# POTENTIATION OF THE TOXIC ACTION OF METHOTREXATE AFTER PRELIMINARY ADMINISTRATION OF COLLOIDAL GOLD TO MICE

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UDC 615.277.3.099.015.21: 615.31 : 546.59

A solution of colloidal gold with particle size 8-24 nm was injected intraperitoneally into mice, and 1 h later the animals received an intramuscular injection of methotrexate solution in a dose of 10-50 mg/kg body weight. Preliminary administration of colloidal gold potentiated the toxic action of the methotrexate: in the experimental mice the toxic effects developed sooner and the mice died from toxicosis about 4 times more frequently than in the control.

A few years ago Svet-Moldavskii and Pavlotskii [2-4] suggested the principle of selective protection of vitally important organs and tissues of the organism in the chemotherapy of cancer and placed it on a firm experimental basis. In their investigations the chemotherapeutic agent used was methotrexate (MTX), and the selective radio protector was folic acid, adsorbed on ink particles.

In the present writers' experiments, colloidal gold solution was used instead of ink to adsorb the folic acid. During the very first experiments along these lines a paradoxical phenomenon was observed: in some experiments, instead of the expected protection of the animal against the toxic action of MTX, the preliminary administration of folic acid adsorbed on colloidal gold had the opposite effect, for the toxicosis was aggravated. This was reflected in the more frequent appearance of toxic manifestations (general weakness, diarrhea, stomatitis) and the higher mortality among the experimental animals than among the unprotected controls, receiving MTX only. Since the folic acid was used in these experiments in doses (25-50 mg/kg) which were nontoxic to mice, it had to be assumed that the observed effect was due to administration of colloidal gold as a carrier of the folic acid.

To test this hypothesis the present investigation was undertaken.

## EXPERIMENTAL METHOD

Male (18-20 g in weight) noninbred mice from the "Kryukovo" nursery were used for the experiments. Commercial MTX (Lederle) of batch 4567-89 was used for injections. The contents of each ampule were dissolved before injection in 2 ml sterile distilled water. The colloidal gold was prepared in the radiochemistry laboratory of the Institute of Biophysics, Ministry of Health of the USSR. The gold content of the preparation was 3 mg/ml. The particle size varied from 8 to 24 nm. Gelatin was used as the stabilizer. The colloidal gold preparation was sterilized in the autoclave (1 atm, 15 min, 120°C).

The scheme of the experiments was as follows. The experimental mice received 3 intramuscular injections of MTX, each dose consisting of 10-50 mg/kg body weight. The intervals between injections

Laboratory of Virology, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow. Laboratory of Radiochemistry, Institute of Biophysics, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, L. F. Larionov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 12, pp. 72-73, December, 1971. Original article submitted April 30, 1971.

TABLE 1. Potentiation of Toxicity of Methotrexate after Preliminary Injection of Colloidal Gold ($Au^{197}$)

| Material injected | Number of mice | | Mortality, in % |
|---|---|---|---|
| | total | no. dying from toxicosis | |
| $Au^{197}$ + MTX 1 h later | 37 | 28 | 75.7 |
| $Au^{197}$ + MTX 24 h later | 17 | 6 | 35.3 |
| MTX | 31 | 6 | 19.3 |
| $Au^{197}$ | 18 | 0 | 0 |

were of 2 days. An intraperitoneal injection of 0.5 ml colloidal gold was given to the mice 24 h or 1 h before the MTX injections. Animals receiving MTX only or colloidal gold only were used as the controls.

Death of the experimental and control animals was recorded for 20 days after the last injection of MTX.

## EXPERIMENTAL RESULTS

Altogether 3 experiments were carried out by the above scheme, and they yielded similar results which are summarized in Table 1.

The results indicate that preliminary injection of colloidal gold 1 h before MTX considerably potentiated the toxic action of the latter. Mice injected with colloidal gold and MTX died from toxicosis about 4 times as often as the controls receiving MTX only. The difference between the mortality rates among the animals of these groups was statistically significant ($P < 0.01$). It was also noted that the toxic manifestations developed sooner in the experimental animals than in the controls. At autopsy on the mice receiving colloidal gold the liver and spleen were stained a dark color. Injection of colloidal gold 24 h before MTX also potentiated the toxic action of the compound, but the difference between the mortality rates in the animals of this group and the control was not statistically significant ($P > 0.05$). Injection of colloidal gold alone into the mice caused no toxic effects.

The injection of a colloidal solution into an animal may give rise to blocking or stimulation of the reticulo-endothelial system [1, 6, 7, 9, 10]. These states are characterized by changes in reactivity of the body, reflected in changes in the level of antibody formation [8, 14], changes in sensitivity to harmful agents such as carbon tetrachloride [11, 15, 16], chloroform [12], and radiation [13], and also in disturbances of metabolism and the removal of biologically active compounds from the blood [5]. According to Murray [9], colloidal gold in doses close to those used in the present experiments caused blocking of the reticulo-endothelial system. Consequently, the increased toxicity of MTX could have been closely connected with changes in the reactivity of the animals resulting from blocking of the reticulo-endothelial system by the colloid gold.

These results must be taken into account when further work is undertaken to study selective protection of vitally important organs and systems of the body during the chemotherapy of tumors.

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