

Short communication

Contamination of heparin by histamine: measurement and characterization by high-performance liquid chromatography and radioimmunoassay

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Plasma samples which were collected in the presence of heparin contained 12.83 ± 0.34 ng/ml histamine-immunoreactive material ($n = 10$). In contrast, histamine-immunoreactive material in plasma samples which were collected in the presence of EDTA/phenanthroline contained 0.32 ± 0.01 ng/ml ($n = 10$). Histamine-immunoreactive material was found as a contaminant in three different heparin formulations with unrelated batch numbers. The concentrations of the histamine-like material were 32.82, 81.93, and 280.23 ng/ml, respectively. Octadecasilyl-silica (ODS) cartridges were used to purify histamine from other constituents in the heparin preparations. The histamine-immunoreactive material in the three preparations could be characterized as histamine with cation-exchange high-performance liquid chromatography (HPLC) on a TSK SP-5 PW column with NaH_2PO_4 , pH 5.0, as a mobile phase and gradient elution. For the accurate measurement of histamine in plasma samples with heparin as an anticoagulant, one should be aware of a possible contamination of heparin formulations by histamine.

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Heparin is a polar mucopolysaccharide with a mol. wt. of 20 000, which contains carboxylic and sulfuric acid residues as functional groups. Besides its application in the prophylaxis and treatment of cardiac infarction, thrombophlebitis, and embolic disease, it is widely used as an anticoagulant in blood sampling.

Histamine (H) is produced and stored in mast cells and basophil leukocytes, and its role as a mediator of immediate-type hypersensitivity in both allergic and pseudoallergic reactions has been well established (2, 5–7). Therefore, the determination of H can be used as a marker for these reactions. However, the accurate and reproducible measurement of H in biologic samples such as blood is hampered by methodologic problems including blood sampling and the use of anticoagulants which may contain impurities of H or H-like material. Indirect evidence

of the contamination of H in various heparin formulations comes from animal studies. Injections of heparin formulations in anesthetized cats showed significant vasodepressor activity. This effect was reduced by the administration of an H_1 -antagonist, indicating the involvement of H (8).

In this study we report the contamination of heparin by H-immunoreactive material. Cation-exchange high-performance liquid chromatography (HPLC), in combination with a specific radioimmunoassay (RIA) for H, was used to characterize the H-like material.

Material and methods

Collection of blood

Human blood was collected from the cubical vein in the presence of either heparin or an inhibitor cock-

tail. For 20 ml blood, 0.5 ml heparin or 1.0 ml inhibitor cocktail was used. The inhibitor cocktail was a mixture of 0.125 M EDTA- Na_4 and 0.025 M 1.10 *o*-phenanthroline in 0.9% sodium chloride solution (1). Blood was transferred to plastic tubes kept at 4°C, and plasma was prepared by centrifugation (6000 g, 4°C, 5 min). Plasma samples which were collected in the presence of heparin or the inhibitor cocktail (EDTA/phenanthroline) were pooled separately and stored in aliquots of 0.5 ml at -20°C before the radioimmunologic measurement of H.

Purification of histamine in heparin formulations

H was purified from three heparin formulations with octadecasilyl-silica ODS cartridges (Sep Pak C₁₈, Millipore Waters, Eschborn, Germany). The cartridge was first rinsed with 3 ml methanol and then 3 ml 0.01 N HCl. Heparin samples (1.0 ml) were applied to the cartridge, and approximately 1 ml eluate was collected. The cartridge was then washed once with 1 ml 0.01 N HCl, and the eluting fraction was collected. The two eluted fractions were combined and dried in a vacuum centrifuge. The dried residues were dissolved in 1.0 ml HPLC water and spun at 20°C, 10000 g for 2 min. H was measured radioimmunologically in the reconstituted samples. The heparin formulations were as follows: formulation no. 1: 25000 IE heparin/5 ml, no. 2: 25000 IE heparin/5 ml, and no. 3: 20000 IE heparin/ml.

Radioimmunoassay (RIA)

RIA for H was done with a test kit delivered by IBL (Immuno-Biological Laboratories, Hamburg, Germany). Briefly, 0.1 ml samples or standards were incubated for 30 min at room temperature to form an H derivative which was measured radioimmunologically with ¹²⁵I-labeled H and specific anti-H-antiserum. The cross reactivity of the antibody with acylhistamine was 100% and with *N*-methylhistamine (MH) 0.01%. L-histidine, imidazole acetic acid, *N*-methylimidazole acetic acid (MIAA), serotonin, and 5-hydroxyindole acetic acid did not cross-react with the antibody.

High-performance liquid chromatography (HPLC)

An ERC HPLC equipment model ABEO 88 (ERC, Alteglofsheim, Germany) was used. All separations were done at room temperature on a cation-exchange column, TSK SP-5 PW (75 × 7.5 mm; TosoHass, Stuttgart, Germany) with gradient elution. The mobile phases were solution A: HPLC water; and solution B: 0.5 M NaH_2PO_4 solution in HPLC water, adjusted to pH 5.0 with concentrated phosphoric acid. Initially, 10% of solution B correspond-

ing to 0.05 M was delivered for 9 min; this was followed by an exponential increase of solution B to 60%, corresponding to 0.325 M for 16 min. At 25 min, the column was flushed isocratically with 60% of solution B. The column was equilibrated under the initial conditions for 20 min. The separations were done at a flow-rate of 0.6 ml/min. The column was calibrated with H, MH, L-histidine, and MIAA with UV detection at 225 nm and a sensitivity of 0.1 absorbance units full scale (AUFS). The mobile phases were passed through an RC 55 membrane filter (Schleicher and Schüll, Dassel, Germany) before use.

For the characterization of H-like material, 0.8 ml of the samples which were purified on ODS cartridges were injected into the TSK SP-5 PW column. Fractions of 0.6 ml were collected. Aliquots of 0.1 ml of each fraction were withdrawn for the radioimmunologic measurement of H.

Chemicals

All chemicals used were of analytic grade. L-histidine, H, and MIAA were purchased from Sigma Chemie, Deisenhofen, Germany. MH was delivered by Calbiochem, Frankfurt, Germany. All other chemicals, including HPLC water, were delivered by Merck AG, Darmstadt, Germany.

Results

Histamine in pooled plasma samples

H was measured radioimmunologically in plasma pools which were collected in the presence of heparin or EDTA/phenanthroline. Significant differences in the H-immunoreactivity were noted between the heparin and the EDTA/phenanthroline plasma samples. The H-immunoreactivity was 40-fold

Table 1. Radioimmunologic measurement of H in heparin and EDTA/phenanthroline plasma pools. Blood collected in presence of heparin showed 40 times higher H levels than blood samples collected with EDTA/phenanthroline

Histamine immunoreactivity (ng/ml)	
Heparin plasma pool (n= 10)	EDTA plasma pool (n= 10)
14.96	0.35
12.38	0.39
12.04	0.31
14.24	0.29
12.48	0.30
12.51	0.31
12.13	0.34
11.34	0.29
13.42	0.29
12.77	0.32
12.83±0.34	0.32±0.01

Table 2. Radioimmunologic measurement of H in three different heparin formulations. In samples before ODS purification (pre-ODS), concentrations of H-immunoreactivity varied between 33 and 280 ng/ml. In samples after ODS purification (post-ODS), H-immunoreactivity ranged from 19 to 279 ng/ml

	Histamine immunoreactivity (ng/ml)	
	Pre-ODS	Post-ODS
Formulation no. 1	32.82	28.04
Formulation no. 2	81.93	279.24
Formulation no. 3	280.23	19.29

higher in heparin plasma samples than in EDTA/phenanthroline plasma samples. H-immunoreactive material in samples which were collected in the presence of heparin was measured as 12.83 ± 0.34 ng/ml, as compared with 0.32 ± 0.01 ng/ml in plasma samples with EDTA/phenanthroline (Table 1).

Histamine in heparin preparations

H-immunoreactivity could be identified in three different preparations of heparin. The concentrations

were 32.82 ng/ml in formulation no. 1, 81.93 ng/ml in no. 2, and 280.23 ng/ml in no. 3. In the same samples which were purified on ODS cartridges, the concentrations of H-like material were 28.04, 279.24, and 19.29 ng/ml (Table 2).

HPLC characterization

HPLC characterization of the H-like material present in heparin after purification on ODS cartridges on a cation-exchange TSK SP-5 PW column and gradient elution showed the presence of H in all three formulations (Fig. 1). The highest H concentration was found in formulation no. 2 followed by no. 1. The lowest concentration was found in formulation no. 3.

Discussion

This study was suggested by the finding of various plasma H concentrations depending upon the anti-coagulant used for the collection of blood. Various H concentrations in the plasma of healthy volunteers have been reported. In one study where EDTA was used, the plasma H concentrations were 0.23 ng/ml (3). This agrees with our finding of 0.32 mg/ml. However, another study, in which it is not clear whether EDTA or heparin was used for blood sampling, reported values of 4.35 mg/ml (4). In our study, the radioimmunologic determination of H in plasma samples which contained heparin showed H concentrations 40 times higher than samples collected in the presence of EDTA/phenanthroline. This confirms our previous findings and data from the literature. We always measured higher H concentrations in heparin-containing plasma samples with three RIA kits from three different manufacturers (unpublished data). These results suggest the presence of H or H-like material in heparin. H is stored in granules of mast cells and basophil leukocytes, where it is ionically bound to the acidic groups of the heparin molecule. The source of most commercial heparin preparations is the intestines of slaughtered animals, which are rich in mast cells. Therefore, it is very likely that these preparations contain H unless specific attempts are made to remove the H.

In fact, various concentrations of H-immunoreactivity could be identified in three unrelated heparin formulations from the three different manufacturers. H-immunoreactivity was also present in these formulations when H was partially abstracted from other constituents with ODS cartridges.

A combination of HPLC and RIA was utilized to characterize H-immunoreactivity. In all three heparin formulations examined, the H-immunoreactive material could be clearly identified as H. The find-

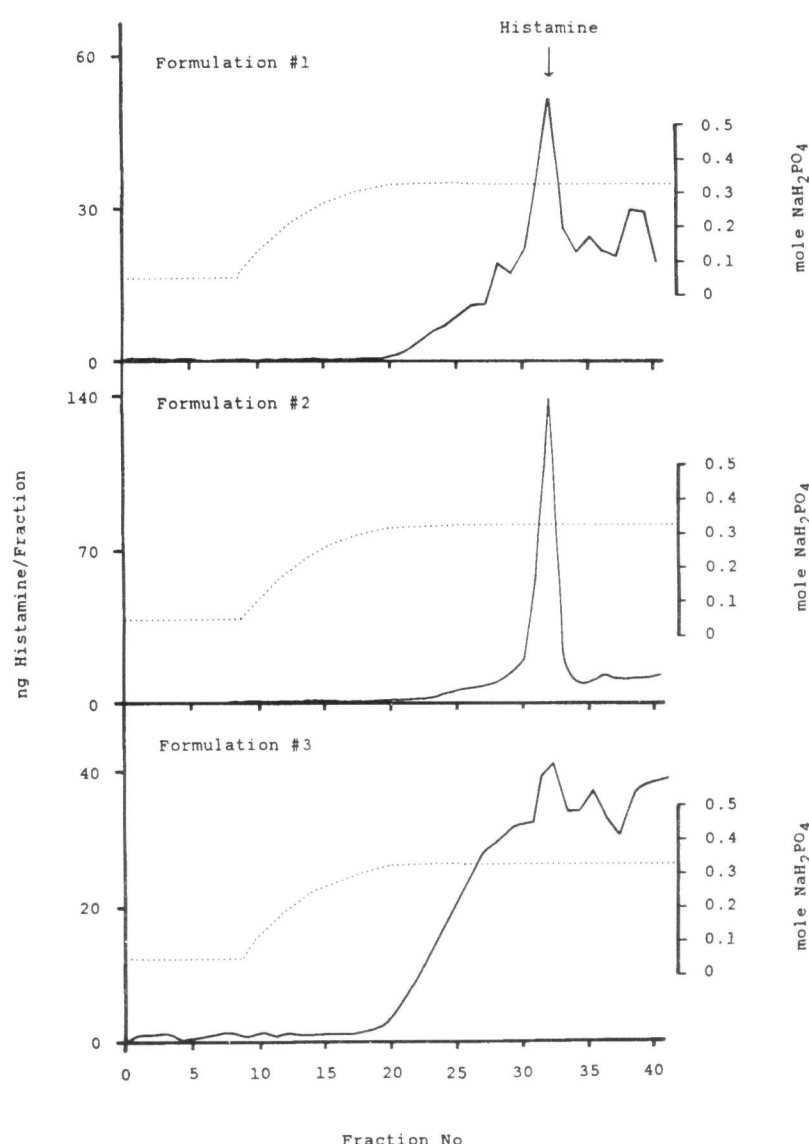


Fig. 1. HPLC characterization of H-immunoreactivity in three heparin formulations on cation-exchange TSK SP-5 PW column and gradient elution. H-immunoreactive material could be characterized in all three formulations as H with highest concentrations in formulation no. 2 and lowest in no. 3.

ing of H as a contaminant in heparin agrees with data reported by Sjodin & Svensson (8). Administration of heparin in cats showed vasodepressor activity which could be blocked by an H_1 -antagonist, indicating the involvement of H. This was found in 34 batches of heparin formulations. The contamination by H was estimated to be as much as 100 ng H/5000 IU of heparin.

In summary, H was found as a contaminant in heparin. The H-like material could be characterized on HPLC as H. These results may have some clinical implications not only for the therapeutic use of heparin, but also for its function as an anticoagulant in blood sampling. Special attention should be paid when heparin is administered as a therapeutic agent in H-sensitive patients who may develop anaphylactic reactions. There is also the potential risk of overestimating the plasma H content when heparin is used as an anticoagulant.

For the accurate and reproducible measurement of H in plasma as a diagnostic factor for immediate-type hypersensitivity reactions, one should be aware of this risk. In addition, H is known to prime basophil leukocytes. Therefore, the use of heparin as an anticoagulant in the preparation of these cells may lead to inconclusive results in the basophil H-release. In conclusion, the presented data provide support for the use of EDTA instead of heparin as an anticoagulant in studies of plasma H and H-release.

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