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Short communication

Protection from ifosfamide-induced alopecia by topical thiols in young rats

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Summary. An animal model for testing substances that might prevent alopecia induced by oxazaphosphorines has been developed. It is based on the fact that the rat pups experience virtually total hair loss following administration of oxazaphosphorines. Using this model, we showed that epicutaneous treatment with sodium thioglycollate prevented ifosfamide-induced hair loss on the treated area. These experiments indicate that prevention of oxazaphosphorine-induced alopecia in man may be achieved by topical thiols.

Introduction

Alopecia is a frequent and distressing side effect of most anticancer agents, often leading to a refusal of chemotherapy by the patient. Measures taken in the clinic to prevent hair loss have been unsatisfactory [6]. Systematic animal experiments are obviously needed but have been hampered by the lack of suitable models. Recently, however, Angora rabbits were used to study doxorubicin-induced hair loss [5], and in the present experimental investigation into prevention of oxazaphosphorine-induced hair loss, rat pups were studied. In contrast to adult animals, suckling rats completely lose their hair after a single injection of ifosfamide or cyclophosphamide [7].

Systemic administration of thiols can reduce the general toxicity of cyclophosphamide and its analogues [3] by neutralizing their toxic metabolites. Since the latter are probably responsible for the hair loss as well, it seemed logical to use SH-compounds for preventing the oxazaphosphorine-induced alopecia in young rats. In our animal experiments, the thiols were applied epicutaneously to avoid systemic effects of SH-groups that could interfere with the cytostatic action of oxazaphosphorines [1 – 4].

Materials and methods

Animals. Suckling Sprague-Dawley rats of both sexes (breeder: Zentralinstitut für Versuchstierzucht, Hannover, FRG) were used throughout this study. The animals were kept under specific pathogen-free (SPF) conditions.

Drugs and treatment. Ifosfamide (ASTA Pharma AG, Frankfurt, FRG) was given subcutaneously as a 2% solution in normal saline. Sodium thioglycollate (STG) was obtained by dissolving 2 ml thioglycollic acid (Merck, Darmstadt, FRG) in 1 *N* NaOH to pH 8.0 and adjusting it to 100 ml with distilled water. Commercially available L-cysteine, thiourea, L-cysteine ethylester hydrochloride, cysteamine hydrochloride, and dithioerythritol were dissolved in phosphate buffer (pH 8.0).

All thiol solutions were freshly made for each experiment. A total of 12 pups from the same litter (aged 11 – 12 days and weighing about 20 g) were randomly assigned to 3 experimental groups consisting of 4 animals each. All animals were treated with ifosfamide (100 mg/kg, given s. c. in the abdominal region). One group received no further treatment and served as ifosfamide controls. Prior to ifosfamide treatment, the dorsal regions of animals belonging to the other two groups (thiol- and phosphate-buffer-treated pups, respectively) were washed with soap and warm water to remove the fat layer on the coat, which would otherwise prevent the thiols from penetrating the skin. Thereupon, the defatted back area was painted for 2 h with either one of the thiols under test or phosphate buffer (pH 8.0) (placebo group), using a smooth paintbrush made from special plastics. After brief washing, ifosfamide was injected, whereupon the painting was continued for a further 3 h.

During epicutaneous treatment the animals were kept in separate cages under infrared light. To prevent their licking the thiol or phosphate buffer solution, suitably formed tubes made from a soft plastic material were put loosely over the heads of the pups. Finally, the solutions were carefully washed away with water. At 12 days after ifosfamide administration, the effect of the topical treatment was photographically documented. Remaining animals from the litter were left without treatment, thus supplying data on body weights of healthy, untreated pups for comparison with data on the treated groups. In all, 3 – 4 litters were used for testing each of the thiols.

Results and discussion

At 5 days after s. c. administration of 100 mg/kg ifosfamide, the hair loss commenced, starting on the top of the head and proceeding slowly toward the tail. Within 5 days the animals grew almost completely bald except in a small area around the eyes and nose. Retardation of growth rela-

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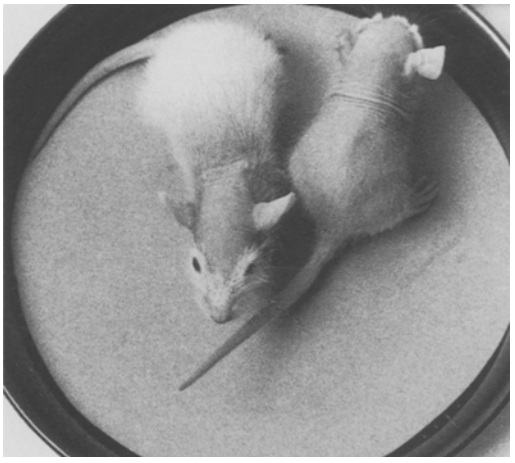

Fig. 1. Painting the back area with sodium thioglycollate protected against ifosfamide-induced hair loss (*left rat*; cf. control animal on the *right*). Both rats were treated with ifosfamide (100 mg/kg, 12 days after birth) and belonged to the same litter. The photo was taken 12 days after ifosfamide administration

tive to the untreated controls became apparent simultaneously with the hair loss and was always very pronounced (12 days after treatment, the body weight amounted to only about 50% of that registered for untreated rats). At 3 weeks after ifosfamide injection, regrowth of the hair was observed.

Topical application of cysteine (10%), cysteine ethylester hydrochloride (10%), dithioerythritol (5%), thiourea (10%), or cysteamine hydrochloride (5%) proved to have no effect; there was no indication of any protection against ifosfamide-induced hair loss.

STG was the only thiol that proved to be effective after topical application in our model. After the administration of 100 mg/kg ifosfamide in the pups treated topically with STG, the loss of hair from the head and neck (i.e. in the nontreated regions) began at the same time as in controls, leading likewise to complete hair loss. However, the whole treated region from the scapula to the tail retained its hair, although its distribution was slightly thin (Fig. 1), evidencing the protective action of STG. On the other hand, retardation of the gain in body weight was as strongly pronounced in half of the animals as in pups treated with ifosfamide alone, whereas in the remaining animals slightly higher body weights were observed.

The pups in the placebo group did not display any difference from the controls treated with ifosfamide alone, thus evidencing that the mechanical procedure (i.e., washing and painting the coat) had no protective effect of its own. The marked difference in appearance between topically treated animals and controls persisted until the beginning of hair regrowth.

Although successful in preventing hair loss, epicutaneous treatment with STG showed little, if any influence on the slowdown in body growth that has always been observed after ifosfamide treatment. Taken together, these findings suggest that little, if any, of the epicutaneously applied STG was absorbed through the skin into the general circulation, although this substance obviously does penetrate the skin. Thus, the protection against hair loss must be attributed to the local action of STG. For the same reason, one may expect that epicutaneously applied STG would not interfere with the antineoplastic action of ifosfamide. However, this remains to be proved.

Since the epicutaneous treatment with several other thiol compounds proved to be ineffective in protecting suckling rats from ifosfamide-induced hair loss, it seems probable that under the experimental conditions chosen, sufficient penetration through the skin is achieved only by STG.

A drawback of the use of STG for topical detoxification is its typical smell, which is difficult to overcome and thus does not ensure patient compliance. The present experimental model should, however, lead to the identification of more acceptable compounds for prophylaxis of alopecia.

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