Effects of Oral Administration of D-Penicillamine on T- and B-Lymphocytes in Peripheral Blood of Rheumatoid Patients

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KOSAKA, S. Effects of Oral Administration of D-Penicillamine on T- and B-Lymphocytes in Peripheral Blood of Rheumatoid Patients. Tohoku J. exp. Med., 1979, 129 (3), 233-239 — The effects of D-penicillamine on T-lymphocytes (rosette forming cells) and B-lymphocytes (surface IgG- and surface IgM-bearing cells) in peripheral blood of 13 patients with classical or definite rheumatoid arthritis were investigated at 4 weeks, 12 weeks and 24 weeks of treatment. At the same time, the determination of the concentrations of serum immunoglobulins and RA tests were carried out, and the rheumatoid activity index was calculated. The administration of D-penicillamine caused a gradual increase in the percentage of T-lymphocytes and a concomitant decrease in the percentage of B-lymphocytes with time. It was found that the tendency toward reduction of the percentage of surface IgM-bearing lymphocytes was more remarkable and accelerated than that of surface IgG-bearing lymphocytes. A decrease in the concentrations of serum IgM and IgG was observed in association with these findings. The agglutination in RA-test became significantly weaker in about half of the cases. Also the rheumatoid activity index became gradually smaller after treatment. The marked decrease in the percentage of B-lymphocytes and in the concentration of serum immunoglobulins may be related to the inhibition of the production of abnormal immunoglobulins. D-penicillamine; T-lymphocytes; B-lymphocytes; serum immunoglobulins; rheumatoid factor

Penicillamine was used in the treatment of rheumatoid arthritis first by Jaffe in 1964 and 1970. In 1973 the multicentre controlled trial (Multicentre Trial Group 1973) confirmed the effectiveness of D-penicillamine in the treatment of active rheumatoid arthritis (RA). Subsequently the effects of D-penicillamine were shown to be similar to those of gold (Huskinson et al. 1974).

In a previous communication, the in vitro penicillamine-treated serum of the patients with RA showed a significant fall in titers of rheumatoid factor and also depolymerization of the macroglobulin containing the rheumatoid factor as evidenced by the ultracentrifugal patterns. These findings suggested the possibility that the immune response is suppressed by penicillamine (Kosaka 1969).

The lymphocytes, which play a primary role in immunologic response, is divided into B-lymphocytes related to humoral immunity and T-lymphocytes related to cell-mediated immunity. Previously the author demonstrated a

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significant increase in percentage of B-lymphocytes and a decrease in percentage of T-lymphocytes in the peripheral blood of the patients with RA in comparison with the healthy subjects (Kosaka 1977).

The question whether the primary action of penicillamine in the patients with RA is exerted on B or T-lymphocytes has remained unanswered. Thus the present study was designed to clarify the effect of penicillamine on the proportion of circulating B- and T-lymphocytes. Furthermore, the serum immunoglobulins, rheumatoid factor and clinically rheumatoid activity index during the course of treatment with d-penicillamine were evaluated.

Patients and Methods

Patients

D-Penicillamine, Metalacaptase supplied by Taisho Pharmaceutical Co., was administered orally to 13 adult patients (2 males, 11 females) with classical or definite RA in a daily dose of 300 mg for the first 4 weeks and then in a daily dose of 600 mg during the period of treatment. All the patients received 30 mg of pyridoxine hydrochloride per day in conjunction with d-Penicillamine. Most of the patients also received non-steroid anti-inflammatory drugs such as indomethacin.

The determination of T- and B-lymphocytes from the peripheral blood of the patients was carried out before treatment and at 4 weeks, 12 weeks and 24 weeks of treatment. At each determination, the levels of serum immunoglobulins (IgG and IgM) were determined and RA tests were carried out. The rheumatoid activity index with an aim for the evaluation of the clinical effects was calculated at 4-weeks intervals until the 24th week and then at intervals of 1 month.

Separation of lymphocytes

Peripheral blood lymphocytes were isolated from the fresh heparinized blood from healthy donors and patients, respectively. The lymphocytes for experiments were prepared using a method of Ficoll-Isopaque centrifugation of Jondal et al. (1972).

Each blood sample was laid over an equal volume of a mixture of 24 parts of 9% Ficoll and 10 parts of Isopaque in 10-ml test tubes and was centrifuged for 30 min at room temperature. The lymphocyte-rich fraction thus obtained from the Ficoll-Isopaque-plasma interface was aspirated and washed three times with phosphate-buffered saline (PBS) of pH 7.2.

T-lymphocyte marker

The T-lymphocytes were identified by the criterion of spontaneous rosette formation with sheep red blood cells (SRBC) according to a modification of the method of Jondal et al. (1972). The lymphocytes (1.2 × 10⁶) in 0.3 ml of medium TC 199 were mixed with an equal volume of a 0.5% suspension of washed SRBC in PBS containing 10% of fetal calf serum absorbed previously with SRBC. The resulting mixture was centrifuged at 1,000 rpm for 5 min and then kept at room temperature for 1 hr. The pellet of the cells was resuspended carefully. Then an aliquot of the resuspended cell layer was withdrawn and the percentage of the rosette-forming lymphocytes (with a minimum of 5 attached SRBC) was counted microscopically.

B-lymphocyte marker

The B-lymphocytes were identified by the presence of surface immunoglobulins according to a modification of the method of Papamichail et al. (1971).

Approximately 1–2 × 10⁶ lymphocytes were suspended in 0.05 ml of a 1:10 dilution of fluorescein-conjugated rabbit anti-human IgG (λ-chain) or anti-human IgM (μ-chain)
Penicillamine on Lymphocytes of Rheumatoid Patients

(Behring-Werke AG, Marburg-Lahn). The incubation at 37°C lasted for 30 min. The cells were then washed three times with PBS and resuspended in 0.05 ml of PBS containing 50% of glycerol. A drop of the cell suspension was placed on a slide and overlaid with a coverslip.

The membrane immunofluorescence was examined with an Olympus FLM fluorescence microscope. The number of immunofluorescent cells was counted and then the total number of cells in the same field was counted without a primary filter. At least 500 cells were examined from each preparation.

**Immunoglobulins and rheumatoid factor**

The serum concentration of IgG and IgM respectively was measured with a Partigen plate-kit (Behring-Werke) by the technique of single radial immunodiffusion. In the detection of rheumatoid factor, the commercially available RA-test kit (Hyland) was used. In all the patients the rheumatoid factor was positive before treatment.

**Measurements of percent reduction of B-lymphocytes or serum immunoglobulins, and percent increment of T-lymphocytes**

The percent reduction and percent increment were calculated by the following formula:

\[
\text{Percent reduction or increment} = \frac{(B/A - 1) \times 100}{A}
\]

A: percent before treatment

B: percent after treatment

**Rheumatoid activity index (%)**

The rheumatoid activity index (Lansbury 1958) was calculated on the basis of the following findings: 1) duration of morning stiffness, 2) grip strength, 3) number of clinically active, painful joints, 4) ESR (1 hr value by Westergren method).

**Results**

The percentage of T-lymphocytes was found to be increased in almost all the cases. There was a gradual increase of the percentage of T-lymphocytes with time of treatment. The mean of percent increment was 17% at 4 weeks, 23% at 12 weeks and 37% at 24 weeks of treatment in comparison with the percentage of T-lymphocytes before treatment (Fig. 1).

As to the percentage of B-lymphocytes, there was a decrease in percentage of both surface IgG-bearing lymphocytes and surface IgM bearing lymphocytes. It was found that there was a tendency for the number of B-lymphocytes to become

![Fig. 1. Effect of D-penicillamine on T-lymphocytes in patients with RA.](image-url)
normal by treatment. The mean of percent reduction of the surface IgG-bearing lymphocytes was 6% at 4 weeks, 16% at 12 weeks and 19% at 24 weeks of treatment. On the other hand, the mean of percent reduction of surface IgM-bearing lymphocytes was 14% at 4 weeks, 23% at 12 weeks and 23% at 24 weeks of treatment. Thus the tendency toward reduction of the surface IgM-bearing lymphocytes was more remarkable and accelerated than that of the surface IgG-bearing lymphocytes (Fig. 2).

The concentration of serum IgG was found to be decreased in almost all the cases. The mean of the percent reduction was 15% at 4 weeks, 27% at 12 weeks and 18% at 24 weeks of treatment. Also the concentration of serum IgM was found to be decreased in almost all of the cases. The mean of the percent reduction was 17% at 4 weeks, 26% at 12 weeks and 42% at 24 weeks of treatment. Thus, the rate of decrease of IgM was more remarkable than that of IgG (Fig. 3).

The results of the RA-test after treatment became negative in 3 of 13 cases. In 3 cases the results of the RA-test became less strongly positive. The results of the RA-test have remained unchanged in 7 of 13 cases. These results indicate that
the degree of agglutination of RA-test became weaker in about half of the cases investigated.

The rheumatoid activity index on an average became smaller with time in almost all of the cases. However, when the dosis of D-penicillamine was decreased because of the improvement of clinical symptoms after 12 months of treatment, there was a transient elevation of rheumatoid activity index (Fig. 4).

![Fig. 4. Effect of D-penicillamine therapy on rheumatoid activity index in patients with RA.](image)

**DISCUSSION**

The rationale for the application of D-penicillamine in the treatment of RA is the observation that this drug acted as a dissociative agent on disulfide bonds of the rheumatoid factor (Jaffe 1962; Kosaka 1969, 1970).

Then the effect in vitro of penicillamine on cultured human lymphocytes which were stimulated with phytohaemagglutinin was investigated by Roath and Wills (1974). The drug in this system proved to be an effective inhibitor of lymphocyte transformation and, presumably, of immunoglobulin synthesis. They also demonstrated that the effect of D-penicillamine seemed to be dose-related and that D-penicillamine had no cytotoxicity. Thus the effect on the function of lymphocytes was interpreted as a clear reflection of the immunosuppressive effects of D-penicillamine. Similar results were reported by Schumacher et al. (1975). In their experiments the metabolism of lymphocytes stimulated by different agents was dose-dependently inhibited by D-penicillamine. The influence of D-penicillamine on the metabolism of lymphocyte, once the stimulatory effect had been established, decreased with time. When preincubated with D-penicillamine, the inhibitory effect on metabolism of lymphocytes was dose-dependent. The inhibitory effect observed in the mixed lymphocyte cultures was much less weaker after preincubation of the stimulator cells than of the responder cells. The inhibitory effect of D-penicillamine on the growth of HeLa-cells was also dose-dependent.

Despite these experimental evidences, Huskisson and Berry (1974) found no evidence of any in vivo change which could be correlated with the effect of D-penicillamine on T-lymphocytes. They found no suppression of the responsiveness
to intradermal injection of tuberculin or keyhole limpet hemocyanin in their patients who were treated with D-penicillamine. On the other hand, it was claimed that in the treatment of RA the effect of penicillamine was due to the suppressive effect on lymphocytes (Brandt and Svensson 1975). The number of lymphocyte was significantly smaller in patients treated with penicillamine with a reduction of T-lymphocytes and other lymphocytes.

It is to be emphasized that the present study dealt with the effect in vivo of D-penicillamine on the function of T- and B-lymphocytes. In a previous communication, the present author demonstrated a significant decrease in percentage of T-lymphocytes and an increase of percentage of B-lymphocytes in the peripheral blood of patients with RA in comparison with the healthy persons (Kosaka 1977). In the present experiment, the administration of D-penicillamine caused an increase of the percentage of T-lymphocytes and a decrease of the percentage of B-lymphocytes in the peripheral blood of the patients. It was found also that the decrease of the percentage of B-lymphocytes was associated with a decrease in serum concentration of immunoglobulins, particularly of IgM. Thus there was a tendency for the percentage of B-lymphocytes and the concentration of serum IgM to return to the normal level. The change in the concentrations of serum immunoglobulins in the present findings is in accordance with the reduction of the level of serum IgM and IgG that, as already reported, occurs in RA-patients when treated with penicillamine (Bluestone and Goldberg 1973).

Presumably D-penicillamine acts not only on humoral antibody, but also on antibody-producing lymphocytes, and thus inhibits the production of abnormal immunoglobulins, especially that of IgM.

When treated with penicillamine, the degree of agglutination in RA tests became weaker. The weaker agglutination seemed to be related to the reduction of serum IgM and with the dissociation of rheumatoid factors in vitro in the previous report of the present author (Kosaka 1969).

Many investigators agree that D-penicillamine is slow-acting on rheumatic activity, whereas corticosteroids and anti-inflammatory agents are rapidly-acting on clinical symptoms of RA. Similar results were obtained also in this study. A significant improvement of the rheumatoid activity index was first seen at the end of 4 weeks and the improvement observed became more apparent with time during treatment. The association of the improvements in clinical findings with the return to normal of the percentage of T-, B-lymphocytes and the levels of serum immunoglobulins after treatment is of particular interest.

On the other hand, the occurrence of side effects was a problem in the clinical use of D-penicillamine. In this study, skin eruptions or pruritus were observed in 3 cases and gastrointestinal disturbances in 2 cases. But most of these symptoms were transient and could be relieved with antihistaminics or digestives.
References


