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CO-N reaction – a new serological activity index – on Wegener's granulomatosis

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Abstract

Cordyceps ophioglossoides is one of the Japanese old 'Kanpoh' drugs used for metrorrhagia as a decoction. We found that CO–N, a galactosaminoglycan from *Cordyceps ophioglossoides*, reacted with sera from patients with some collagen diseases. By using CO–N, we made a new serological activity index (CO–N reaction). In the present study, we investigated CO–N reaction on patients with Wegener's granulomatosis (WG). The aggregation titres of CO–N (CO–N numbers) displayed a possible correlation with their clinical activity. CO–N reaction might also serve an important role in supporting the diagnosis of active WG and in helping to assess the degree of disease activity. The purpose of this report is to introduce this new serological activity index for Wegener's granulomatosis.

Key words: Wegener's granulomatosis; Serodiagnosis; Collagen diseases

Introduction

In Japan, there are many traditional drugs which are called 'Kanpoh' (Chinese ethical drugs). *Cordyceps ophioglossoides* (Fig. 1) is a kind of *Ascomycetes* fungus that has been used for metrorrhagia as a decoction for hundreds of years. Since 1986 Ohmori and his colleagues have investigated its anti-cancer effects. They have reported that the protein-bound anti-tumour polysaccharide SN–C obtained from *Cordyceps ophioglossoides* can be fractionated into two different types of anti-tumour polysaccharide, CO–1 and CO–N, after sonic treatment [Ohmori *et al.*, 1986; Ohmori *et al.*, 1988 (a,b); Ohmori *et al.*, 1989]. CO–N has shown a host-mediated anti-tumour effect, whereas CO–1 appeared to have direct cytotoxicity [Ohmori *et al.*, 1988 (b); Ohmori *et al.*, 1989]. CO–1 is a β-(1–3)-D-glucan with (1–6) linked side chains and has a helical higher structure [Yamada *et al.*, 1984 (a)]. CO–N is a protein bound α-(1–4) galactosaminoglycan [Yamada *et al.*, 1984 (b); Kawaguchi *et al.*, 1986]. As an anti-tumour agent, both of them are not useful because of their severe side effects. However, CO–N aggregates with the sera from patients with advanced malignant diseases and also aggregates with the sera of some collagen diseases. By using CO–N, we made a new serological activity index (CO–N reaction). We have already reported that the aggregation titre of CO–N with the sera of rheumatoid arthritis (RA) corresponded well with the Lansbury indexes (Ikeda *et al.*, 1989).

In the present study, we evaluated its usefulness as a serological activity index of Wegener's granulomatosis (WG). For several years, the determination of anti-neutrophil cytoplasmic antibodies (ANCA) has been shown to serve an important role in supporting the diagnosis of active WG and helping to assess the degree of disease activity (Rasmussen *et al.*, 1988). It might be helpful for our clinic to introduce this newly developed laboratory guide as a serological activity index of collagen diseases including WG.

Materials and methods

Patients

Blood samples were obtained from 12 patients with Wegener's granulomatosis (WG) from several hospitals in the Tokyo, Osaka and Kyushu areas (Table I). All cases were Japanese (Mongolian) and diagnosed according to the criteria of the WG research group of the Ministry of Health and Welfare. Their mean age was 37.2 years, with a range of 18 to 52 years. There were seven males and five females. Five cases were clinically stable. Their clinical signs and symptoms included sinusitis, fever, otitis, rhinitis or nasal symptoms in all cases, subglottic stenosis in eight cases, renal failure in four cases, and pulmonary infiltrate in five cases. Follow-up years ranged from 0 to 10 years.

Patients were treated with prednisolone, initially 0.5–2.0 mg/kg body weight, combined with cyclophosphamide 1.0–4.0mg/kg body weight. In the acute phase of illness, these immunosuppressive drugs were increased. These drugs were gradually tapered off and discontinued.

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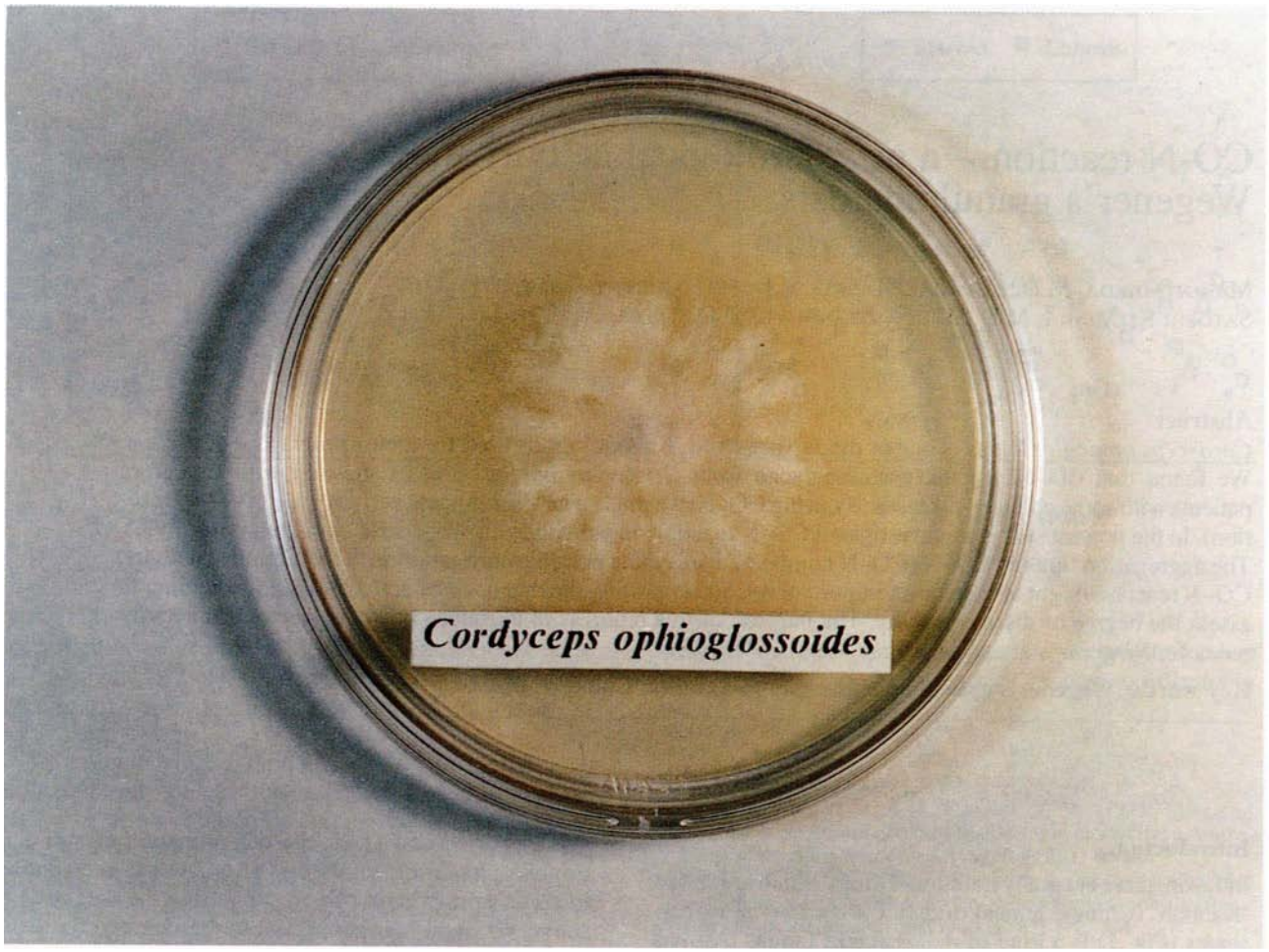

Fig. 1
Cultivated *Cordyceps ophioglossoides*. It is a kind of *Ascomycetes* fungus.

Immunosuppressive medications were generally continued for more than one year after remission.

CO–N Reaction

CO–N was received from the Research Institute of Life Science, Snow Brand Milk Products Co. Ltd. (Tochigi, Japan). The precise isolation method of CO–N was described in our former reports [Ohmori *et al.*, 1988 (b); Ohmori *et al.*, 1989].

Cordyceps ophioglossoides culture was centrifuged to remove mycelia. Charcoal was added to the filtrate. The resulting precipitate (SN–C) was collected and SN–C was obtained by drying *in vacuo*. SN–C was resuspended in acetic acid to a concentration of 0.5 per cent, sonicated at 4°C and then centrifuged. The supernatant solution was incubated at 60°C for 30 minutes and then centrifuged to remove a white precipitate (CO–1) and then 10 per cent aqueous ammonium hydroxide was added to make a pH of 6.0–9.0. The resulting precipitate was redissolved in acetic acid and reprecipitated with ammonium hydroxide. The precipitate was washed with water to achieve a purified galactosaminoglycan (CO–N).

CO–N reaction was carried out according to the method described in our former reports [Kawaguchi *et al.*, 1986 (a); Ikeda *et al.*, 1989] (see Fig. 2). CO–N was prepared as 2.4 mg/ml N-galactosamine solution in the succinate buffer and used after incubation at 50°C for 15 minutes. One ml of serum was diluted 4.5 times with succinate buffer. The diluted serum was centrifuged at 300 × **g** for 15 minutes and filtrated through a 0.22 μm filter. The serum was incubated at 50°C for 10 minutes and added to 0.27 ml of the prepared CO–N reagent. The aggregation of serum and CO–N was measured at 900 nm by a spectrophotometer after incubation at 50°C for 20 minutes. CO–N reaction titre (CO–N number) was obtained by the following formula: CO–N number = value of optical density × 1000. We analysed the CO–N number of 150 healthy subjects and their average was 27 ± 20 (mean ± SD). Based upon three standard deviations, we selected less than 90 as the normal range of the CO–N number.

Results

Table I shows the CO–N number of our subjects, which ranged from 20 to 1560. CO–N numbers of the inactive group ranged from 20 to 120 with the average value being 63.0 ± 34.3 (mean ± SD). Those of the active group ranged from 230 to 1560 and the average was 789.9 ± 544.3 (mean ±SD), which is higher than the inactive group ($p<0.05$). We obtained the sera of two patients before therapy, (CO–N numbers were 1560 and 1530). Six months after the initiation of therapy, their numbers were decreased to 1400 and 460 respectively. Since we do not have an appropriate index for more pre-

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TABLE I

CLINICAL DATA AND CO–N NUMBERS AT THE TIME OF INVESTIGATION OF 12 PATIENTS WITH WEGENER'S GRANULOMATOSIS

Patients	Sex	Age (years)	Disease duration (years)	Disease activity	Organ involvement	Treatment before investigation	CO–N number
1. Y.S.	M	46	0 0.5	Active Active	ENTLK ENTLKEy	None Ps 60 mg/day Cy 200 mg/day	1560 1400
2. T.I.	M	40	0 0.5	Active Active	NLK NLK	None Ps 30 mg/day Cy 100 mg/day	1530 460
3. K.M.	F	40	8	Active	NK	Ps 30 mg/day	140
4. K.M.	F	50	2	Active	NTL	Ps 30 mg/day	400
5. H.T.	M	28	3	Active	NTKEy	Ps 30 mg/day Cy 50 mg/day	230
6. O.N.	M	35	3	Active	NTLK	Ps 30 mg/day Cy 50 mg/day	1010
7. T.M.	M	40	3	Active	NTL	Ps 30 mg/day Cy 50 mg/day	460
8. S.N.	M	18	3	Inactive	NT	Ps 10 mg/day	45
9. T.O.	M	52	8	Inactive	N	None	50
10. T.S.	F	27	6	Inactive	N	Ps 5 mg/day	80
11. Y.K.	F	29	1	Inactive	NT	Ps 5 mg/day	120
12. M.Y.	M	41	10	Inactive	NTEyB	Ps 10 mg/day	20

M = male; F = female; E = ears; N = nose; T = larynx and trachea (including subglottic stenosis); L = lung; K = kidneys; Ey = eyes; B = brain; Ps = prednisolone; Cy = cyclophosphamide.

cise statistical evaluation of clinical activity of WG, such as the Lansbury index for RA, we were unable to do a more precise analysis, such as coefficient of correlation between their clinical activity and their CO–N number.

Discussion

We have been investigating the CO–N reaction on the sera of other collagen diseases, such as polyarteritis nodosa (PN), thromboangitis obliterans (Buerger's disease), systemic lupus erythematosis (SLE), aortitis syndrome (Takayasu's disease) and rheumatoid arthritis (RA). Based on these observations, it seems that the CO–N reaction may be useful for the activity index of PN, Buerger's disease and RA (Ikeda *et al.*, 1989). For example, CO–N numbers of RA corresponded well to Lansbury indexes (r = 0.8) (Ikeda *et al.*, 1989). One of the other benefits of CO–N reaction may be a relative stability which corresponds to a patient's clinical activity, different from the erythrocyte sedimentation rate (ESR) which can be influenced by medication. If a clinical activity index on WG including general condition, laboratory findings, CO–N number etc. could be developed, it would be advantagous in the therapy of WG.

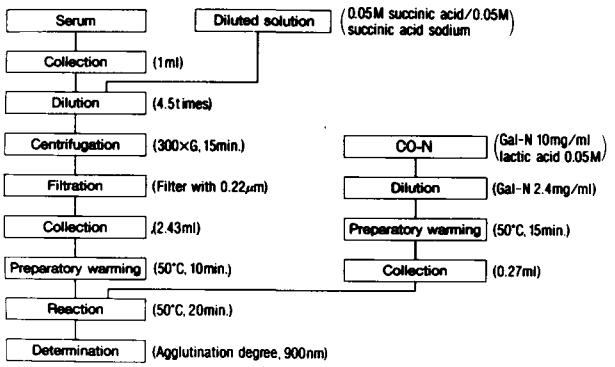

Fig. 2

The procedure for CO–N assay.

Kawaguchi *et al.* (1986 b) reported that the aggregate of CO–N reaction on cancer patients consisted of albumin, haptoglobin, alpha$_1$-antitrypsin, alpha$_1$-acidglycoprotein, haemopexin, CO–N itself and other unspecified substances. All the four proteins except albumin are known as acute phase reactants produced in an inflammatory process. In addition, they reported that this reaction was induced by the electrical aggregation between CO–N and acidic compound in sera of cancer patients but the aggregation was not observed when N-acetylated CO–N, chitosan, or diethylaminoethyl-dextran instead of CO–N were added to the serum. Therefore, they speculated that this reaction required the protein-portion of CO–N to have a molecular weight greater than 10000. A precise analysis in WG was not done because of the difficulty of obtaining sera. The method for assay of CO–N is too complicated at this time for clinical use. A more simplified method needs to be developed.

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