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# Cytogenetical aspects of buprenorphine maintenance treatment program – preliminary report

## Cytogenetyczna ocena substytucyjnej terapii buprenorfiną – badania wstępne

The study was aimed at a preliminary evaluation of the effect of buprenorphine, a synthetic opioid used as a substitute in the therapy of dependence on psychoactive substances, on the structure of the genetic material of patients receiving such treatment. The material for the study were lymphocyte cultures from peripheral blood, analysed by a cytogenetic micronuclear test. The obtained results were compared with the literature data on the mutagenic action of methadone.

Celem pracy była wstępna ocena wpływu buprenorfiny, syntetycznego opioidu stosowanego jako substytut w leczeniu uzależnienia od substancji psychoaktywnych, na strukturę materiału genetycznego pacjentów poddanych takiej kuracji. Materiałem do badań były hodowle limfocytów z krwi obwodowej, analizowane przy pomocy cytogenetycznego testu mikrojądraowego. Wyniki porównano z danymi literaturowymi, dotyczącymi mutagennego działania metadonu.

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### Introduction

The main objective of substitution treatment programmes is to create conditions helping psychoactive drug addicts to get out of the drug habit. Such programmes are particularly effective in the case of patients with drug addiction of many years' duration, bad health condition, or in the case of addicts who have failed in their attempts to successfully get out of the habit [21]. A substitution therapy considerably diminishes or wholly eliminates the tendency to turn to opiates by reducing their euphoric effect, and by curbing the craving for narcotics [20]. At present two parallel therapeutic programmes have been carried out involving two different substitutes: methadone and buprenorphine.

Methadone, used since 1964, is a synthetic opioid whose properties are similar to those of morphine. It is quickly absorbed from the alimentary tract, thanks to which it can be administered orally in single daily doses of 80 - 100 mg. Methadone saturates the majority of  $\mu$  receptors during 24 - 36 hrs [12]. It is a relatively safe drug, as the tolerance to it does not increase even when it is used for a few years; moreover, its efficacy has been confirmed by a number of literature data [1,10,11,13,14,18,21,26].

Buprenorphine, an oripavine derivative, is a relatively new drug used as a substitute in the treatment of drug dependence. It shows agonistic properties towards the  $\mu$  receptor and (slightly weaker) towards the  $\kappa$  receptor. Buprenorphine is well absorbed in the alimentary duct and in the oral cavity, so it can be administered either as a solution or sublingually (tablets). It has a long half-life period in the organism, due to which it can be taken every three days.

Buprenorphine treatment, alternative to methadone one, is directed mainly to young patients, often without a long history of drug dependence and with high motivation for going into therapy [9,15,19,24].

The mutagenic action of psychoactive opioid substances has been commonly known [6,16,22,25]; also genotoxic effects of the therapeutic doses of methadone have been examined [7,8,23], hence an attempt was made at a cytogenetic assessment of buprenorphine administration. The micronuclear test, used routinely to monitor the effect of agents evoking changes in the human DNA structure, was regarded as the most suitable for studies of this type.

### Micronuclear (MN) test

Micronuclei (MN) are formed during mitosis from acentric fragments or complete chromosomes, which have not been included in one of the daughter nuclei in the telophase [3,4,5]. The direct cause of their development may lie in chromatid breaks (acentric fragments), or in the lack of possibility of attaching a chromosome to mitotic spindle fibres due to centromere or cleavage spindle lesions or as a result of changes in the structure of kinetochoric proteins. The micronuclear test reveals these lesions in the structure of chromosomes.

### Materials and methods

The study was conducted on lymphocyte cultures from peripheral blood, collected – on the occasion of routine clinical tests – from 16 patients of the Detoxification Ward. The patients were participants of a substitution programme using buprenorphine. Before going into therapy, the subjects had been opiate (mainly heroin) addicts for 3-25 years.

Seventy two-hour lymphocyte cultures were pre-

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pared according to the modified method of Moorhead et al. [17] using the RPMI 1640 medium ("BIOMED" Kraków) with an addition of a calf serum ("SIGMA") and penicillin. To stimulate mitoses, phaseoline LF-7 ("BIOMED" Kraków) was used, while cytochalasin B ("SIGMA") - added 24 hrs before the termination of culture - was applied as a cytokinesis blocker. After the designed time was up, the cultures were treated with a hypotonic solution of KCl and fixed with acetic alcohol. The slides were stained with Giemsa dye and were analysed under a light microscope. In each culture, 1000 binuclear cells were inspected, having indicated in how many of them micronuclei were present.

Results and discussion

The data on the patients examined are shown in table I. The group under study consisted of 13 men and 3 women, but no differentiation with respect to sex was further made due to a significant difference in the numbers; some literature data [2,8] also suggested a lack of dimorphic differences in frequencies of micronuclei.

Besides sex, the group under examination was also differentiated with respect to such variables as age, period of participation in the substitution programme, and buprenorphine dosage. A preliminary evaluation of the impact of the above-mentioned parameters on the frequency of micronuclei occurrence was carried out by a regression method. It was found that the patient's age and dosage were positively correlated with the frequency of micronuclei ( $r = 0.5$ ), while the length of the period of buprenorphine treatment had the most pronounced effect. In that case, the correlation coefficient  $r = 0.81$ , and the intercept and slope coefficients calculated for the regression line were significant on level  $p < 0.001$ . It should be noted, however, that these are provisional data only, and that the results of the statistical analysis have to be verified in the future using a quantitatively larger group of patients.

Buprenorphine is a preparation that has fairly recently been introduced to the substitution treatment of opiate dependence [9,15,19,24]; earlier, methadone was used in therapies of this type. In order to evaluate a possible mutagenic activity of buprenorphine, an analysis of the frequency of micronuclei was performed, having compared the group of patients under examination with an artificially collated (on the basis of literature data) control [7,8,23]. While selecting persons for that group, the number of both sexes (3 women, 13 men), the patients' age and the duration of treatment were taken into account. Using an analysis of variance, both those groups were compared in respect of the variables which were of fundamental importance for the choice of patients for the control; however, no statistically significant differences were found. The data on the control group are shown in table II.

The analysis of variance was also applied to compare the frequency of occurrence of micronuclei observed in the examined patients and in the control. The value of coefficient  $F$  ( $F = 0.1$ ;  $p$ -Value = 0.75) indicates that these two groups do not significantly differ in this respect, either.

The conducted preliminary cytogenetic

Table I  
Characteristics of studied group.  
Charakterystyka badanej grupy.

No.	Gender	Age	Day of therapy	Dose of buprenorphine [mg]	% MN
1	m	26	3	1.6	16
2	m	52	3	4.8	20
3	f	32	4	3.6	19
4	m	18	24	3.2	18
5	m	27	30	1.6	18
6	f	21	90	4.0	16
7	m	18	120	1.6	9
8	m	19	181	1.6	9
9	f	42	188	4.0	19
10	m	27	217	2.4	9
11	m	22	224	1.6	12
12	m	19	242	3.2	11
13	m	21	259	0.8	12
14	m	20	419	1.6	9
15	m	21	579	2.4	5
16	m	26	761	2.4	3

Table II  
Characteristics of control group (methadone therapy).  
Charakterystyka grupy kontrolnej (terapia metadonem).

No.	Gender	Age	Day of therapy	% MN
1*	m	27	3	20
2*	m	33	4	22
3*	f	22	7	20
4**	m	21	21	11
5***	m	33	30	17
6**	f	28	132	8
7**	m	28	122	5
8***	m	28	134	14
9***	f	30	207	14
10*	m	31	243	6
11***	m	33	253	13
12*	m	44	263	13
13***	m	30	297	21
14***	m	29	475	11
15*	m	26	560	10
16***	m	29	703	10

\* Gorazd et al. [8]; \*\* Skowrońska et al. [23]; \*\*\* Furtak et al. [7]

evaluation of buprenorphine activity has shown that this drug does not differ from methadone in respect of genotoxicity, and that both these treatments of drug dependence pose a lower risk of inducing lesions in the genetic material when compared with opiates taken by drug addicts not enrolled for the programmes of substitution therapies.

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