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Disodium N-(2-Carboxyphenyl)-4-chloroanthranilate: Enhancement of Immune Response in Mice[1)]

Effects of disodium N-(2-carboxyphenyl)-4-chloroanthranilate, which was newly synthesized, on the immune response in mice were investigated. An oral administration of this compound increased the production of splenic plaque forming cells against both a thymus-dependent (sheep red blood cells) and a thymus-independent antigen (bacterial lipopolysaccharide).

Keywords——new diphenylamine; immune response; adjuvant activity; splenic plaque forming cell; sheep red blood cell; bacterial lipopolysaccharide

During investigations of biological activities of a new series of diphenylamine derivatives, disodium N-(2-carboxyphenyl)-4-chloroanthranilate (CCA) was found to have an immunoenhancing activity. In this article, we will describe that an oral administration of CCA stimulates immune response against sheep red blood cells (SRBC) and bacterial lipopolysaccharide in mice.

Five to 6 weeks old male inbred mice of IV CS strain, maintained in our laboratory, were used in all the experiments.

Mice were intravenously injected with 1×10^7 SRBC (Toshiba Kagaku Kogyo). Number of hemolytic plaque forming cells (PFC) in the spleen was determined 48-hr after immunization, according to the method of Cunningham, *et al.*[2)]

As shown in Table I, CCA increased the number of splenic PFC at a dose of 10, 50, or 100 mg/kg, but did not at 2 mg/kg, when orally administered 24-hr after immunization.

Table I. Effect of Oral Administration of Various Doses of CCA on Anti-SRBC PFC Response in Mice

Dose (mg/kg)	No. of mice	PFC/spleen	PFC/10^8 spleen cells
0	16	298±34	371±43
2	7	218±40	357±71
10	18	437±49[a)]	609±82[a)]
50	15	422±44[a)]	577±75[a)]
100	6	410±71	472±82
Non-immunized	6	64±20[b)]	103±34[c)]

Mice were intravenously injected with 1×10^7 SRBC. Fourty-eight hours later, splenic PFC number was determined. CCA was orally administered 24 hr after immunization. Value indicates mean ± S.E.
Statistically significant difference from controls; *a*) $p<0.025$, *b*) $p<0.005$, *c*) $p<0.05$.

Table II shows a relationship between the effect and the timing of administration of CCA. An oral dose of 50 mg/kg of CCA showed an enhancing effect when given simultaneously with, 6 or 24-hr after immunization, but did not when administered 24-hr before.

Subsequently, the effect of CCA on primary immune response against thymus-independent antigen, E. coli 055: B5 lipopolysaccharide (LPS) (Difco Lab.), was investigated. Mice were intraperitoneally injected with 5 μg of LPS, and killed 4 days later for the splenic PFC

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2) A.J. Cunningham and A. Szenberg, *Immunology*, **14**, 599 (1968).

Table II. Ralationship between Enhancing Activity of CCA on Immune Response to SRBC and Timing of Administration

Timing of administration	No. of mice	PFC/spleen	PFC/10^8 spleen cells
Control	26	297±29	368±32
24 hr before	21	283±30	360±47
Simultaneous	19	455±54[a]	637±85[b]
6 hr after	19	422±61[a]	561±74[a]
24 hr after	21	420±49[a]	614±61[c]
Non-immunized	8	60±17[c]	96±31[c]

Mice were intravenously injected with 1×10^7 SRBC. PFC assay was performed 48 hr a ter immunization. CCA of a dose of 50 mg/kg was orally administered at various intervals from antigen injection. Value indicates mean ± S.E.
Statistically significant different from controls: *a*) $p<0.05$, *b*) $p<0.005$, *c*) $p<0.001$.

assay. LPS-coated SRBC were prepared according to the chromium-chloride coupling method.[3]

As shown in Table III, an oral administration of CCA 24-hr after antigen injection resulted in an increase of splenic PFC number at a dose of 10 or 50 mg/kg, in comparison with control.

Table III. Effect of CCA on Primary Immune Response to LPS in Mice

Treatment	No. of mice	PFC/spleen	PFC/10^6 spleen cells
Control	10	90530± 4978	612±54
CCA 10 mg/kg	10	112940± 6304[a]	796±69[a]
CCA 50 mg/kg	9	139955±11444[b]	941±69[b]

Mice were intraperitoneally injected with 5 μg of LPS. CCA was orally administered 24-hr, and splenic PFC assay was performed 4 days after immunization. Value indicates mean ± S.E.
Statistically significant difference from controls: *a*) $p<0.05$, *b*) $p<0.005$.

From these results, we concluded that CCA stimulated the immune responses against both a thymus-dependent and a thymus-independent antigen. Previously reported immunostimulating substances with relatively low molecular weight, are antibiotics such as diketocoriolin,[4] amphotericin B,[5] or nystatin,[6] and vitamin A[7] or E.[8] Tetramisole is well known as the chemically synthetic immunostimulant, which is used for the immunotherapy of cancer and infectious diseases in mice,[9] and rheumatoid arthritis in human.[10] CCA is a newly

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synthesized simple chemical, and has a new basal skeleton of chemical structure which is quite different from that of the known immunostimulants hitherto reported. Investigations for the medicinal application of this chemical are now undergoing in some experimental models for human diseases.

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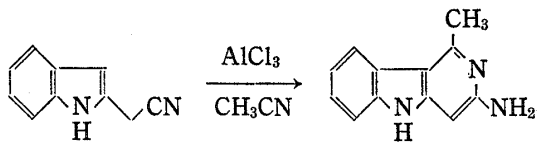
Synthesis of a Mutagenic Principle isolated from Tryptophan Pyrolysate

A potent mutagen, 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole isolated from tryptophan pyrolysate was synthesized.

Keywords——tryptophan pyrolysate; mutagen; amino-5H-pyrido[4,3-*b*]indole; γ-carboline; tar constituent

In the course of our study[1] on biologically active tar constituents, we isolated two potent mutagens, named Trp-P-1 and Trp-P-2, from tryptophan pyrolysate.[2] The structure of Trp-P-1 was determined by X-ray crystallography as 3-amino-1,4-dimethyl-5H-pyrido-[4,3-*b*]indole, and that of Trp-P-2 was deduced as 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole mainly by a spectral comparison with Trp-P-1. In the present communication, we wish to report a synthesis of Trp-P-2 in a quite simple way.



To a solution of 2-cyanomethylindole[3] (500 mg) in acetonitrile (10 ml) was added aluminum trichloride (5.0 g), and the mixture was refluxed for 12 hr. After addition of water, neutral and acidic products were removed by ether, and the basic fraction was extracted, after basification by solid K_2CO_3, with methylene chloride and then ethyl acetate. The crude basic product (173 mg) was dissolved in a small amount of methanol and few mililiters of ethyl acetate, and a drop of acetic acid was added. The crystalline precipitates (67 mg) were collected and recrystallized from ethyl acetate containing methanol to give the acetate, plates, mp 250—260° (the crystal form changed at about 220°.) The chromatography (silica gel, ethyl acetate–ethanol) of the mother liquor yielded an additional crystalline (54 mg). The identification with the authentic sample (Trp-P-2 acetate) was performed by the com-

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