

Sensitization of guinea pigs to trivalent and hexavalent chromium compounds

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الملخص:

لفرض دراسة الحساسية التلامسية على الحيوانات، فقد أجريت التجارب على خمسين خنزيراً غينيا بوزن ٢٥٠ - ٣٠٠ غراماً للواحد، وذلك بتحفيظ تلك الحيوانات باستعمال ١٪ من مادة ثاني كرومات البوتاسيوم أو ١٪ من مادة كبريتات الكروم القاعدية. لقد اتضح بعد اجراء اختبار الحساسية ان نسبة حدوث الحساسية تكون عالية (١٠٠٪) عند استعمال ثاني كرومات البوتاسيوم وتقل الى (٨٠٪) عند استعمال محلول كبريتات الكروم وهذا يدل على ان محلول كبريتات الكروم لها قابلية فعالة وقوية لاجداث الحساسية. لقد وجد عند تحفيز الحيوانات بكبريتات الكروم القاعدية ان نسبة حدوث الحساسية في الحيوانات هي ٨٠٪ بعد اجراء الاختبار عليها باستعمال نفس المادة. نستنتج من هذا ان كبريتات الكروم القاعدية هي محفز قوي للحساسية ويمكن ان يكون لها نفس قوة تحفيز ثاني كرومات البوتاسيوم.

Summary

Fifty guinea pigs initially weighing 250-300 gm were divided into groups; some were sensitized with 1% potassium dichromate, others with 1% basic chromium sulphate and yet others with 0.5% basic chromium sulphate solution. Animals sensitized with potassium dichromate showed 100% positive skin reaction following epicutaneous challenge with potassium dichromate. However, the percentage decreased to 80% when the animals were challenged with chromium sulphate solution. This may indicate that chromium sulphate solution is a strong elicitor for the hypersensitivity reaction. The percentage of the positive skin reaction was 80% in guinea pigs induced and challenged with basic chromium sulphate which may indicate that chromium sulphate is also a good sensitizer and has almost the same sensitizing capacity as potassium dichromate.

Introduction

The ability of chromium to induce a hypersensitivity reaction is extensively documented^(1,2). This type of hypersensitivity is a cell mediated, contact type of reaction^(3,4). However, hexavalent chromium com-

pounds are thought to be more powerful sensitizers than trivalent chromium compounds^(5,6,7). During our study in the Iraqi tanneries we found a high percentage of workers with dermatitis. Trivalent chromium sulphate was used in the tanning process.

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In order to define the ability of chromium sulphate to induce and elicit a hypersensitivity reaction, this work was done on guinea pigs. The results were compared with the ability of hexavalent potassium dichromate to induce and elicit a hypersensitivity reaction. Concentrations as those used in the tanning process were also applied for sensitization and eliciting of the hypersensitivity response.

Materials and Methods

Fifty guinea pigs (Dunkin-Hartley albino guinea pigs), weighing initially 250-300 gm were used for this experiment. They were divided into five groups; each of ten animals. Chromium compounds used for sensitization were 1% basic chromium sulphate (taken from the powder added to the rotating drums during the tanning process) and 1% potassium dichromate. The method of Magnusson and Kligman⁸ was used as follows:

1- Induction:

An area of 4x6 cm. over the shoulder of guinea pigs was shaved. Induction was in a two stage operation. In the first stage a row of three intradermal injections was made on each side of the midline of the shoulder as follows:

-0.1 ml. of Freund's complete adjuvant	For the first and fourth groups
-0.1 ml. of 1% potassium dichromate in liquid paraffin	
-0.1 ml. of 1% potassium dichromate in adjuvant.	
-0.1 ml. of Freund's complete adjuvant	Second group
-0.1 ml. of 1% basic chromium sulphate in liquid paraffin.	
-0.1 ml. of 1% basic chromium sulphate in adjuvant.	
-0.1 ml. of Freund's complete adjuvant	Third group
-0.1 ml. of 0.5% of basic chromium sulphate solution (taken from the drums) in liquid paraffin.	
-0.1 ml. of 0.5% basic chromium sulphate in adjuvant.	
-0.1 ml. of Freund's complete adjuvant	Fifth group
-0.1 ml. of liquid paraffin **	
-0.1 ml. saline.	

In the second stage, one week after the injections, the same area was shaved and pre-treated with 10% sodium lauryl sulphate in white soft paraffin. Twenty-four hours later 1% potassium dichromate in white soft paraffin for the first and fourth groups, 1% chromium sulphate in white soft paraffin for the second and third groups, was spread over a 2x4 cm. patch of filter paper and applied to the skin. The patch was covered by an overlapping impermeable plastic adhesive tape. This was in turn firmly secured by elastic adhesive bandage, and left in place for forty-eight hours.

2- Challenge:

Animals were challenged two weeks after topical induction. Hair was removed from a 5x5 cm. area on the flank by shaving, then 0.5% potassium dichromate incorporated in white soft paraffin was applied for first and fifth groups while 0.5% chromium sulphate in white soft paraffin was applied for the second and third groups.

The fourth group was challenged with chromium sulphate solution. A 2x2 cm. filter paper was dipped in solution and applied in the same fashion as for topical induction. The patch was sealed to the flank for twenty-four hours under a four cm. strip of plastic adhesive tape, which in turn was secured by elastic adhesive bandage.

3- Readings:

The challenged site was examined 24hr. after removal of the patch and 24hr. later to detect weak, slowly developing reactions. The reactions were graded according to the specification of Magnusson and Kligman as follows:

- 0 No reaction
- +1 Scattered mild erythema
- +2 Moderate and diffuse erythema
- +3 Intense redness and swelling

Results

1-Skin reactivity of guinea pigs sensitized to either potassium dichromate or basic chromium sulphate (Table 1):

In the group of guinea pigs sensitized and challenged with potassium dichromate, three out of ten sho-

wed intense redness and swelling (+3), six showed moderate and diffuse erythema (+2) and only one showed scattered mild erythema (+1).

The results of induction and challenge with basic chromium sulphate are shown in group two. In general the severity of reaction is less than that observed in group one where only one animal showed (+3) reaction, two animals showed (+2), five animals (+1) reaction and two animals gave no reaction.

Group three showed the results of reactivity when guinea pigs were sensitized with chromium sulphate solution (taken from the tanning drums), and challenged with chromium sulphate powder. This is done to verify the capability of chromium sulphate solution used in tanning process to induce a contact type of hypersensitivity. The severity of a reaction was less than in group two; six animals gave negative reaction.

Group four of guinea pigs were sensitized with potassium dichromate and challenged with chromium sulphate solution. This is done in order to show the capability of the solution of chromium sulphate to elicit a hypersensitive reaction induced by strong sensitizer. The severity of the reaction was similar to group two and less than group one. Control guinea pigs which received saline showed negative skin reactions.

2- Histological findings in the skin of sensitized and non-sensitized guinea pigs:

The main histological changes in the skin of sensitized guinea pigs were observed in the epidermis and upper part of the dermis. Severe histological changes were observed in the skin of the sensitized guinea pigs exposed to potassium dichromate (Table 1, Group one). Both epidermis and upper dermis showed extensive infiltration with white blood cells mainly lymphocytes (Fig.2).

The epidermis showed acanthosis, spongiosis and hyperkeratosis clearly demonstrated by increase in thickness and edema (Fig.3). The dermis layer showed accumulation of lymphocytes around hair follicles and blood vessels (Fig.4). The hypodermis showed accumulation of fat droplets, lymphocytes, neu-

trophils and some eosinophils together with macrophages; accumulation of red blood cells and necrotic areas were also observed in this layer (Fig.5). Fig. 6 showed the effect of chromium sulphate on skin of sensitized guinea pigs, (Table 1, Group 2,3). The epidermis again showed acanthosis, spongiosis and hyperkeratosis but at lower intensity than that observed with potassium dichromate. The infiltration of white blood cells and macrophages in the epidermis and dermis layers were less than that observed with potassium dichromate. In general the severity of reaction was less than that observed in potassium dichromate.

The main histological changes in the skin of guinea pigs (Table 1, Group four) after induction with potassium dichromate and challenge with basic chromium sulphate solution are shown in Fig.7. The epidermis again showed acanthosis, spongiosis and hyperkeratosis but at a lower intensity than that observed in potassium dichromate challenged guinea pigs. However, the reaction was more severe than that observed in guinea pigs challenged with basic chromium sulphate. One animal showed severe ulceration and necrotic area in the epidermis which may indicate a granuloma case (Fig.8). The normal layers of the skin are shown in Fig.1.

Discussion

Chromium dermatitis is one of the most serious occupational dermatoses⁹ and in industrialized countries chromate is the most common sensitizer in males¹⁰. In the course of our study in a tannery in Baghdad, we observed a high incidence of dermatitis among workers in the chrome department (to be published later). This has led us to investigate whether the chromium sulphate liquid which was used in the tanning process could induce and elicit contact dermatitis. For this purpose we used an animal assay, the guinea pig maximization test as proposed by Magnusson and Kligman⁸. These authors believe that there seem to be no instance in which a substance sensitizing the guinea pig fails to do so in man.

Two groups of guinea pigs were sensitized with potassium dichromate (Table 1). All of the 10 animals in

group one gave a positive reaction following epicutaneous challenge with potassium dichromate. Thus the latter hexavalent chromium compound can be considered as a very strong sensitizer. On the other hand, 80% of the animals in group four showed a positive reaction when challenged with chromium sulphate liquid. This indicates that this trivalent chromium compound is a rather strong elicitor of contact dermatitis. However, the intensity of the reaction was strong in group one as compared with group four animals. This is in agreement with the finding of Polak, Turk and Frey⁹ who used trivalent chromium chloride and hexavalent potassium dichromate. This could have been due to the lower skin penetrating capacity of trivalent chromium compounds in comparison to hexavalent chromium compounds. However, it should be mentioned that the important statistic in maximization testing is the frequency of sensitization and not intensity⁸.

Results obtained from tests performed on group two guinea pigs (Table 1) indicate that trivalent chromium sulphate is not only a good elicitor of contact dermatitis but is also a good inducer of sensitivity since 80% of the animals gave a response. The intensity of the response was comparable to that of group four guinea pigs. But, when chromium sulphate solution was used for sensitization and chromium sulphate powder for challenge a rather weak response was elicited in only 40% of the animals (Table 1, Group three). This could have been due to lower concentration of elementary chromium in the solution that was used for sensitization. In clinical practice, both trivalent and hexavalent chromium compounds can provoke a positive patch test reaction when applied to the skin of chromium sensitive patients, trivalent

chromium being used at a much higher concentration¹¹. The requirement for a higher concentration of trivalent chromium compounds may be explained by the finding that these compounds bind strongly to proteins^{12,13}. If these proteins are irrelevant then most of the chromium will be converted into a non-immunogenic form.

In this study, skin biopsies were taken from lesions induced by different chromium compounds in the sensitized guinea pigs. Apart from some seemingly quantitative differences, the histological changes seen upon challenge with potassium dichromate, chromium sulphate powder, or chromium sulphate solution were qualitatively similar. These changes are not specific for chromium and can be seen in allergic contact dermatitis due to other metals and even in irritant contact dermatitis. This also applies to the human situation since light microscopy studies of skin biopsies taken from areas of positive patch test reactions do not generally allow a differentiation between an allergic reaction and an irritant one. In assessing cellular changes seen in skin biopsies taken 72 hour after patch test of chromium sensitive patients, Forslind and Wahlberg¹⁴ found that it was difficult to differentiate between the effect of an alkaline vehicle (pH12) as such and the specific effect of chromate solution. Thus it is not unexpected to find similar qualitative histological changes in the affected areas of the skin of guinea pigs in the various groups.

From the results of this study we could establish that the chromium sulphate solution used in the tanning process can induce and elicit an allergic contact hypersensitivity reaction in guinea pigs. This finding infers that this same solution could impose the same effect on workers in the tanning industry which is the subject of another study.

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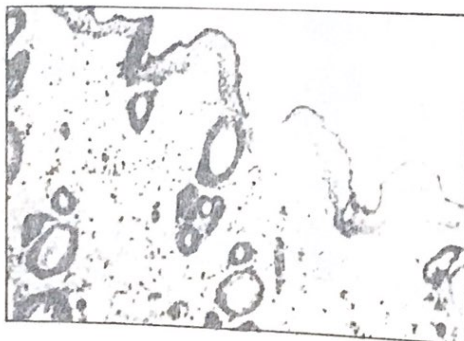
Table 1 - Skin reactivity of guinea pigs sensitized to different chromium compounds

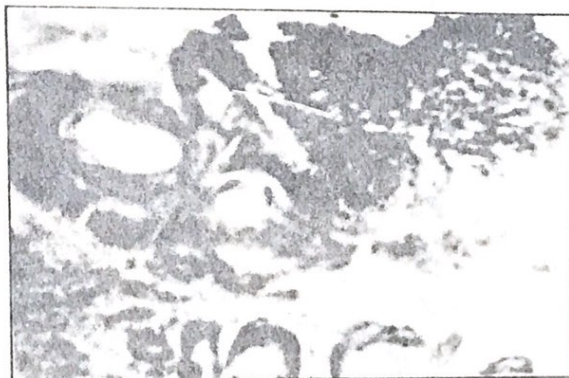
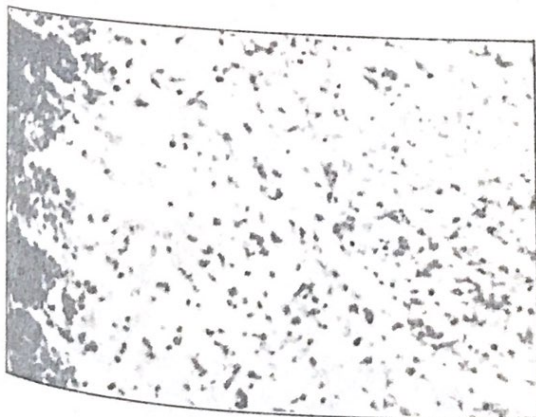
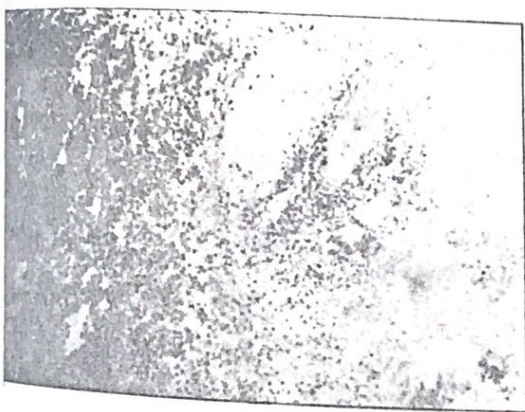
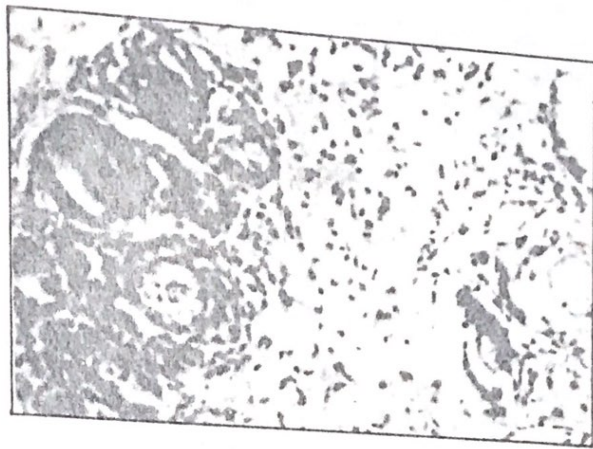
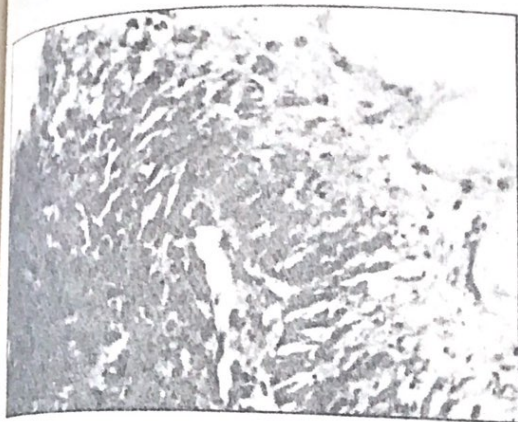
Group No.	Induction	Challenge	No. of animals	Grade*
1	Pot. dichromate 1%	Pot. dichromate 0.5%	3	+++
			6	++
			1	+
2	Chromium sulphate 1%	Chromium sulphate 0.5%	1	+++
			2	++
			5	+
			2	0
3	Chromium** sulphate solution 0.5%	Chromium sulphate 0.5%	4	+
			6	0
4	Pot. dichromate 1%	Chromium sulphate solution 0.5%	1	+++
			3	++
			4	+
			2	0
5	Saline	Pot. dichromate 0.5%	10	0

* Patch testing and severity of reactions were graded according to the specification of Magnusson and Kligman⁸.
0 No reaction

+1 Scattered mild erythema
+2 Moderate and diffuse erythema
+3 Intense redness and swelling

** Chromium sulphate solution taken from the drums during the tanning process (0.5% w/v).





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