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Preclinical Evaluation of Silated Hydroxyethylcellulose – Bacterial Mutagenicity and Toxicity Test

Przedkliniczna ocena krzemowej pochodnej hydroksyetylocelulozy – bakteryjny test mutagenności i toksyczności

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Abstract

Background. Silated hydroxyethylcellulose was created at the Department of Physiochemistry and Polymer Technology Silesian Technical College in Gliwice and the Department of Conservative Dentistry and Periodontology Silesian Medical University in Katowice. The polymer creates a network of hydroxyethylcellulose chains connected by silane coupling agents. Such structure, enabling the penetration of newly formed bone into polymer, can help one treat bone defects. The authors predict the possibility of using polymer as a carrier for such materials as hydroxyapatite, tricalcium phosphate or calcium hydroxide in periodontology, surgery or endodontics.
Objectives. Evaluation of genotoxicity caused by silated hydroxyethylcellulose using short-term Salmonella test.
Material and Methods. Two strains of *Salmonella typhimurium* TA98 and TA100 were used. Aqueous extract of silated hydroxyethylcellulose was estimated in three doses: 125, 250 and 500 µl per plate, twice. The value of mutagenic activity index and percentage of bacterial survival was calculated. Statistical analysis was performed using Bernstein's test ($p < 0.05$).
Results. The mutagenic activity index for both strains was below 1 (ranged from –0.03 to +0.27). The percentage of bacterial survival was over 50 (ranged from 86.1 to 100). The dose-response dependences for silated hydroxyethylcellulose were not statistically significant.
Conclusions. Evaluation of silated hydroxyethylcellulose properties with short-term *in vitro* tests showed no mutagenicity and toxicity for the used bacterial strains (**Dent. Med. Probl. 2005, 42, 1, 27–30**).

Key words: preclinical evaluation, mutagenicity tests methods, Salmonella test.

Streszczenie

Wprowadzenie. Krzemowa pochodna hydroksyetylocelulozy jest polimerem, który powstał w wyniku współpracy Katedry i Zakładu Stomatologii Zachowawczej i Chorób Przyzębia Śl. AM z Katedrą Fizykochemii i Technologii Polimerów Politechniki Śląskiej w Gliwicach. Materiał ten tworzy zawieszoną w środowisku wodnym sieć zbudowaną z łańcuchów hydroksyetylocelulozy połączonych grupami krzemowymi. Taka budowa umożliwia penetrację nowo powstającej kości do wnętrza polimeru. Są prowadzone badania dotyczące zastosowania polimeru jako nośnika takich materiałów, jak hydroksyapatyt, fosforan trójwapniowy czy też wodorotlenek wapnia w leczeniu periodontologicznym, chirurgicznym i endodontycznym.
Cel pracy. Ocena genotoksyczności krzemowej pochodnej hydroksyetylocelulozy krótkoterminowym testem bakteryjnym.
Materiał i metody. Jako organizmy testowe posłużyły dwa szczepy bakterii *Salmonella typhimurium*: TA98 i TA100. Wodny roztwór polimeru zbadano w trzech stężeniach: 125, 250 i 500 µl/płytkę w dwóch powtórzeniach. Obliczono procent bakterii przeżywających i wielkość efektu mutagennego. Przeprowadzono analizę statystyczną metodą Bernsteina z założonym poziomem istotności $p < 0,05$.
Wyniki. Wartość aktywności mutagennej obliczona dla najwyższej badanej dawki mieściła się w przedziale od –0,03 do +0,27, była więc < 1, co świadczy o braku efektu mutagennego. Również procent bakterii przeżywających dla tej samej dawki, który wynosił 86,1–100%, potwierdza brak efektu toksycznego badanego polimeru. Zależności dawka–odpowiedź dla krzemowej pochodnej hydroksyetylocelulozy i obu szczepów testów były nieistotne statystycznie.
Wniosek. Ocena właściwości krzemowej pochodnej hydroksyetylocelulozy krótkoterminowymi testami *in vitro* nie wykazała mutagenności i toksyczności dla zastosowanych szczepów bakteryjnych (**Dent. Med. Probl. 2005, 42, 1, 27–30**).

Słowa kluczowe: badania przedkliniczne, test genotoksyczności, test bakteryjny Salmonella.

Silated hydroxyethylcellulose is one of these materials which can be used as a carrier of drugs or grafts. It was offered to authors by the Department of Physiochemistry and Polymer Technology Silesian Technical College in Poland.

The polymer creates a network of hydroxyethylcellulose chains connected by silane coupling agents suspended in water. Such structure, enabling the penetration of newly formed bone into polymer, can help one treat bone defects. It has good adhesive strength to many substances for example ceramics. The authors predict the possibility of using polymer as a carrier for such materials as hydroxyapatite, tricalcium phosphate or calcium hydroxide in periodontology, surgery or endodontics.

The first stage of preclinical investigations are short-term tests using bacteria and fungi as tested organisms [1]. The Ames test is used world-wide as an initial screen to determine the mutagenic potential of new chemicals and drugs. International guildelines have been developed for use by corporations and testing laboratories to ensure uniformity of testing procedures [2, 3]. Presented examination was conducted according to PN-EN ISO 7405:2000 and ISO 10993-3:1992 standards [1, 4].

The aim of the study was the evaluation of genotoxicity caused by silated hydroxyethylcellulose using short-term Salmonella test.

Material and Methods

Mutagenicity and toxicity of silated hydroxyethylcellulose was estimated using short-term test with *Salmonella typhimurium* strains TA98 and TA100 in two variants: without (–S9) and with enzymatic activation (+S9) [5–7]. S9 is a cofactor-supplemented postmitochondrial fraction prepared from the livers of rats. This mammalian metabolic activation system is used to activate compounds that are not directly mutagenic or toxic.

The reversion mutation Salmonella test from histidine auxotrophy ("his^-") to histidine prototrophy ("his^+"), which produce a histidine independent strain of this organism, was used for indication of mutagenicity. The *S. typhimurium* strains TA98 and TA100 were kindly provided by Prof. Bruce N. Ames (University of California, Berkeley, CA, USA). Each standard tester strain contains a different type of mutation in the histidine operon. TA98 detects mutagens that cause frameshift mutations and TA100 – base-pair substitutions. In mutagenic test the number of spontaneous revertants that is the number of bacteria cells (SR), in which mutation to "*his^+*" form ran spontaneously, was examined. It is a characteristic and constant number in a certain range for a given strain. The number of revertants in negative controls (K1-6 and K1-6+S9) was also verified. The level of revertants induced by direct mutagen – 4-nitroquinoline-N-oxide (NQNO) and indirect benzo(a)pyrene (B(a)P) – positive control was also examined. Colonies of spontaneous revertants, induce revertants in negative and positive controls as well as samples were counted after 48 hours.

Mutagenic effect of tested material was expressed as relative factor called mutagenic rate (MR), including the number of revertant colonies in the appropriate negative control, according to the equation 1 was done:

$$MR = \frac{\text{revertants/sample} - \text{revertants/control}}{\text{revertants/control}}. \quad (1)$$

The sample was considered evidently mutagenic when its mutagenic rate MR ≥ 2.

During toxicity studies L-histidine was added to provide conditions for the growth of all bacteria. Cellular toxicity was determined by mixing of different dilutions of the bacteria culture (10^{-4} for TA98 and 10^{-5} for TA100) with the same concentrations as in mutagenicity assay. These plates were incubated at 37°C for 48 h, then initial number of colonies bacteria, colonies of bacteria in appropriate negative control (T1-3, T6 and T1-3+S9, T6+S9) and number of colonies on plates with samples was evaluated. The percentage of surviving bacteria was considered as the toxicity indicator according to the equation 2:

$$T = \frac{\text{number of bacteria/sample}}{\text{number of bacteria/control}} \times 100\%. \quad (2)$$

The percentage of surviving bacteria lower than 50% confirm the toxic property of tested materials.

Statistical Analysis

Data bases, statistical analysis and graphs were done using STATISTICA for Windows, version 5.0 (StataSoftInc. 1998).

Analysis of linear regression using the method of the least-square was carried out for each sample. The doses of aqueous solution were treated as independent variable while the number of induced revertants (mutagenicity) or colonies of bacteria (toxicity) as dependant variable. The number of revertants or bacteria in suitable negative control was treated as reply for zero dose.

Results

Characteristics of tested strains

Before performing a set of tests the strain genotypes were confirmed: histidine requirement, *rfa* and *uvrB* mutations, presence of pKM 101 plasmid. In each experiment SRR and positive controls using diagnostic mutagens to confirm the revertion properties, the specificity of each strain and the efficacy of the S9 mix were included. Spontaneous reversion rates for strains were in typical range according to literature: 37 ± 11 for strain TA98 and 165 ± 31 for strain TA100.

Mutagenicity

Relationship between doses of tested material and number of induced revertants shows Figure 1. No significant linear correlation was observed between three tested doses of aqueous solution of silated hydroxyethylcellulose and induced number of TA98 revertants. Significant, but negatively linear relationship dose vs. number of revertants was determined for TA100+S9. Mutagenic rate was calculated for highest dose (Tab. 1). Results indicate that silated hydroxyethylcellulose was not mutagenic for both strains of Salmonella: TA98 and TA100, regardless of the enzymatic activation variant.

Toxicity

Relationship between doses of tested material and number of colonies of bacteria shows Figure 2. Considering the study carried out using both strains, no significant linear correlation between three doses of aqueous solution of silated hydroxyethylcellulose and number of colonies of bacteria was stated. Percentage of surviving bacteria in highest dose is presented in Table 2. Results indicate that silated hydroxyethylcellulose was not toxic for both strains of Salmonella: TA98 and TA100, regardless of the enzymatic activation variant.

Table 1. Mutagenic rate (MR) of highest dose of aqueous solution of silated hydroxyethylcellulose tested by Salmonella assay

Tabela 1. Ocena aktywności mutagennej najwyższych badanych dawek krzemowej pochodnej hydroksyetylocelulozy za pomocą testu bakteryjnego Salmonella

Dose of 500 µl (Dawka 500 µl)	Strain TA98 (Szczep TA98)		Strain TA100 (Szczep TA100)	
Activation (Aktywacja)	–S9	+S9	–S9	+S9
MR	0.22	0.27	–0.03	–0.14

Table 2. The percentage of surviving bacteria in highest dose of aqueous solution of silated hydroxyethylcellulose tested by Salmonella bacteria

Tabela 2. Procent bakterii przeżywających w najwyższych dawkach krzemowej pochodnej hydroksyetylocelulozy badany testem bakteryjnym Salmonella

Dose of 500 µl (Dawka 500 µl)	Strain TA98 (Szczep TA98)		Strain TA100 (Szczep TA100)	
Activation (Aktywacja)	–S9	+S9	–S9	+S9
% of surviving (% przeżycia)	86.9	98.2	100	86

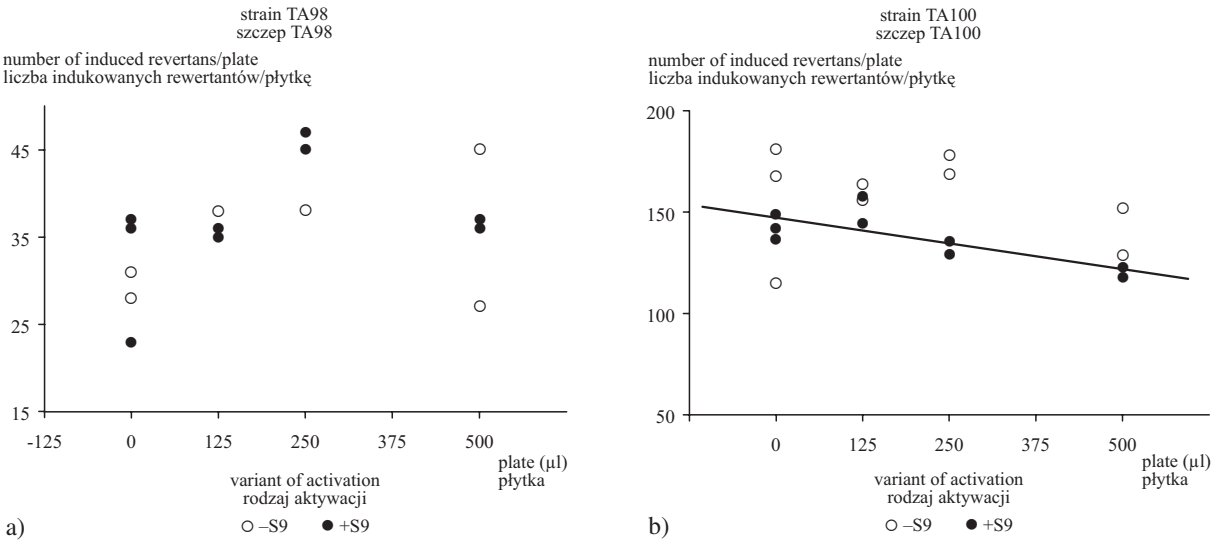

Fig. 1. Mutagenic effect of aqueous solution of silated hydroxyethylcellulose tested by Salmonella assay

Ryc. 1. Efekt mutagenny wyciągu wodnego krzemowej pochodnej hydroksyetylocelulozy badany testem bakteryjnym Salmonella

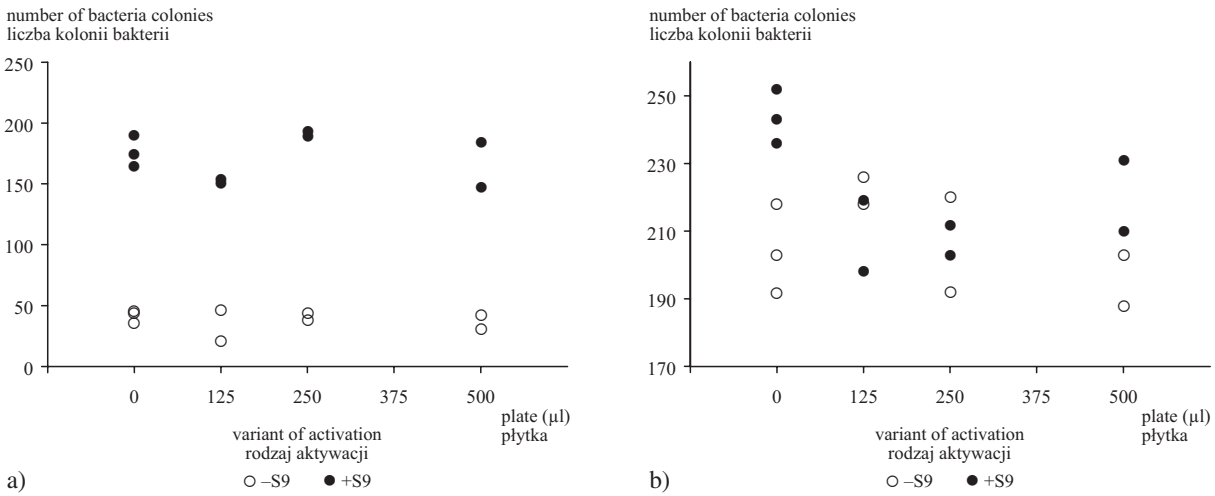

Fig. 2. Toxic effect of aqueous solution of silated hydroxyethylcellulose tested by Salmonella bacteria

Ryc. 2. Efekt toksyczny wyciągu wodnego krzemowej pochodnej hydroksyetylocelulozy badany testem bakteryjnym Salmonella

Discussion

The short-term Salmonella test is conducted to determine whether gene mutation, changes in chromosome structure, or other DNA changes are observed [4]. It is a valuable initial test in the discrimination of mutagens from non-mutagens. For a variety of chemical test substances this assay is easily conducted according to international guidelines for genotoxicity testing. However, Salmonella test may sometimes give false positive results because of presence of histidine or its precursors [3]. In this case the growth promoting constituents in the test sample should be as poor as possible or the results should be verified by more experiments. In presented study testing material, silated hydroxyethylcellulose, showed lack of bacterial mutagenicity and toxicity.

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