The effect of the cytostatics doxorubicin, 6-thioguanine and cytarabine on retinol store in rat liver was examined. When rats were treated with pharmacological doses of the combination of doxorubicin and 6-thioguanine for 10 days, the content of retinol in the liver was reduced by about 33%. In a longer term experiment, doxorubicin and cytarabine given separately reduced the retinol store by 33% and 11%, respectively, while doxorubicin and 6-thioguanine given in combination reduced retinol in liver by 31%. For one of the cytostatics (doxorubicin) the effects on plasma retinol and on acyl CoA:retinol acyltransferase (ARAT) activity in small intestine were also examined. Both were transiently reduced during the experiment.

Key words: Cytostatics, Retinol, RBP, ARAT, Rats, AML, Cancer.

INTRODUCTION

In several in vitro systems, retinoids have been shown to regulate proliferation and differentiation of a variety of cells. Retinoids induce terminal differentiation in, e.g. mouse F9 teratocarcinoma cells and in human promyelocytic leukemia cells in concentrations which can be obtained in vivo. In several animal models, retinoids can prevent tumour promotion when animals are treated with carcinogenic viruses or chemicals. Furthermore, reports from clinical trials also suggest effects of retinoids in human cancers.

Acute myeloid leukemia (AML) accounts for about 20% of all leukemias in children. The prognosis for young patients with AML is poor. However, during the past 10 yr there has been progress in the treatment of the disease, mainly due to intensive remission induction and post remission programs with chemotherapy. But still only about 40% of the patients live 1 yr after diagnosis.

In a single institution study, 20 Norwegian children with AML have been treated according to a protocol including doxorubicin, cytarabine and 6-thioguanine as induction therapy, followed by four courses of high dose cytarabine as consolidation (Lie S, Wathne K O, Petersen L B, Slordahl S, Norum K R: High-dose retinol as maintenance therapy in children with acute myeloid leukemia. Submitted for publication). Eighteen out of the 20 children achieved complete remission. During remission, the children received 52 μmol (15 mg) retinol as retinyl palmitate per square meter daily. Thirteen of the 18 children are still in their first remission with a mean observation time of 36 months. In this study, retinyl ester given in doses up to 30 times the recommended daily allowances, has not caused any side effect for up to 4 yr of therapy.

Ethanol has previously been shown to reduce liver retinol stores. Furthermore, toxins like DDT and TCDD also reduce liver retinol dramatically, and recently Lieber's group reported that patients exposed to the drugs α-methyldopa, prednisone, methadone, phenezine sulfate, phentoixin and phenothiazine had their liver retinol stores reduced to an average of less than 10% of normal values. A post-mortem study of liver retinol store in the U.S.A. showed that one third of the population studied had low reserves of retinol, possibly as a result of drug use.

The aim of the present study was to investigate whether cytostatics included in the Norwegian pro-
tocol for treatment of children with AML also reduced liver retinol store, using rats as a model.

MATERIALS AND METHODS

Animals

Male Wistar rats (150–200 g) were supplied from Dyrlæge Møllegård Hansen Avlslab, Ejby, Denmark. The animals had free access to food and water. They were fed pelleted diet containing about 12.6 μmol (3.6 mg) of retinoid kg⁻¹ (50% retinyl palmitate and 50% retinyl acetate). Blood samples were taken from the heart in anesthetized rats. The liver was removed immediately after the rat had been killed, and was washed in ice-cold isotonic potassium phosphate buffer (pH 7.4).

Cytostatics

6-Thioguanine (Lanvis) was kindly provided by The Wellcome Foundation Ltd, Høvik, Norway, doxorubicin (adriamycin) by Farmitalia, Asker, Norway and cytarabine (Cytosar) from Upjohn, Blommenholm, Norway.

Treatment protocol

Our experiments were designed to resemble the Norwegian protocol for treatment of children with AML. The children included in the Norwegian protocol received three courses of 75 mg doxorubicin m⁻² once weekly and three courses of 100 mg 6-thioguanine every 12 h for 4 days in the induction phase. As maintenance, four courses of 2 g cytarabine every 12 h for six days were given in the consolidation phase. Animals were given the same doses converted per square meter according to Freireich et al.11 However, because of side effects in pilot experiments, the rat doses for doxorubicin and 6-thioguanine were reduced compared to the Norwegian protocol. Tables 1a and 1b present the doses and duration of treatment in the short-term experiment and the long-term experiment, respectively.

Screening for side effects

Cytostatics uniformly have a suppressive effect on bone marrow, and they may also induce liver toxicity and anorexia. Table 2 presents the effect of cytostatics on haematological parameters and liver enzymes. Blood samples were collected for three groups of rats in the short-term experiment on the day of sacrifice. Although dosage requirements in small animals often are higher than in larger animals, the results presented in Table 2 suggest that the cytostatics did have the expected effect. Rats in the treated groups had their body weights reduced by 20–50% in the short-term group and by 11–25% in the long-term group (data not shown).

Chemicals

Retinol (all-trans), palmitoyl CoA, bovine serum albumin, dithiothreitol (DTT), dimethylsulphoxide (DMSO) and N,N-diphenyl-p-phenylenediamine (DPPD) were from Sigma Chemical Co., St Louis, MO, U.S.A. All other chemicals were of standard commercial high purity.