Investigational New Drugs 4: 373-376, 1986

Short communication: Cause and prevention of mafosfamide-induced venous pain

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Key words: acrolein, mafosfamide, mesna, N-acetyl-cysteine, oxazaphosphorines, venous pain

Abstract

An experimental rat model for the study of venous pain induced by 4-hydroxy-cyclophosphamide (4-OH-CP) derivatives was developed and validated. Using various metabolites and chemical variants of 4-OH-CP it was found that pain induction was independent from the compound's alkylating activity but possibly related to the spontaneous generation of minute amounts of acrolein from the 4-OH-CP molecule. Accordingly, the pain could be prevented by the addition of thiol compounds such as mesna or N-acetyl-cysteine.

Introduction

Mafosfamide is a cyclophosphamide derivative which spontaneously hydrolyses in vitro and in vivo to the 4-hydroxy-species and 2-mercaptoethane sulfonate (Fig. 1). The activity spectrum of mafosfamide is similar to that of the parent drug, however, it is independent from the molecule's 'activation' by liver enzymes (1). Throughout the preclinical evaluation of mafosfamide it was observed that it induced concentration-dependent tissue irritation in rats after subcutaneous injections. In comparison to other bifunctional alkylating agents such as mechlorethamine, however, the local tolerance of mafosfamide on a molar basis was approximately 10 times better (2).

During the clinical phase I trials it was found that immediate and reversible pain along the injected vein constituted a severe side effect beginning at a dose of 400 mg/m^2. Escalations beyond 1000 mg/m^2 were impossible even after maximal dilution of the compound (3 and P. Harper and H. Rogers, personal communication). None of the patients showed signs of phlebitis or tissue inflammation at the injection site. In order to further elucidate the nature and specificity of the mafosfamide-induced pain, a simple and predictive animal model had to be developed. The present communication describes such a model and results of studies looking at the mechanism of the described venous pain.

Materials and methods

Experimental design

A 18 gauge needle was placed into the lateral tail vein of outbred Sprague-Dawley rats (180–230 g) and connected to an infusion pump. Thereafter, the animal was placed into a semi-restraining stainless wire-tube which allowed normal sensory contact with the environment and immobilized the animal without pressure. Normal saline (i.e. 0.9% sodium chloride) was infused at room temperature at a rate of 1 ml/hour. Within 30 minutes of infusion the normally night-active animals were physiologically asleep and at this point the infusion was switched to another one containing the test agent. In case of irritation, the animals awoke and demonstrated characteristic escape and defense movements; the

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Table 1. Pain-inducing threshold concentration in the infusion fluid of various compounds (+ = reaction; − = no reaction)

Compound	Reaction	Concentration mg/ml
Normal saline	−	−
Ringer's solution	−	−
HCl pH 4	−	−
Cyclophosphamide	+	30
Ifosfamide	+	>10
Asta Z 7557	+	1
Asta Z 7654	+	1
4-OOH-CP	+	< 1
Dechloro 4-OOH-CP	+	< 1
Phosphoramide mustard	+	> 5
Chloracetaldehyde	+	< 0.1
Acrolein	+	< 0.0001
Anaxirone	+	>30
Asta Z 7654 + 5 m Mesna	+	> 5
Asta Z 7654 + 5 m NAC	+	> 5

Asta Z 7557 = Mafosfamide cyclohexylamine salt
Asta Z 7654 = Mafosfamide lysine salt
4-OOH-CP = 4-Hydroperoxy-cyclophosphamide
NAC = N-acetyl-cysteine

experiment was subsequently terminated.

Test substances

All chemicals listed in the table were either synthesized in the Department of Chemistry, Asta-Werke AG, Bielefeld, or purchased from commercial sources. All reagents tested were diluted with normal saline. In groups of five animals the approximate threshold concentration of each compound which reproducibly induced a reaction was determined.

Results

Table 1 summarizes the results: Neither saline, Ringer's solution, hydrochloric acid (pH 4), cyclophosphamide (below 30 mg/ml) nor ifosfamide (below 10 mg/ml) induced any visible reaction. In contrast, mafosfamide cyclohexylamine salt (Asta Z 7557) and mafosfamide lysine salt (Asta Z 7654) caused irritation at concentrations of 1 mg/ml. 4-Hydroperoxy-cyclophosphamide and dechloro-4-hydroperoxy-cyclophosphamide were at least as irritating as mafosfamide. Among the metabolites of mafosfamide, such as phosphoramide mustard, acrolein and chloracetaldehyde, acrolein was the most effective agent inducing reactions in the animals: the threshold concentration was 10^4 times lower than that of mafosfamide and other related compounds. In a similar experiment various concentrations of sodium 2-mercaptoethane sulfonate (INN: mesna) or N-acetyl-cysteine (NAC) were added to the test agent's solution. As can be seen from the table, a 5 molar mesna or NAC solution completely prevented the pain induced by mafosfamide lysine salt.

Discussion

Clinically many directly acting alkylating agents with high chemical reactivity induce venous irritation resulting in phlebitis or thrombophlebitis but they do not commonly induce pain along the injected vein. In general, this side effect correlated with the unspecific alkylating activity of the compounds. Most likely, the causative factor is alkylation of protein structures on the endothelial cell membrane or within the cell, leading to tissue damage with cell death and an inflammatory or thrombotic response. Mafosfamide, being only the precursor of the ultimate alkylating moiety phosphoramide mustard, is of comparatively low chemical reactivity (4) and consequently its activity as a tissue-irritant was considerably less pronounced than that of directly acting alkylating agents when administered subcutaneously (2). The unusual clinical feature of immediate venous pain already suggested that its cause may be specifically related to the activated oxazaphosphorine ring and not to the initially non reactive alkylating chloroethyl groups of the molecule.

The saline-infused sleeping rat appears to be an adequate experimental model of the clinical phenomenon: mafosfamide reproducibly induced a reaction at low concentrations, whereas saline and other compounds clinically known to be inert, were

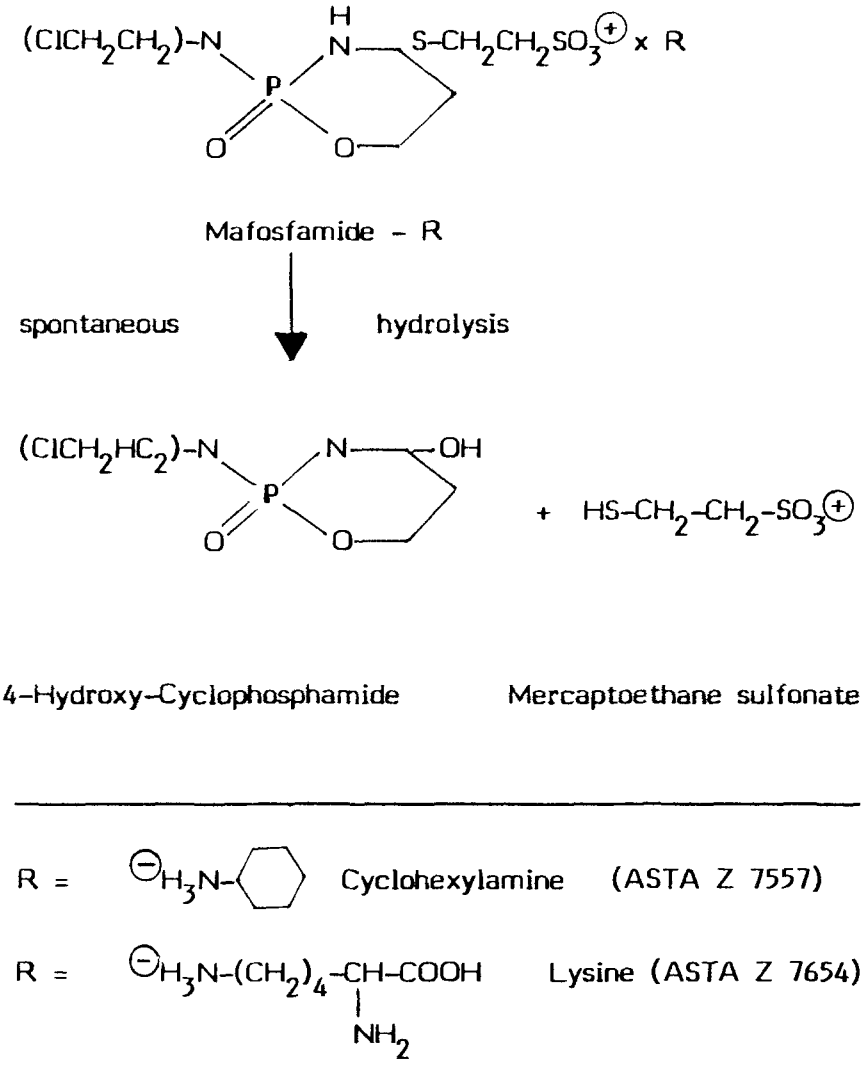

Fig. 1. The hydrolysis of mafosfamide

tolerated without discomfort. Another derivative of 4-hydroperoxy-cyclophosphamide, the dechloro-4-OOH-CP has no alkylating activity at all, but – similar to its parent compound – it undergoes also spontaneous degradation to (dechloro)-phosphoramide mustard and acrolein. The finding that the threshold concentration for pain induction for this compound was identical to that of mafosfamide indicated that the phenomenon of venous pain was not due to the alkylating activity but rather related to the 'activated' oxazaphosphorine ring or to the generation of acrolein. In comparison to the other metabolites of mafosfamide such as phosphoramide mustard and chloracetaldehyde, acrolein demonstrated an extraordinary potency to induce venous pain in our animal model.

The prediction of venous pain of the experimental model described was further suggested by the effects of α/β-triglycidyl-urazol (INN: anaxirone). This alkylating compound is a tri-epoxyde currently in clinical development which frequently caused severe phlebitis in patients, however, it can generally be injected without inducing venous pain (5). In the present study anaxirone was also well tolerated by the sleeping rat and apparently did not induce pain during its infusion.

From the foregoing it appears that pain receptors within the blood vessel are particularly sensitive to

the activated oxazaphosphorine ring and/or acrolein. Since some thiol compounds are able to form a non-toxic complex with 4-hydroxy-cyclophosphamide and/or acrolein it was suggested that their addition to the infusion fluid could prevent or reduce the symptom (6).

Under the experimental conditions described, mesna and N-acetyl-cysteine completely inhibited the mafosfamide-induced pain. Obviously, the present experimental model only gives a semi-quantitative estimate of the minimal thiol concentration necessary to prevent venous pain. The validity of these experimental results was confirmed in a still ongoing clinical phase I study, where the direct addition of 5 molar mesna to mafosfamide infusions abolished the pain reaction at the injection site (P. Harper and H. Rogers, personal communication). However, the overall clinical usefulness of the above approach has still to be determined and will be communicated in due course.

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