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Description of a specific bronchial provocation test for the diagnosis of occupational asthma due to platinum salts

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Key words
Bronchial provocation test; platinum; occupational asthma

Parole chiave
Test di provocazione bronchiale; platino; asma occupazionale

Summary
Background: *Occupational exposure to platinum salts may cause the onset of skin and respiratory disorders with an IgE-mediated allergic mechanism. The diagnosis of occupational asthma due to platinum salts was, in a small number of cases, achieved also via occupational specific bronchial provocation tests (sBPT), which until now were conducted by pouring platinum salt dusts from one tray to another or by direct aerosoling of hexachloroplatinate solutions into the patient's airways.* **Methods:** *Here we describe an original occupational sBPT based on atomization of solutions of ammonium hexachloroplatinate, at increasing concentrations, in a 5 m^3 challenge room: the starting solution is a 1:100 dilution of the preset threshold of the patient's skin reactivity to the substance. In the absence of a bronchoconstrictive response, the following concentration is atomized (each time 10 times higher than the previous one), until the maximum concentration, 10^{-2} M, is reached. The patient is not in the challenge room during atomization of the solutions, but enters when this operation has been completed and remains there for 15 minutes, unless he/she shows signs of respiratory trouble before that time. After each exposure, the patient is clinically monitored, with respiratory function tests at preset times, until at least 8 hours after the end of the exposure.* **Results and Conclusions:** *The test allowed identifying a respiratory hypersensitivity specifically to platinum as cause of asthma in two precious metal workers, with the onset of immediate bronchospasm in one patient and biphasic bronchospasm in the other. Compared to the sBPT by pouring a mixture of platinum salt dusts from one tray to another, the method we designed offers a better standardization of bronchial stimulation and, compared to direct aerosoling of hexachloroplatinate into the patient's airways, it has the advantage of reproducing the respiratory risk conditions occurring in the workplace and offers better safety guarantees for the patient, since it reduces the risk of onset of serious asthmatic or even systemic responses in subjects highly hypersensitive to this metal.*

Riassunto
«Descrizione di un test di provocazione bronchiale specifica per la diagnosi di asma professionale da sali di platino». **Introduzione:** *L'esposizione lavorativa a sali di platino può causare l'insorgenza di patologia cutanea e respiratoria con meccanismo allergico IgE mediato. La diagnosi di asma professionale da platino in non molti casi si*

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è avvalsa anche dell'effettuazione di test di provocazione bronchiale specifica (TPBs) professionale, finora condotti con la metodica del travaso di miscele di polveri di sali di platino o con quella dell'aerosolizzazione diretta di soluzioni di esacloroplatinato nelle vie aeree del paziente. **Metodi:** *Descriviamo una metodica originale di TPBs professionale basata sulla nebulizzazione di soluzioni di ammonio esacloroplatinato, a concentrazioni crescenti, in cabina di esposizione di 5 m^3: la concentrazione di partenza è una diluizione 1:100 della soglia predeterminata in base alla reattività cutanea del paziente alla sostanza; in assenza di risposta broncocostrittiva si procede con l'erogazione in atmosfera della concentrazione successiva (ogni volta 10 volte più elevata rispetto alla precedente), fino a terminare con la concentrazione massima di 10^{-2} M. Il paziente non è presente in cabina durante la nebulizzazione delle soluzioni; vi entra al suo termine e vi staziona per 15 minuti a meno di una più precoce insorgenza di disturbi respiratori. Dopo ogni esposizione egli viene monitorato clinicamente e con prove di funzionalità respiratoria a tempi prefissati, fino ad almeno 8 ore dal termine dell'esposizione.* **Risultati e conclusioni:** *Il test ha consentito di individuare come causa dell'asma di 2 lavoratori di metalli preziosi una condizione d'ipersensibiltà respiratoria specifica a platino, con insorgenza di broncospasmo immediata in uno e difasica nell'altro. Rispetto al TPBs condotto con il travaso di miscela di polveri di sali di platino il metodo da noi messo a punto è caratterizzato da una miglior standardizzazione della stimolazione bronchiale; rispetto all'aerosolizzazione diretta di esacloroplatinato nelle vie aeree del paziente ha il vantaggio di riprodurre le modalità del rischio respiratorio che si verificano sul lavoro e di offrire maggiori garanzie di sicurezza per il paziente, in quanto riduce il rischio d'insorgenza di gravi risposte asmatiche od anche sistemiche in soggetti marcatamente ipersensibili al metallo.*

Introduction

Platinum is a transition precious metal without antigenic properties; nevertheless, many studies on precious metal workers and catalyst production workers (8-1) have shown that platinum salt compounds may cause skin and mucosal sensitivity with type I hypersensitivity mechanism (IgE-mediated) according to the Gell and Coombs classification (2-10). Chlorinated soluble compounds like hexachloroplatinic acid and its ammonium and potassium salts are the chemical formulations that are most dangerous in this sense, because they can cause hives and contact dermatitis, rhinitis, conjunctivitis and also bronchial asthma (13).

In most of the articles available in the literature, diagnosis of occupational platinum-related asthma was based on a positive stop-resume test result, associated with prick tests positive to platinum salts and/or the presence of specific IgE in the serum. Some studies also included specific bronchial stimulation tests, adopting differentiated exposure methods: in the 1960's and 1970's Bijl (3) and Pepys (11) poured mixtures of platinum salt dusts (ammonium hexachloroplatinate, ammonium tetrachloroplatinate and sodium hexachloroplatinate) from one tray to another; in the 1990's Merget (7) used a respiratory stimulation technique similar to the non-specific bronchial provocation test with methacholine, administering increasing concentrations of hexachloroplatinic acid directly into the patient's airways, by means of a jet nebulizer.

The aim of this paper was to describe a specific bronchial provocation test which we designed for the diagnosis of platinum-related occupational asthma, based on the preset threshold of the patient's skin reactivity to the substance.

Case studies and methods

Patients

Two male patients were studied, aged 31 and 45, referred to us for oculorhinitic and respiratory disorders (dry cough, dyspnoea and wheezy breathing), occurring at the workplace.

In the first patient, employed in a company operating in primary and secondary smelting and electrolytic refining of precious metals (silver, gold, platinum and palladium), who had smoked 25-30 cigarettes/day for the last 10 years, symptoms had started about 10 months previously. In another

medical facility he underwent prick-tests against common environmental inhalants, which were positive to mites and cat dander, even though before that time he was asymptomatic; the total serum IgE dosage had shown above average values (199 UI/ml) and a slight hypereosinophilia in peripheral blood.

In the second patient, who was working on platinum refining for the subsequent production of medals and coins by plating, smelting and cold molding processes, who had been a smoker (11p/y) until 3 years before, respiratory symptoms had started 18 months before, preceded by about 6 months by pruritic dermatosis in the upper limb areas not protected by gloves or clothing. At the time of the onset of the first symptoms he was working in the refinery shop; he was then moved to the plating shop, where symptoms improved at first, but then started to worsen. In the meantime, he underwent prick-tests against the most common environmental inhalants, with negative outcome and normal dosage of total serum IgE (84 UI/ml)

At the time of our assessment, both patients were being treated by inhalation of an association of cortisone and long-acting beta2 agonist drugs and orally with antihistamines.

Study methods

Allergologic investigation: we performed the basic study consisting of prick-tests with commercially available preparations against common environmental inhalants (Lofarma Allergeni Milan) only on the second patient, because for the first patient a condition of atopy had already been documented with certainty.

In both patients platinum sensitization was investigated by means of a prick-test with ammonium hexachloroplatinate in water solution, buffered at pH 7 (phosphate), at increasing concentrations starting from 10^{-6} M and up to 10^{-2} M. The test reading, performed after 15 minutes and after 2 hours, was considered inconclusive (+) when the skin reaction was smaller than half the weal caused by histamine, mildly positive (++) if equal to half of the reaction to histamine, clearly positive (+++) if equal to that of histamine, strongly positive (++++) if greater than the diameter of the weal from histamine. As a control of the specificity of the skin reaction, prick-tests against platinum at the same concentrations used in the 2 patients under study were also performed on 15 subjects (6 of whom were atopic), not exposed to metal, after obtaining their informed consent.

Both workers also underwent patch tests against ammonium tetrachloroplatinate 0.25% PET, gold-sodium thiosulfate 0.5% PET, palladium chloride 1% PET, metallic zinc 2.5% PET, potassium dichromate 0.5% PET, metallic ruthenium 0.1% PET, tetrachloroauric acid 0.1% PET (F.I.R.M.A. allergens) and against ammonium hexachloroplatinate 0.2% H_2O, silver nitrate 1% H_2O, copper sulfate 4% H_20, selenium dioxide 0.1 % H_2O, cobalt chloride 1% H_2O, cadmium sulfate 1% H_2O, ammonium molybdate 1% in H_2O, prepared in our laboratory of Industrial Hygiene and Biotoxicology. The reading of the patch-tests was performed after 20 minutes to check for urticarial reactions and then after 48 and 72 hours, with the SIDEV/GIRDCA 1999 interpretation criteria (5).

Basal respiratory functional study: this was performed following the performance and interpretation recommendations of ATS-ERS (9) and included the measurement of static and dynamic lung volumes (FVC, VC, FEV1) and maximum expiratory flows at 75%, 50%, 25% of FVC.

Study of non-specific bronchial reactivity to methacholine: the non-specific bronchial provocation test was carried out one week before the specific one with platinum salts, by means of an ME.FAR inhaler dosimeter, with the method suggested by the ATS-ERS protocol (6). The 2 workers underwent a second bronchial provocation test also the day after the specific bronchial test with platinum. The patient with a PD_{20} lower than 1600 μg of the substance was considered hyper-reactive, as indicated by ATS-ERS (6).

Occupational specific bronchial provocation test (sBPT) with platinum salts: in the method we designed, aliquots of 2 ml of increasing concentra-

tions of the ammonium hexachloroplatinate water solution used for the prick-tests are administered in 2 minutes in a challenge room (measuring 5 m^3). This is done by atomization with a PTFE cross-flow nebulizer (Venturi System) (14) that is able to produce particles mostly smaller than 10 micron. The patient is not in the challenge room during aerosoling, but enters and remains there for 15 minutes, unless respiratory disorders occur before this time, associated with a FEV1 reduction equal to or higher than 20% of the basal value. After exposure, FEV1 is monitored at 5, 10, 15, 30, 60, 90 and 120 minutes (4); if it does not drop by at least 20% of the basal value, ammonium hexachloroplatinate solutions are atomized at progressively increasing concentrations. In the case of a bronchoconstrictive response, monitoring of FEV1 is continued, at least every hour, up to at least 8 hours after the end of the exposure. The concentration of the first atomization is made up of the first 1:100 dilution of the solution that triggered the first definite skin reaction (prick-test the same size as the histamine weal); any subsequent atomization is each time 10 times more concentrated than the previous one, until the maximum 10^{-2} M concentration of the solution is reached. Measurement of platinum exposure is made during the 15 minutes the patient stays in the challenge room after atomization, by means of atmospheric sampling with 3 l/min flow and ICP-MS analysis.

Test specificity was assessed by also performing it, after obtaining their informed consent, on 2 subjects not exposed to platinum salts, hyper-reactive to methacholine (one with a reactivity threshold of 200 μg, and the other 1450 μg), and asthmatic for other causes.

Results

Table 1 reports, for the 2 patients, the outcomes of the allergological skin tests against platinum, the basal respiratory test and the bronchial reactivity test with methacholine measured before performing the bronchial provocation test with hexachloroplatinate.

In both patients the prick test was clearly positive for ammonium hexachloroplatinate; conversely, the patch tests against platinum and the other metals were negative, while basal respiratory conditions and non-specific bronchial reactivity were normal. In patient No. 2 the prick-tests against common environmental inhalants were confirmed negative, excluding atopy.

The prick-tests against ammonium hexachloroplatinate in the 15 controls were negative, also at the highest concentration (10^{-2} M).

The results of the occupational sBPT to ammonium hexachloroplatinate are shown in figure 1 and table 2.

In the first patient exposure started at the 10^{-7} M concentration; after atomizing 2 ml of the 10^{-4} M solution, his stay in the challenge room was discontinued after 10 minutes because he developed respiratory problems and a significant FEV1 drop (-32%) compared to the pre-test value; FEV1 serial monitoring then showed a spontaneous return to basal values within 2 hours. In the second patient exposure started at a 10^{-6} M concentration; a significant bronchoconstrictive reaction occurred after the 15 minutes' stay in the challenge room after atomizing 2 ml of the 10^{-2} M solution; the reaction was biphasic [dual reaction, as per the classification adopted by Perrin (12)]: first a 27% FEV1 drop, followed by a spontaneous return to basal values within 90 minutes after exposure, and by a second

Table 1 - Personal and clinical characteristics of the 2 patients occupationally exposed to platinum suffering from oculorhinitic and respiratory disorders at the workplace

	Age Yrs	Atopic state	Prick-test against $(NH_4)_2PtCl_6$	Basal FEV_1 lt (% of predicted - CECA)	PD_{20} methacholine before sBTP μg
Patient 1	31	yes	+++ (10^{-5} m)	5.77 (101%)	2250
Patient 2	45	no	+++ (10^{-4} m)	4.24 (112%)	>3200

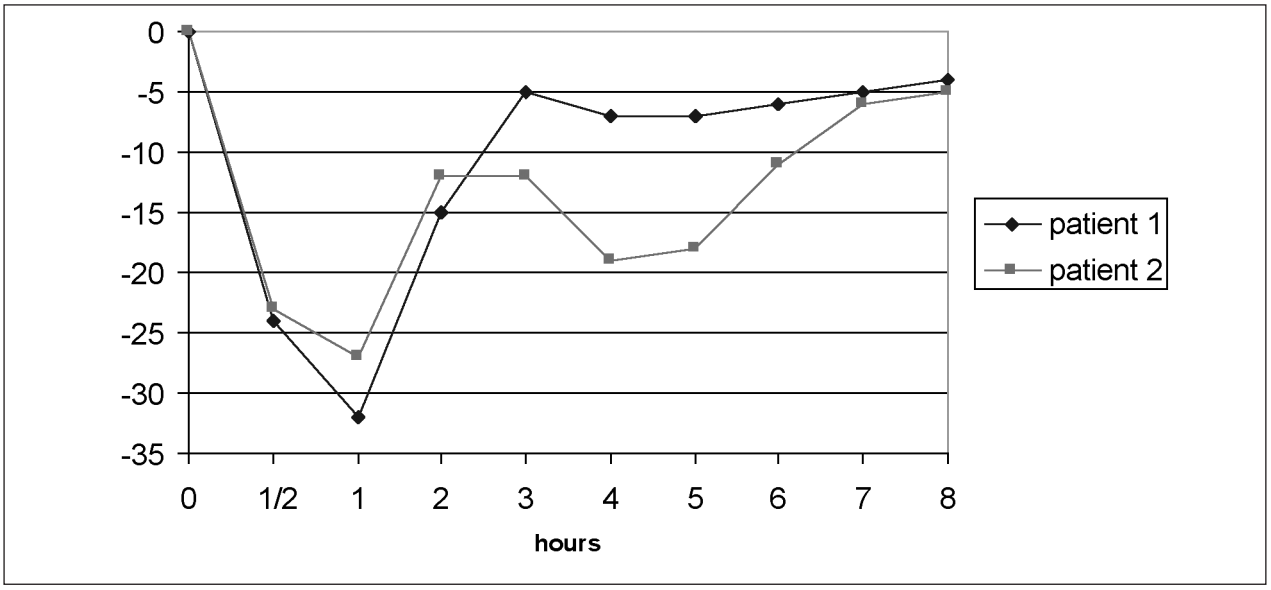

Figure 1 - Changes in lung function of the 2 patients occupationally exposed to platinum in the specific bronchial provocation test with ammonium hexachloroplatinate

Table 2 - Lung function values of the 2 patients occupationally exposed to platinum in the specific bronchial provocation test with ammonium hexachloroplatinate

	Basal FEV_1	30'	1	2	3	4	5	6	7	8
Patient 1	5770	4390	3930	4900	5480	5360	5360	5425	5480	5540
		-24%	-32%	-15%	-5%	-7%	-7%	-6%	-5%	-4%
Patient 2	4240	3270	3100	3730	3730	3435	3480	3770	3990	4030
		-23%	-27%	-12%	-12%	-19%	-18%	-11%	-6%	-5%

less intense bronchial constriction starting from the 4th hour (up to -19% FEV1 drop), with a spontaneous resolution by the 6th hour.

The platinum concentration to which the 2 patients were exposed during their stay in the challenge room changed from 0.0003 μg/m^3 when the initial 10^{-7} M concentration was atomized, to 30 μg/m^3 when the highest concentration 10^{-2} M was atomized.

Therefore, calculating a ventilation of the patient at rest of 8 litres of air per minute, the amounts of platinum inhalable during a 15 minute stay started from 0.36 ng (atomization of the 10^{-7} M solution) and increased until they reached 3.60 μg (atomization of the 10^{-2} M solution).

In both patients sBPT also caused dry cough, wheezy breathing and sneezing crises starting from the same concentration of the metal salt which caused the FEV1 drop.

Only patient No. 1 accepted repeating the bronchial provocation test with methacholine the day after sBPT, and it was possible to observe that he developed non-specific hyper-reactivity of the airways (his PD_{20} to methacholine dropped from 2250 to 400 μg).

The sBPT in the two controls, who were hyperreactive to methacholine but not occupationally exposed to platinum, were negative.

Discussion

In the sBPT we designed to assess airways reactivity to platinum salts in subjects exposed occupationally and suffering from asthma, patients freely inhale amounts of the substance aerodispersed in a challenge room. It is therefore a bronchial stimulation that reproduces "in the laboratory" the respiratory risks occurring in the workplace. The administration of platinum salts in the challenge room, based on the preset threshold of the patients' skin reactivity to the substance, is performed so as to obtain a preset and low stimulus dose even with the most concentrated solution (10^{-2} M); as a matter of fact, in a work environment complying with the 2 μg/m^3 (ACGIH) TLV the total amount of platinum inhaled over a work shift is about 10 μg (2 μg/m^3 x approx. 5 m^3 of ventilated air during the 8-hour shift, assuming that the worker does not perform a physically demanding job), therefore about three times the maximum quantity of platinum inhalable by the patient in the challenge room. The decision to expose the patient in the challenge room starting only from the end of atomization of the solutions is a further safety precaution, taken to minimize the risk of serious asthmatic disorders or systemic responses in highly sensitive people and to reduce mucosal irritation due to aerosol acidity. Moreover, the artificial ventilation system of the booth guarantees a homogeneous distribution of the aerosol before the patient goes inside.

Regarding the bronchial stimulation techniques against platinum used by others, it should be noted that no information on the degree of atmospheric pollution produced during the sBPT conducted with platinum salt dusts manipulation was given by Bijl (3), or Pepys (11), while the amounts of hexachloroplatinate inhaled by patients in Merget's technique (7) were similar to those in our test, but they were aerosoled directly into the subject's airways and are therefore potentially irritating or dangerous.

The test we performed properly identifies subjects specifically sensitive to platinum; it is therefore possible to rule out that the elicited asthmatic reactions are non-specific, because it did not cause bronchial constriction in asthmatic controls sensitive to methacholine but not exposed to platinum.

The sBPT proved to be essential in identifying the causal agent of occupational asthma in the 2 patients studied: the medical history and the positive reaction to the specific prick-test, although corroborating, were not able alone to prove that platinum was the causal agent of bronchial disorders, since they also suffered from rhinitis. Moreover, both subjects showed normal bronchial reactivity to methacholine except for the dyspnoeic crises related to their work. In this respect, it should also be noted that Merget's (8) large study group included people not hyper-reactive to methacholine but specifically reactive in sBPT to platinum salts. Lastly, the patterns of the induced bronchial reactions (immediate in one patient, dual in the other), were similar to those already described by other authors who investigated platinum-related asthma, who documented both immediate asthmatic reactions (the most frequent), and dual and late bronchoconstrictive responses.

No potential conflict of interest relevant to this article was reported

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