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HEPARIN - COMPOUND 48/80 INTERACTION

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Heparin - Compound 48/80 İnteraksiyonu

Özet: Heparin - Compound 48/80 interaksiyonu araştırıldı. Elde edilen sonuçlar göstermiştir ki, Compound 48/80 rat mezenteriumu mast hücreleri granüllerinin ve ticarî heparinin Toluidin mavisi ile metakromatik boyanmasını önlemektedir. Ayrıca, Compound 48/80 heparinin antikoagülan etkisini de inhibe etti. Bu sonuçlara dayanılarak Compound 48/80 ile heparin arasında kimyasal bir interaksiyonun bulunduğu söylenebilir.

Birçok araştırıcılar Compound 48/80 ile mast hücrelerini degranüle ederek bu hücrelerdeki heparinin özellikle lipid metabolizması ve atherosclerosis üzerine olan etkisini incelemişlerdir. Heparinin bir kısmının Compound 48/80 tarafından inaktive edileceğinin gözönünde tutulması ve Compound 48/80'in toksik etkisinin heparin tarafından önlenmesi mekanizmasına açıklık getirmesi, bu araştırmanın önemli yönünü teşkil etmektedir.

Summary: Heparin - Compound 48/80 interaction was investigated. It was found that Compound 48/80 inhibited the metachromatic staining of mast cell granules of mesenteric tissue of the rat and commercial heparin by toluidine blue. Compound 48/80 has also inhibited the anticoagulant effect of heparin. The results indicate that a chemical interaction between heparin and Compound 48/80 may be present.

It is concluded that, some of the heparin may be inactivated by Compound 48/80 when this substance is used to degranulate mast cells. The findings support the idea that heparin protects the organism against the toxic effect of Compound 48/80.

Introduction

Compound 48/80,a synthetic polyamine, degranulates mast cells and releases histamine and heparin. It is well known that the injection of heparin into the blood releases clearing factor lipase (CFL) and that this clears the chylomicra from the blood [6, 14]. It is also known that there is a relation between faulty fat metabolism and the incidence of atherosclerosis.

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The animal body has its own heparin-producing system, the tissue mast cells, and it has been claimed that there might be a relationship between the population of tissue mast cells and the incidence of atherosclerosis [4, 7]. Several investigators have used Compound 48/80 to degranulate or deplete mast cells in order to investigate the relation between heparin released from mast cells and fat transport or atherosclerosis [5, 16, 17].

During our histochemical study of the effect of mast cell heparin on CFL in rat mesentery, Compound 48/80 was injected intraperitoneally to degranulate mast cells and release heparin. One ml. of saline containing 200 ug Compound 48/80 was injected intraperitoneally; control animals received 1 ml saline alone. Intraperitoneal injection of Compound 48/80 did not increase the activity of CFL. When, however, 1 ml distilled water was injected intraperitoneally, an increase in CFL activity was observed. This fact suggested the possibility that released heparin might interact with Compound 48/80 and thus be inactivated. Distilled water disrupts mast cells and released heparin increases CFL activity.

Experiments were therefore designed to detect heparin-Compound 48/80 interaction and the resulting inactivation of heparin.

Methods

1. Treatment of Rat Mesenteric Tissue With Compound 48/80.

Rats (Wistar Strain) were decapitated, and whole intestines with attached mesentery were removed and placed in ice cold Ringer's solution. The mesentery was tied to a 1 cm. high plastic collar, which had been cut from a round plastic bottle having a diameter of 4.5 cm., and the attached intestine trimmed off.

Three collars of mesenteric tissue were prepared from the same animal. This method of preparation was found to facilitate later fixing, washing and staining of the tissue.

The collars of mesenteric tissue were placed in petri dishes which contained 10 per cent formalin in saline and fixed for one hour. At the end of fixation period, the tissues were washed with distilled or deionized water and placed on glass slides, and the plastic collars were removed. The tissues were left to dry on the slides.

One of the slides containing mesenteric tissue was covered with a solution of 2 mg/ml. Compound 48/80 (Burroughs-Wellcome and Co.) and left for 10 minutes. The tissue was next washed with distil-

led or deionized water. Control tissues not treated with Compound 48/80 were washed as well. All tissues were then stained with a 1:2000 aqueous solution of toluidine blue for 2 minutes and washed with deionized water.

2. Staining Reaction of Heparin With Toluidine Blue.
The following solutions were individually prepared:

Aqueous solution of heparin	0.2 mg/ml.
Aqueous solution of Compound 48/80	0.5 mg/ml.
Aqueous solution of toluidine blue	1:1000

(Heparin sodium injection U. S. P., Lot No. 402–1, Connaught Medical Research Laboratories, Toronto; Toluidine blue, British Drug Houses, Canada Ltd., Toronto).

The following experiments were done with the above solutions (refer to Table 1):

TABLE 1.
INTERFERENCE WITH THE COLOR REACTION OF HEPARIN BY COMPOUND 48/80.

Tube 1	Color reaction	Tube 2	Color reaction
1 ml. Heparin solution 1 ml. Distilled water 0.5 ml. Toluidine blue solution	Typical purple color	1 ml. Heparin solution 1 ml. C. 48/80 solution 0.5 ml. Toluidine blue solution	No purple Color developed. Mixture remained blue

The following experiments were done to detect what concentration of Compound 48/80 completely inhibits the color reaction of heparin with toluidine blue (refer to Table 2):

TABLE 2.
EFFECT OF CONCENTRATION OF COMPOUND 48/80 ON THE COLOR REACTION OF HEPARIN

Tube No.	1	2	3	4	5
Compound 48/80 (aqueous sol.)	—	200 μg in 2 ml	400 μg in 2 ml	800 μg in 2 ml	400 μg in 2 ml
Heparin (aqueous sol.)	400 μg in 5 ml	400 μg in 5 ml	400 μg in 5 ml	400 μg in 5 ml	—
Distilled water to make up to 10 ml	5 ml	3 ml	3 ml	3 ml	8 ml
Toluidine blue (aqueous sol. 1:2000)	0.5 ml	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Color reaction	Purple	Purple	Blue, slight tinge of purple	Blue	Blue

3. Blood Coagulation Experiments.

The following experiments were performed to detect the inactivation of heparin by Compound 48/80 (refer to Table 3):

TABLE 3.

INACTIVATION OF HEPARIN BY COMPOUND 48/80.

Tube No.	1	2	3	4	5	6	7
Compound 48/80 (aqueous sol.)	—	200 μg in 2 ml	400 μg in 2 ml	800 μg in 2 ml	400 μg in 2 ml	—	Blood alone
Heparin (aqueous sol.)	400 μg in 5 ml	400 μg in 5 ml	400 μg in 5 ml	400 μg in 5 ml	—	—	
Distilled water to make to 10 ml	5 ml	3 ml	3 ml	3 ml	8 ml	10 ml	
0.5 ml mixture from each tube were mixed with 1.5 ml dog blood in glass tubes.							
Whole blood clotting time	No clotting in 3 hrs.	No clotting in 3 hrs.	No clotting in 3 hrs.	12′	10′	9′	9′

Results

In mesenteric tissue treated with a solution of Compound 48/80 (Method 1), mast cells remained unstained and could not be observed by light microscopy; control tissues contained many mast cells typically stained with toluidine blue. This result indicated that Compound-48/80 probably binds to heparin and prevents the staining of mast cell granules with toluidine blue.

Commercial heparin was tested *in vitro* to see if its color reaction with toluidine blue prevented by Compound 48/80. The results are given in Table 1. Heparin without Compound 48/80 gave a typical purple color with toluidine blue. When, however, heparin solution was first mixed with a solution of Compound 48/80, no purple color developed (Table 1).

The concentration of Compound 48/80 just necessary to prevent the development of purple color was examined and the results are shown in Table 2. An aqueous solution containing equal amounts (400 μg) of Compound 48/80 and heparin exhibited a blue color with a purplish tinge barely detectable when toluidine blue was added (Table 2, tube 3). Tube 4 and 5 remained blue.

When 800 μg Compound 48/80 interacted with 400 μg heparin, heparin was inactivated and its anticoagulant action abolished (Table 3, tube 4). Compound 48/80 itself does not effect blood coagulation as seen in Table 3, tube 5.

Discussion and Conclusion

Heparin, a highly negatively charged anticoagulant substance, interacts with cationic dyes one of which is toluidine blue. When a solution of toluidine blue is mixed with heparin a purple color develops. This metachromasia is due to an interaction of polyanion (heparin) and a cationic site of the dye [1]. Electron transfer occurs during this reaction between dye and anionic site of polyanion [1].

Compound 48/80 at a concentration of 800 μg almost completely inhibited the color reaction and anticoagulant effect of heparin at 400 μg. This result indicates that Compound 48/80 occupies the anionic site of heparin which can no longer react with the cationic site of the dye. The protamine-like property of Compound 48/80 was reported bu Mota *et al.* [12] and heparin protection against the toxic effect of Compound 48/80 by Higginbotham and Daugherty [8].

The exact nature of binding of Compound 48/80 and heparin requires investigation by appropriate methods, e. g. measurement of absorption spectra[18] of mixture of polyanion - Compound 48/80 and pulse radiolysis [11].

The interaction of Compound 48/80 and heparin is important with respect to the fact that Compound 48/80 may inactivate some of the heparin it has released and thus inhibits the heparin effect expected. There are other means to degranulate mast cells e. g. bee venom [2, 3] n-decylamine [2, 3], Polibrene [10], extract from Ascaris suis [15] and extract of Ascaris lumbricoides [13]. It has not yet been investigated, however, whether these mast cell degranulating agents have any interaction with heparin or not. Mast cells are disrupted by hypotonic salt solutions. Bloom and Haegermark [3] reported that 5-fold dilution of physiological salt solution caused the release of 91 per cent of the histamine from the peritoneal mast cells of rats. Since Compound 48/80, and probably other mast cell degranulating agents, interacts with heparin released from mast cells, hypotonic salt solution or even distilled water could be used, where applicable, to disrupt mast cells. It must be considered, however, that the actions of Compound 48/80 and distilled water on mast cells are different. Compound 48/80 selectively degranulates the mast cells without disrupting cell membranes, while distilled water disrupts cell membranes [9].

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