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In vivo cytogenetic studies of the genotoxic effects of polymethyl methacrylate employed in orthopaedics

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Polymethyl methacrylate (PMMA) is a material employed as a cement in prosthesis that has been found to induce cytogenetic damage in human lymphocytes *in vitro*. This effect is particularly achieved before the 5th day of the exposition, while after this period no significant variations appeared. In the present study, results of cytogenetic tests in humans are reported. Sister chromatid exchange analysis and micronucleus tests have been performed on lymphocytes of patients who had undergone prosthesization with cemented prosthesis (group A) or with biological anchorage prosthesis, as control (group B). DNA damage was investigated before implantation and 5 d after surgery in both groups. Cytogenetic tests did not show any significant increase in the number of micronuclei and sister chromatid exchanges with respect to control values in patients with PMMA cemented prosthesis.

1. Introduction

Synthetic materials employed for biomedical applications must meet severe acquisites in order to prove their real biocompatibility. The tests usually performed to evaluate the biocompatibility primarily include an *in vitro* stage, and eventually tests on animals and man. Short- and long-term pathological effects caused by the introduction of the biomaterial into the human organism must be investigated, because systemic effects or local reactions, related to biochemical and/or histological variations of tissues next to the biomaterial must occur.

The cytogenetic tests represent an effective approach to complete the picture of the compatibility tests. In fact, they can reveal the exposure to mutagenic and cancerogenic substances, quantifying the frequency of chromosomal damage in subjects exposed to the biomaterial.

Polymethyl methacrylate (PMMA, CAS No 9011-17-7) is an acrylic resin widely used in orthopaedy, odonthology and in ophthalmology, owing to its physical attributes such as transparency, lightness and hardness which make it useful for several applications. The carcinogenicity assay performed in man to test methyl methacrylate (MMA), the component that, when polymerized, fixes the orthopaedic prosthesis to the bone tissue, did not provide sufficient information, even though recent data reported by IARC (1994) [1] classified the MMA into group number 3, in which potential cancerogenic substance are included. The evaluation of data reported in the literature concerning the unreact MMA monomer reveals that this material produces some systemic toxic effects in man and in laboratory animals, even if the substance is rapidly metabolized by hydrolysis and oxidation to CO_2, *in vivo* [2]. Successively, CO_2 is largely eliminated by breathing, while a small part is secreted in urine as thioether.

Here we report the results concerning the genotoxic effect of PMMA, *in vivo*, performed by the sister chromatid exchange analysis and the micronucleus test, applied on peripheral lymphocytes of patients in which a PMMA cemented prosthesis has been implanted.

Micronuclei (MN) are formed during cell division from acentric fragments or whole chromosomes that have not been incorporated into the main nuclei at cell division and provide a measure of both chromosome breaks and whole chromosome loss [3]. Sister chromatid exchanges (SCEs) represent symmetrical exchanges between sister chromatids; they do not result in alterations of the chromosome optical morphology nor genetic information [4].

A 5 d follow up has been chosen because previous *in vitro* results [5–7] showed no significant increase of DNA damage over longer periods. Furthermore, toxic products carry on their effects *in vivo* just for a short time, as the rapid metabolism they undergo greatly reduces the time of their activity in the body.

2. Materials and methods

2.1. MMA and PMMA

The cement was the Surgical Simplex P (Howmedica International Ltd, London UK). It is provided as a combination of powder and liquid, the powder particles ranging from 30–150 μm diameter. The powder comes in two forms: radiopaque and non-radiopaque. The radiopaque powder consists of 88% PMMA, mostly polystyrene copolymer, 10% barium sulphate, 2% benzoyl peroxide. The non-radiopaque form lacks barium sulphate and is 98% PMMA, with the remainder the same. The chemical composition of the liquid is 97.5% methyl-methacrylate, 2.5% dymethyl-*p*-toluidine as activator, and hydroquinone as inhibitor. The inhibitor prevents polymerization of the monomer during storage, whereas the activator causes benzoyl peroxide to initiate polymerization after mixing. When the liquid and powder are mixed, the monomer softens the polymeric beads to form a gel. The dymethyl-*p*-toluidine in the liquid reacts with the benzoyl peroxide in the powder to produce reactive benzoyl-free radicals. As these radicals react with the monomer, breaking the double bonds, polymerization is started.

The amount of heat produced during polymerization is directly related to the amount of monomer polymerizing, but different conditions can modify the rise in temperature. Peak polymerization temperatures are higher when the ratio of powder to monomer is lowered, when the mixing temperature is raised, and when the cement is thicker. Under standard conditions, the temperature in the centre of the cement may exceed 100 °C when the mass is thick (about 1 cm). However, the temperature at the surface of the cement mass in a clinical situation is much lower.

Immediately after polymerization, approximately 3% residual monomers remain in the cement; this amount falls to 2% within a few hours and to 1.5% after 4 mon.

The amount of methyl methacrylate that reaches the systemic circulation during and shortly after the polymerization causes no significant toxicity, but does have an observable effect on several constituents of normal serum.

2.2. Description of samples

Lymphocyte cultures were collected using whole blood of each patient drawn before and 5 d after surgery.

The group under observation was composed of 10 subjects in which a PMMA-cemented prosthesis has been implanted (group A). The control group (group B) was formed by patients who received the same diagnostic and surgical treatment reserved for the patients of group A, except for the biological anchorage prosthesis.

The two groups were comparable for age, sex, life behaviour, cause of surgical therapy (coxarthrosis mostly) and pharmacological treatment. All the patients were females, except for one male subject of group B; the mean age was 66.5 ± 8.55 for group A, and 61.6 ± 6.85 for group B. The sample was also homogeneous concerning smoking behaviour, as it was composed of only one smoker in each group.

2.3. Cell culture

Heparinized whole blood belonging from each patient was cultured in RPMI-1640 (Sigma Chimica, Milan, Italy) supplemented with 30% foetal calf serum (FCS, Sigma), 3% phytohaemagglutinin (PHA-M, Sigma) and antibiotics (100 U/ml penicillin and 50 μg/ml streptomycin).

Bromodeoxyuridine (5 μg/ml BrdU, Sigma) and colchicine (0.25 μg/ml Colcemid, Sigma) were added to cultures at 24 and 70 h, respectively for SCEs analysis. Cytocalasin B (6 μg/ml, Sigma) was added to the flasks for MN test at 44 h. After 72 h incubation in the dark and at 37 °C, all the cultures for SCEs and MN tests were treated with a hypotonic solution and then routinely fixed (methanol and acetic acid 3 : 1). After centrifugation, the supernatant was fully removed and splashes of lymphocytes were made.

Slides of micronucleated cells (MC) were stained with Giemsa 5% [8]. Slides of SCEs were stained with the fluorescence + Giemsa (FPG) technique according to standard procedures [9].

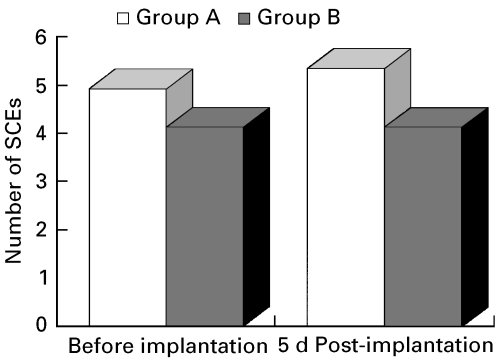
The frequency of SCEs was counted in 50 metaphasic plates/sample, while the frequency of MC was estimated by counting 1000 binucleated/sample.

2.4. Statistical analysis

The mean values of SCEs have been compared by the *t*-test, after the normalization of the data, while the chi-square test (χ^2 test) has been employed for the evaluation of MC frequency.

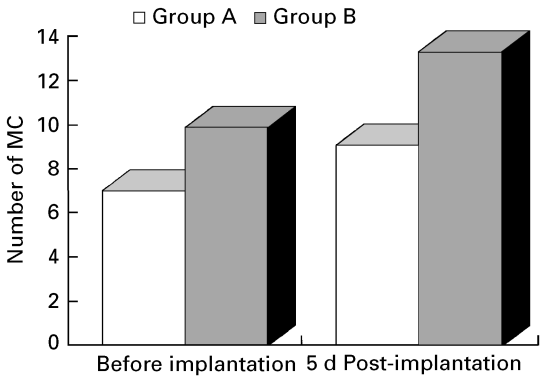
3. Results

The results of SCEs analysis performed on the values of group A reveal a mean frequency of 4.93 ± 0.85 SCEs before operation, and of 5.37 ± 1.18 SCEs after.



	Mean of SCEs/patient	
	Before implantation	After implantation
Group A	4.93 ± 0.85	5.37 ± 1.18
Group B	4.14 ± 0.35	4.15 ± 0.42

Figure 1 Mean frequency of SCEs in the patients of group A (PMMA cemented prosthesis) and of group B (non cemented prosthesis) before and after implantation (ten subjects for each group). No significant differences were found.



	Mean of MC/patient	
	Before implantation	After implantation
Group A	7 ± 4.95	9.11 ± 5.69
Group B	9.89 ± 5.37	13.33 ± 8.38*

*Significant difference ($P < 0.05$ χ^2 test).

Figure 2 Mean frequency of MC in group A (PMMA cemented prosthesis) and in group B (non-cemented prosthesis) before and after implantation (nine subjects for each group). The increase of MC 5 d after implantation is significant ($p < 0.05$ χ^2 test) in group B. Two subjects of group B had a higher increase of MC than other subjects, which amplifies the standard deviation.

In the subjects belonging to group B, mean frequencies of 4.14 ± 0.35 SCEs are observed before implantation, and 4.15 ± 0.42 SCEs after (Fig. 1).

The SCEs frequency evaluated before and after the surgical treatment does not result in a significant increase in either group, according to our statistical analysis. The analysis of MC frequency is showed in Fig. 2. Only the values obtained from nine subjects have been examined, because of few data available from one subject of each group. In group A, the mean frequency of MC is 7 ± 4.95 before implantation and 9.11 ± 5.69 5 d after operation. In group B, at the same observation time, the mean frequency of MC is 9.89 ± 5.37 and 13.33 ± 8.38. The increased values observed in group B is significant per 5%.

4. Discussion

Professional or chirurgical exposition to MMA, the monomeric form of the bone cement PMMA, produces some negative effects, such as contact dermatitis [10] and asthma [11], as well as gastrointestinal [12], cardiovascular [13, 14] or respiratory consequences [15]. Furthermore, the monomer interacts with the endocrine system modifying the level of insulin, prolactine and somatotrope hormone [16].

The monomer, which is responsible for the main toxic effects observed in the use of cemented prosthesis implants, is the ingredient which is released in the largest amount; in fact, 2%–5% monomer is not polymerized.

Studies of the genotoxic effect, according to the IARC data [1], reveal that MMA does not induce any increase of reversion in stocks of *Salmonella typhimurium*, either in the absence or presence of metabolic activators, while there is an increase of mutations if metabolic activators are added [17]. Indeed, in the cytogenetic tests, the MMA has been proved to increase the CAs and the MN in murine lymphoma cell lines, as well as to produce an increase of CAs and SCEs in Chinese hamster ovary cells, *in vitro*. Research on experimental stocks did not prove a significant increase of MN frequency in the bone marrow cells of mice treated by MMA [18], while an increase of CAs frequency in the bone marrow cells of rats was noticed, under the same experimental conditions [19].

Our results show that the frequency of SCEs, analysed in humans before and after implantation of PMMA-cemented prosthesis, does not undergo any significant variations, thus confirming, *in vivo*, the results already obtained *in vitro* on human lymphocytes exposed to PMMA soon after its polymerization [3]. Furthermore, significant differences in SCEs frequency, before and after surgery, do not take place either in the subjects of group B, showing in this way that no other factors related to surgical treatment, for example anaesthesia, increase SCEs.

On the contrary, the bone cement had induced an increase of MN in human lymphocytes, *in vitro* [4]. Such a difference is probably due to the fact that the unreacted MMA monomer released in small amounts during and after the polymerization, is quickly eliminated *in vivo*, while *in vitro* it accumulates in the medium and could carry on a genotoxic effect. In group B (non-cemented prosthesis), the MN test showed an unexpected increase of MC after implantation, that could be explained by the presence, in this group, of two subjects with a greater increase of MC at follow up, that amplifies the standard deviation and may produce the significant χ^2 result in this case. As every factor was comparable (i.e. anaesthesia, surgical technique, smoking behaviour, age), unknown individual characteristics of the two patients are presumably responsible for the stronger increase of MC.

5. Conclusion

The cytogenetic tests performed have not shown any chromosomal damage caused by PMMA or by other substances released (during or after polymerization) up to 5 d after operation. Yet, either the contradictory results reported in the literature concerning the possible genotoxic or cancerogenic effect of this biomaterial, or mostly the important complications of the cemented prosthesis, once again advise against their use in young patients, in which the biological anchorage prosthesis is more suitable.

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