, in concordance with the data reported by Seiper (26) and by Barnett (27). Only in the latter study was the response rate significantly higher in collagen-treated patients, as evaluated by the Paulus criteria, and this was limited to patients treated with the lower dosage (200 mg). Seiper's results differ qualitatively in that a higher (but still not statistically significantly different) rate of responders was observed in patients treated with higher doses (10 mg) of CII.

As regards the efficacy variables, neither Sieper nor Barnett reported significant differences between their collagen and placebo-treated patients. In our study several endpoint variables showed a greater tendency toward improvement in the collagen-treated patients as compared to the placebo-treated patients, but no statistically significant difference was recorded by intention to treat analysis. According to the ITT, no clinical variable was significantly reduced in the collagen group versus the placebo group at the final follow-up (Table III). This finding is in contrast with the physician (bias toward objective findings) and patient (bias toward subjective symptoms) assessments of treatment efficacy (Table IV). Moreover, a significant reduction in the number of swollen and tender joints with oral collagen treatment was reported by Trentham (25).

Several factors may have contributed to the different results reported by different authors, such as the origin of the collagen, the length of the treatment, the mean duration of the illness, and the different dosage of CII administered.

Concerning the type of CII, Sieper employed bovine collagen, and therefore the results of his study cannot be compared directly with other studies where chicken collagen was employed. Concerning the duration of treatment, a progressive increase in the number of responders to oral collagen was reported during 12 weeks of follow-up (26), leading Sieper to hypothesise that significant differences might emerge only after a longer follow-up. In our study, substantial increases in the cumulative percentage of responders were observed up to the 16th week of treatment, being higher than in the Sieper study (35.5% vs 21.6%) at the 12th week, further increasing at the 16th week (45.2%) and showing no significant increase at the 24th week (48.4%). As for the duration of illness, it has been suggested that the response to CII treatment might be more pronounced in patients with advanced, cartilage-destroying disease, and in patients with antibodies to CII (26-27).

We did not find a significant relationship between the response rate to treatment and the presence of IgG CII antibodies. The variation in antibody titer during the study was unrelated to changes in disease activity. The overall percentage of patients with CII IgG antibodies in our study (8 pts, 13.3%) is similar to the percentage reported by Barnett (18%). In the collagen group, only 1 out of 5 patients presenting CII antibodies at baseline responded to treatment, without showing significant changes in the antibody level, and the only case of disappearance of CII antibodies was in a non-responder. Therefore, we were unable to confirm the findings of previous studies reporting correlations between the presence of antibodies to CII and the likelihood of response to treatment (29), or between decreases of antibody titers and the response to treatment (41).

Treatment with oral CII is usually considered to be safe, and no major toxicity has been reported. The non-appearance of anti-CII antibodies during treatment was reported as evidence against a sensitization to the fed antigen (25). However, in our study 3 patients showed a definite worsening of the subjective and objective indices of disease activity during treatment, the number of swollen and tender joints increasing ≥ 40% compared to baseline values. Anti-CII antibodies were absent at baseline in these patients, and the worsening of their disease was not accompanied by increasing antibody levels. In all 3 patients disease activity returned to baseline values within one month after their treatment was interrupted, without any changes in their pharmacological treatment.

Our finding of a definite worsening of disease in a subset of patients treated with oral CII should be considered as anedoctal at this time. Nevertheless, a reanalysis of the available clinical data by other authors might be justified.

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