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# Induction of antibodies to methadone during methadone maintenance treatment of heroin addicts and its possible clinical implications

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**Abstract**

By means of two different types of enzyme-linked immunosorbent assay (ELISA) techniques, antibodies to methadone were detected in blood plasma of heroin addicts on methadone maintenance treatment. In 11–15% of cases immunoglobulin (Ig) M antibodies were detected, while IgG antibodies were observed in 33–40%. At least two types of antibodies to methadone were induced—antibodies with high affinity to methadone and low-affinity antibodies more specific for morphine than for methadone. The methadone antibody-positive group of patients had a significantly higher plasma methadone concentration—440 ng/ml, than the antibody-negative group—250 ng/ml ($P < 0.005$) despite almost the same mean therapeutic doses of methadone. Of patients with all types of antibodies to methadone 52% were human immunodeficiency virus (HIV)-positive, whereas in the group without antibodies, HIV-positive reactions were observed in 10.5% only ($P < 0.002$). Alternatively, 87.5% of HIV-positive patients had antibodies to methadone, a fact which should be taken into consideration during methadone dose adjustment. 

*Keywords:* Methadone; Antibody; Blood plasma; Immunodeficiency virus infection; (Human)

## 1. Introduction

Induction of drug-specific antibodies as a possible cause of resistance to a drug has been reported during long-term parenteral administration of polypeptide drugs, such as, for instance, insulins (Waldhaus et al., 1985) and calcitonins (Woodhouse et al., 1977). Despite of their much lower molecular weight and conceivably low immunogenicity, in the 1970s, there appeared some paper reports of an immune response to opiates in animals treated with morphine solution or subcutaneously implanted morphine pellets (Ringle and Herndon, 1975; Beranek et al., 1976). Antibodies were detected by formation of immune complexes with radiolabelled morphine.

Attenuation of morphine effects in animals immunised with morphine chemically coupled to a large molecular weight carrier has been reported (De Cato and Adler, 1973; Spector et al., 1973; Wainer et al., 1973). This could be explained by changes in drug pharmacokinetics in the presence of such antibodies: the prolonged persistence of the drug in blood circulation, its delayed clearance and diminished penetration into the brain (Berkowitz et al., 1974; Hill et al., 1975). A similar phenomenon was observed in the case of antibodies to insulin (Berson et al., 1956) and digoxin (Schmidt et al., 1974).

If an immune reaction occurs with repeated doses of a drug, the order of the reaction could be as follows: a primary portion of a drug binds covalently to a protein; this antigen elicits antibody production. Subsequent drug doses will be partially bound in an antigen–antibody reaction instead of reacting with appropriate 'receptor' sites. The resulting tolerance to drug effects then develops (Herndon et al., 1976).

The initial report of Ryan et al. (1972) of [$^3$H]morphine binding globulins detectable in 40% of sera from heroin addicts was not confirmed by other researchers (Hill et al., 1973; Weksler et al., 1973), though they applied similar methods for antibody detection. Only the recent introduction of enzyme-linked immunosorbent assays (ELISA) has provided researchers with a tool able to detect, not only

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high-affinity antibodies, but also low-affinity binding. This allowed independent groups of investigators in Russia and the USA (Myagkova et al., 1989; Biagini et al., 1990; Gamaleya et al., 1991, 1993a,b) to confirm the presence of specific antibodies to morphine in a relatively high percentage of heroin addicts.

Concerning possible specific immune consequences of prolonged treatment of opiate addicts with the potent analgetic, methadone, no data are available, though there was one negative report about the absence of globulin binding to [$^{14}$C]morphine or [$^{14}$C]methadone in rabbits with subcutaneously implanted methadone pellets (Herndon et al., 1976). However, it is well documented that methadone, when added to normal human serum in therapeutic blood levels ($9.3 \times 10^{-8}$ M) binds to almost 60%, so that endogenous antigen could easily be formed. The highest percent binding was found with β-globulin (Judis, 1977). Tocque et al. (1980) found two types of methadone binding sites: (1) more specific, but few and rapidly saturated and (2) less specific, but in greater number and not saturable.

The formation of such methadone–protein complexes in vivo could trigger the induction of methadone-specific antibodies with concomitant changes in pharmacokinetics of the drug. The production of antibodies to methadone coupled to bovine serum albumin was reported earlier for rabbits (Liu and Adler, 1973). Also, data are available on the stimulation of albumin synthesis by methadone (Rothschield et al., 1976).

The aim of the present study was to investigate the possibility of induction of antibodies to methadone during methadone maintenance treatment of opiate addicts and, in the case of a positive immune response, to evaluate their possible role in the changes of pharmacokinetics of the drug (drug plasma concentrations) and the development of resistance to therapy (therapeutic daily doses).

## 2. Materials and methods

### 2.1. Subjects

There were 54 plasma samples from 46 heroin addicts (32 men and 14 women (mean age 37.9 years, S.D. 5.2 years) with different duration of methadone treatment (mean duration 4.1 years, S.D. 4.9 years, range 1 month–23 years) investigated for the presence or absence of antibodies to methadone and morphine by means of an ELISA technique. Most of the patients were infected with hepatitis viruses A, B and C; 16 were HIV (human immunodeficiency virus)- positive. Plasma samples from nine healthy controls (medical staff): three men and six women (mean age 37.7 years, S.D. 6.0 years) without chronic diseases who did not abuse opiates or use methadone were taken for comparison. All the experiments were conducted in accordance with the appropriate ethical guidelines.

### 2.2. Methods

Antibodies to methadone were detected with two types of ELISA techniques.

#### 2.2.1. 1st type of ELISA

Methadone was conjugated to protein (chicken lyzosime, Serva, Germany) according to Liu and Adler (1973) with slight modifications and served as antigen. The conjugate contained 1.5 mol methadone/1 mol protein. A total of 100 μl of the 10-μg/ml solution of methadone–lyzosime conjugate in 0.05 M carbonate buffer, pH 9.5, was dispensed into the wells of microtiter plates with flat bottom (Nunc, Denmark) and incubated overnight at 4°C. After six to seven washings by means of a Handiwash (Dynatech) with 0.05 M phosphate buffered saline (PBS), pH 7.4, containing 0.05% Tween 20 (PBS-T), 100 μl of blood plasma samples was added. Several 2-fold dilutions of plasma samples in PBS, supplemented with 0.1% Tween 20 and 0.1% bovine serum albumin (PBS-AT) were tested (1:25–1:12 800). After 1-h incubation at 37°C, the plates were washed as described above and 100 μl of goat anti-human immunoglobulin G (IgG), γ-chain specific, or immunoglobulin M (IgM), μ-chain specific, peroxidase conjugates (Sigma, USA) diluted 1:1000 in PBS-AT were added to each well. The plates were incubated at 37°C for 1 h and washed as described previously (except that the number of washings was 10). A total of 100 μl of substrate mixture (0.04% orthophenylendiamine with 0.012% $H_2O_2$ in 0.05 M citrate–phosphate buffer, pH 5.0) was added afterwards to each well. After 15–30 min incubation at room temperature in the dark, the reaction was stopped by adding 50 μl of 10% $H_2SO_4$. Absorbance ($A$) at 492 nm wavelength was read on a microplate photometer Multiscan (Labsystems, Finland). The presence of antibodies to methadone in the plasma samples was established if the A in these samples was not less than 2 S.D. higher than the mean $A$ in the control group samples.

The antibody titers were established as plasma dilution in the wells, the absorbency in which was 3 times greater than the $A$ in the blank wells (with addition of PBS-AT instead of plasma).

Similar experiments were performed for detection of antibodies to morphine. Morphine conjugated to protein (chicken lyzosime) as described earlier (Gamaleya, 1993; Gamaleya et al., 1993a,b) served as an antigen.

The inhibitory ELISA was performed for evaluating the specificity of antibodies. Methadone hydrochloride and morphine hydrochloride served as inhibitors and were added to the reaction mixture in different concentrations, simultaneously with the plasma aliquot. The inhibitory ELISA was conducted with two types of antigen coating: methadone–protein and morphine–protein. Each type of reaction was performed with anti-human IgG and IgM

secondary antibodies coupled to horseradish peroxidase. In the inhibitory experiments, two different plasma dilutions were used: 1:100 and 1:400. The inhibitory curves were built and $IC_{50}$ was calculated as the inhibitor concentration that caused 50% inhibition of antibody binding (decrease of the absorbance). The approximate affinity constant $K_a$ was calculated as $IC_{50}^{-1}$. In some cases, Scatchard plots of the binding were drawn and the true affinity constants, $K_d$ values, were calculated according to Friguet et al. (1985).

*2.2.2. 2nd type of ELISA*

A total of 10 μg/ml concentrations of antibodies to human IgG and IgM (Sigma, USA) in PBS (pH 7.4) was absorbed initially to the solid phase (Nunc immunoplates) at 4°C overnight. After six to seven washings as described above, 100 μl of different plasma sample dilutions in PBS-T (0.1% Tween 20) were added to each well. After 1-h incubation at 37°C 100 μl of methadone–bovine serum albumin conjugate (14 mol methadone/1 mol of protein) coupled to horseradish peroxidase was added in optimal dilution in PBS-T, pH 7.4. The final step of the reaction was performed after 1 h incubation at 37°C and 10 successive washings by adding 100 μl of substrate mixture (0.04% orthophenylendiamine, 0.012% $H_2O_2$ in 0.05 M citrate–phosphate buffer, pH 5.0). Absorbance was measured at 492 nm after 50 μl of 10% $H_2SO_4$ had been added to each well.

The main difference of these two approaches is that the first type of ELISA (in which the plates are sensitised with methadone–protein conjugate with low drug–protein ratio) allows the detection of free antibodies, not bound in immune complexes, mainly. The second type of ELISA, in which the concentration of methadone conjugated to horseradish peroxidase is rather high, allows the detection of free antibodies as well as antibodies with their active sites occupied by methadone. The last circumstance is very important, since patients on methadone maintenance therapy practically always have methadone in their blood.

Statistical analysis of the data was performed by calculating the group means ($M$) and standard deviation (S.D.). In all cases, the significance of the differences between group means was calculated using Student's $t$-test. Differences between frequencies were evaluated by application of $\varphi$-rearrangement, where $\varphi = 2 \arcsin\sqrt{P}$ and $u$-criterion (taking into account that $u = |\varphi_1 - \varphi_2| \sqrt{(n_1 n_2)/(n_1 + n_2)}$, $u_{0.05} = 1.96$; $u_{0.01} = 2.58$; and $u_{0.001} = 3.29$), as well as chi-square statistics. The Pearson product–moment correlation coefficient $r$ was used for evaluation of the correlation between the figures.

## 3. Results

Two series of experiments were performed. In the first series, 20 plasma samples from methadone patients were examined. In the second series, 34 more samples were tested. Each plasma sample was examined for the presence of antibodies to methadone by means of the first and second types of ELISA and, for each sample, antibodies were determined in the IgM and IgG class. Different plasma dilutions were tested, starting from 1:100 and higher. For most screening purposes, the 1:100 dilution was used. The sample was considered immunologically positive if absorbance in the ELISA of this sample was 2 or 3 S.D. (in most cases) higher than the mean value in the group of healthy controls.

Table 1 summarises the data for the number of positive cases in the groups studied. The coincidence between the two methods was 17% for IgM and 50% for IgG antibodies, which meant that the two methods detected different types of antibodies to methadone. All the samples were examined simultaneously for the presence or absence of antibodies to morphine, because all the patients were severe heroin addicts and, as we found earlier, antibodies to morphine could be detected up to 2 months or even longer after the last drug intake (Gamaleya, 1993; Gamaleya et al., 1993a,b). The sample was considered positive for antibodies to morphine if its absorbance in the ELISA with morphine–protein conjugate was 3 S.D. higher than the mean absorbance in the control sample group. Of 54 studied, 18 plasma samples of methadone-treated patients had antibodies to morphine (33.3%), among them 9.3% of IgM, 18.5% of IgG and 5.6% of both immunoglobulin classes. Table 2, constructed as a $2 \times 2$ contingency table,

Table 1
Occurrence of immunologically positive cases in combined group of plasma samples

| | Occurence of antibodies to methadone detected by | | | |
|---|---|---|---|---|
| | 1st type of ELISA | | 2nd type of ELISA | |
| Class of antibodies | IgM | IgG | IgM | IgG |
| Number of samples in the group | 53 | 53 | 54 | 54 |
| Number of samples with antibodies to methadone | 8 | 21 | 6 | 18 |
| Percent number | 15.1 | 39.6 | 11.1 | 33.3 |
| Coincidence between the two types of ELISA: number of cases | | | 1 | 9 |
| (%) | | | (16.7) | (50) |

Table 2
Relationship between blood plasma methadone and morphine antibodies

| 1st type of ELISA | Antibodies to morphine are present | Antibodies to morphine are absent | Total |
|---|---|---|---|
| Antibodies to methadone are present | 15 | 14 | 29 |
| Antibodies to methadone are absent | 3 | 21 | 24 |
| Total | 18 | 35 | 53 |
| $\chi^2 = 7.34,\ P < 0.01$ | | | |

| 2nd type of ELISA | Antibodies to morphine are present | Antibodies to morphine are absent | Total |
|---|---|---|---|
| Antibodies to methadone are present | 10 | 14 | 24 |
| Antibodies to methadone are absent | 8 | 22 | 30 |
| Total | 18 | 36 | 54 |
| $\chi^2 = 0.76$, not significant | | | |

can give information concerning a possible relationship between antibodies to methadone and morphine in terms of chi-square statistics. As can be seen from the table, the relationship was established in case when methadone antibodies were detected with the 1st type of ELISA.

To investigate the possibility that antibodies to methadone and morphine can cross-react, several inhibitory ELISA experiments were conducted. Three plasma samples were chosen randomly for the purpose. Table 3 gives the pattern of antibodies detected in these samples. The level of antibodies to methadone was evaluated by measuring the absorbance of the patient's plasma sample compared to the mean $A$ in the control group as well as by establishing the titer. The level of antibodies to morphine was evaluated by measuring absorbance only. The titers of antibodies to methadone give better information on the amount of specific antibodies. As can be seen from Table 3, all the samples chosen for inhibition analysis had antibodies to methadone of one or both classes studied, which could be detected by both types of ELISA.

The results of the experiments on inhibition are given in Table 4. To characterise the inhibition, the concentration of the inhibitor which caused 50% reduction of the absorbance in ELISA was calculated, ($IC_{50}$). The true affinity constants determined as $K_d$ values were commonly 2–3 times less than the $IC_{50}$ values. The Scatchard plot of the binding was typical for the presence of at least two types of antibodies with different $K_d$ values.

The next step was to detect a possible relationship between the presence or absence of antibodies to methadone in patients' blood and the trough plasma methadone levels. Methadone in blood plasma was detected by fluorescence polarisation immunoassay (Beck et al., 1990). Table 5 shows the plasma concentrations in two groups of methadone- treated patients: with and without antibodies to methadone. IgM and IgG antibodies were considered. A significant difference was found with antibody detection by means of the 2nd type of ELISA only. In the latter case, a significant positive correlation ($P < 0.05$) was found between the levels of IgM antibodies to methadone (in percent increase of absorbance over the negative control) and the plasma methadone concentrations (Pearson correlation coefficient $r = 0.370;\ n = 45$).

The relationship between the plasma methadone concentration and the presence or absence of methadone antibodies detected by the 2nd type of ELISA is also evident from Fig. 1 which shows the percentage of patients with and without antibodies to methadone for different ranges of plasma methadone concentrations. For this purpose, all plasma concentrations of methadone were divided into 100-ng/ml ranges and the percentage was calculated for each range. The total number of patients was 45, among them 15 with antibodies to methadone and 30 without. As seen from the figure, low plasma methadone concentrations (101–200 ng/ml) were found in patients without methadone antibodies only. The percentage of patients

Table 3
Pattern of antibodies detected in randomly chosen plasma samples

| Sample no. | Antibodies to methadone | | | | Antibodies to morphine | |
|---|---|---|---|---|---|---|
| | 1st type of ELISA | | 2nd type of ELISA | | IgM | IgG |
| | IgM | IgG | IgM | IgG | | |
| 10 | − (< 1:100) | + (1:3200) | + (> 1:12 800) | + (> 1:12 800) | − | + |
| 11 | + (1:1600) | + (< 1:1600) | − (< 1:100) | ± (1:6400) | + | + |
| 18 | + (1:1600) | + (1:800) | − (< 1:100) | + (1:12 800) | + | − |

(+) Antibodies are present ($A_{sample} \geq$ mean $A_{control} + 3$ S.D.) in sample dilution 1:100.
(±) Antibodies are present ($A_{sample} \geq$ mean $A_{control} + 2$ S.D.) in sample dilution 1:100.
(−) Antibodies are absent ($A_{sample} <$ mean $A_{control} + 2$ S.D.) in sample dilution 1:100.
Antibody titer is indicated in brackets and equals the last sample dilution with absorbance ($A$) exceeding that in the blank by 0.1.

Table 4
Inhibition in ELISA (1st type)

| Plasma sample | Antibody class | Antigen on solid phase | Inhibitor | $IC_{50}$ (M) | |
|---|---|---|---|---|---|
| | | | | Plasma dilution | |
| | | | | 1:100 | 1:400 |
| No. 10 | IgM | Methadone–lysozyme | Methadone | – | – |
| | | | Morphine | $5 \times 10^{-6}$ | – |
| | | Morphine–lysozyme | Methadone | – | – |
| | | | Morphine | $5 \times 10^{-6}$ | $5 \times 10^{-6}$ |
| | IgG | Methadone–lysozyme | Methadone | $4 \times 10^{-5}$ | $2 \times 10^{-8}$ |
| | | | Morphine | $3 \times 10^{-6}$ | – |
| | | Morphine–lysozyme | Methadone | $2 \times 10^{-5}$ | – |
| | | | Morphine | $2 \times 10^{-5}$ | – |
| No. 11 | IgM | Methadone–lysozyme | Methadone | – | $10^{-4}$ |
| | | | Morphine | $3 \times 10^{-6}$ | $3 \times 10^{-6}$ |
| | | Morphine–lysozyme | Methadone | – | – |
| | | | Morphine | $3 \times 10^{-6}$ | $3 \times 10^{-6}$ |
| | IgG | Methadone–lysozyme | Methadone | $3 \times 10^{-5}$ | – |
| | | | Morphine | $3 \times 10^{-6}$ | – |
| | | Morphine–lysozyme | Methadone | – | – |
| | | | Morphine | $3 \times 10^{-6}$ | – |
| No. 18 | IgM | Methadone–lysozyme | Methadone | – | $10^{-4}$ |
| | | | Morphine | $3 \times 10^{-6}$ | $3 \times 10^{-6}$ |
| | | Morphine–lysozyme | Methadone | – | $10^{-4}$ |
| | | | Morphine | $3 \times 10^{-6}$ | $6 \times 10^{-7}$ |
| | IgG | Methadone–lysozyme | Methadone | – | – |
| | | | Morphine | $2 \times 10^{-6}$ | – |
| | | Morphine–lysozyme | Methadone | – | – |
| | | | Morphine | $6 \times 10^{-6}$ | – |

Inhibition is absent with all ranges of inhibitor concentrations tested ($10^{-4}$ to $10^{-12}$ M).

without antibodies was 28.9% in this case, and the difference was significant ($P < 0.05$; $\varphi$-rearrangement was applied, $u = 2.33$). Methadone concentrations in the 201–300 and 301–400 ng/ml ranges were detected more often in patients without antibodies, though the difference was not significant ($\varphi$-rearrangement was used, $u = 0.61$ and 0.65, respectively). Only high plasma methadone concentrations (401–500 and $> 500$ ng/ml) were found in a greater percent of methadone antibody-positive (6.7%), than of antibody-negative (2.2%) patients in the case of both concentration ranges, but the difference was not significant ($\varphi$-rearrangement, $u = 0.83$).

To find some possible explanation for the higher plasma methadone concentrations found in the antibody-positive group, the daily doses of the drug used for treatment in patients with and without antibodies to methadone were compared (Table 5). As can be seen from the table these doses did not differ significantly though they were slightly higher in the antibody-positive group. The total duration of methadone treatment was also compared in the groups of patients with and without antibodies to methadone (all types of antibodies were considered). In the immunologically positive group ($n = 24$), the mean duration of treatment was 4.27 years (S.D. 5.52), while in the negative

Table 5
Methadone plasma concentrations ($C$) and therapeutic daily doses ($D$) in patients with and without antibodies to methadone, mean (S.D.)

| Group of patients | ELISA method used for detection of antibodies to methadone | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1st type | | | 2nd type | | | 1st and 2nd types | | |
| | $n$ | $C$ (ng/ml) | $D$ (mg) | $n$ | $C$ (ng/ml) | $D$ (mg) | $n$ | $C$ (ng/ml) | $D$ (mg) |
| With antibodies to methadone | 17 | 307 (S.D. 134) | 79.1 (S.D. 22.3) | 15 | 438 (S.D. 289) | 79.7 (S.D. 22.0) | 25 | 367 (S.D. 247) | 76.8 (S.D. 20.8) |
| Without antibodies to methadone | 24 | 304 (S.D. 254) | 75.2 (S.D. 24.9) | 30 | 249 (S.D. 110) | 73.5 (S.D. 24.0) | 19 | 251 (S.D. 121) | 74.7 (S.D. 27.1) |
| Difference between the groups | | NS | NS | | $P < 0.005$ | NS | | $P < 0.1$ | NS |

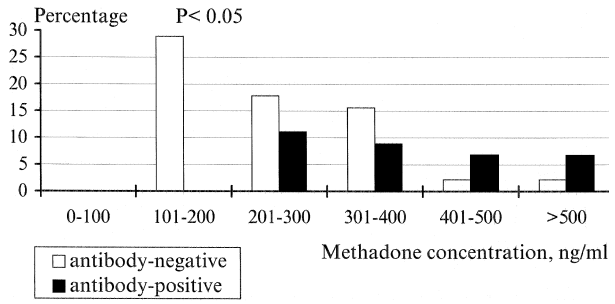


Fig. 1. Percentage of methadone antibody-positive and antibody-negative patients for various ranges of plasma methadone concentrations.

group ($n = 16$) it was 2.4 years (S.D. 2.92), but the difference was not significant ($t = 1.24$).

In the group of patients with antibodies to methadone detected with both types of ELISA ($n = 25$) 14 ($56.0 \pm 10.1\%$) were HIV-positive, whereas in the group without antibodies ($n = 19$) only two patients ($10.5 \pm 7.2\%$) were HIV-positive and the difference was highly significant ($P < 0.001$; $\varphi$-rearrangement and $u$-criterion were applied). For comparison, in the group of patients with antibodies to morphine ($n = 23$) $56.5 \pm 10.6\%$ were HIV-positive and in the group without such antibodies only $16.7 \pm 7.8\%$ were HIV-positive, $P < 0.01$ ($\varphi$-rearrangement and $u$-criterion were applied). Among 16 HIV-positive patients, 14 (87.5%) had antibodies to methadone and 2 (12.5%) had not. For comparison, antibodies to morphine were found in 75% (12 patients) of HIV-positive patients. The mean concentration of methadone in the plasma of HIV-positive patients was 398 ng/ml (S.D. 295), whereas it was 265 ng/ml (S.D. 122), $t = 2.011$, $P < 0.1$ in HIV-negative patients.

## 4. Discussion

Therapeutic drug monitoring is commonly used to monitor patient's condition and to adjust drug dosages to a desired plasma drug level. Adequate maintenance has been found to require different plasma methadone concentrations ranging from 100 to 400 ng/ml (Kreek, 1973; Tennant, 1987; Bell et al., 1990; Loimer and Schmid, 1992). Differences in patterns of methadone metabolism and in absorption from the gastrointestinal tract can lead to a wide range of plasma levels at constant doses of a drug, not only between patients, but in the same patient over time (Tennant, 1987). Although the majority of methadone maintenance patients can maintain an adequate 24-h plasma concentration, some apparently cannot do so even at the high dose of 80–100 mg daily. On the other hand, there are reports that some patients complain of insufficient dosage inspite of high trough levels of plasma methadone. These cases could be explained by the fact that the methadone concentration detected in plasma with a routinely used technique does not correlate with the truly active methadone concentration. There could be several explanations for this phenomenon. One of them is the presence of antibodies, which could bind the drug in the circulation and prevent its action at the appropriate receptor site. According to the present data, these could be antibodies to methadone as well as to morphine.

As can be seen from the data in Table 2, production of antibodies to methadone, detected by the 1st type of ELISA was significantly related ($\chi^2 = 7.34$, $P < 0.01$) with the production of antibodies to morphine. This fact could be the result of at least two possibilities. One possibility is that the production of antibodies to methadone occurs mainly in patients who have developed antibodies to morphine. Such a possibility appears justifiable because, according to other and our own data, heroin and opiate addicts are characterised by the presence of elevated levels of different kinds of antibodies, especially autoantibodies. Among these antibodies, the rheumatoid factor (antibodies against own immunoglobulins) was detected in 36% of heroin addicts (Spiera et al., 1974). Husby et al. (1975) detected antibodies to smooth muscle and lymphocytotoxic antibodies in 46% out of 102 heroin addicts. In morphine-treated rats (Tronnikov et al., 1992) and patients with opiate dependence (Gamaleya et al., 1992) antibodies to neurotransmitters were observed. The appearance of such antibodies could be the result of impaired T-cell regulation of B-cells (Martinez and Watson, 1990). In fact, in morphine-treated animals as well as in opiate addicts, induction of antibodies to morphine was accompanied by the reduction of immune reactivity and functional activity of T-cells and activation of B-cells (Gamaleya et al., 1996).

Another possibility is that some types of antibodies to morphine and to methadone can cross-react, which is plausible as both drugs are used for stimulation of the same type of receptor (opioid). The results shown in Table 4 can be interpreted so that at least two types of antibodies to methadone appear to be present in the blood plasma of methadone-treated patients. In some cases (plasma sample no. 10), antibodies of the IgG class with a rather high affinity to methadone ($IC_{50}$ $2 \times 10^{-8}$ M) and no cross-reaction with morphine were found. It is noteworthy that such high specific antibodies could be detected in the higher plasma dilution (1:400) only. In most cases, however, especially in the lower plasma dilution (1:100), antibodies of the IgM and IgG class, more specific for morphine ($IC_{50}$ $10^{-6}$ to $10^{-7}$ M) than for methadone ($IC_{50}$ $10^{-4}$ to $10^{-5}$ M), and characterised by a rather low affinity were found.

As the physiologically achieved therapeutic blood concentrations of methadone are not less than $10^{-6}$ M (because the average trough plasma concentrations of methadone and the mean levels associated with good social stability and recommended for the rehabilitation of heroin addicts with methadone maintenance treatment are about 100–200 ng/ml and 400 ng/ml, respectively (Beck et al., 1990)), we can suppose that the first type of

antibody is more likely to interfere with the pharmacokinetics of the drug, binding both to free or to protein–conjugated methadone. However, we cannot exclude a certain role of the second type of antibodies in the pharmacokinetics of methadone as well. The significant positive correlation ($P < 0.05$) found between the level of IgM antibodies to methadone (2nd type of ELISA) and the plasma methadone concentration serve as additional evidence for the latter statement because its known that IgM antibodies have low affinity.

The finding of significantly higher plasma methadone concentrations in the group of patients with antibodies to methadone (2nd type of ELISA) while almost the same therapeutic doses of the drug were used leads to the conclusion that such antibodies do contribute to the pharmacokinetics of the drug. Comparison of the percentage of methadone antibody-positive and of antibody-negative patients for various ranges of plasma methadone concentrations showed that concentrations of 101–200 ng/ml were not seen in patients with antibodies to methadone detected by the 2nd type of ELISA. This suggests that the observed difference between the plasma methadone concentrations in the groups of antibody-positive and antibody-negative patients (Table 5) can be largely attributed to the lack of antibody-positive patients in the 101–200 ng/ml concentration range. The close relationship between the presence of antibodies to methadone detected by the 2nd type of ELISA and the levels of plasma methadone can be explained by the fact that this type of the method detects antibodies already bound to methadone.

These data serve as indirect evidence for some role of antibodies to methadone in the pharmacokinetics of the drug, particularly in the maintenance of elevated concentrations of methadone in the blood circulation. Given the similarity in mean daily methadone doses between patients with and without methadone antibodies, and the considerable difference in mean plasma methadone concentrations between these two groups, there is a strong likelihood that the presence of methadone antibodies prevents methadone binding to the brain receptors, resulting in a pronounced loss of biological activity of the drug. However, the significance of such a mechanism for the clinical state of the patients and for their satisfaction with the methadone maintenance dose remains to be established.

The total duration of methadone maintenance treatment seems to play some role in the development of antibodies against methadone. There was a tendency to a longer duration of treatment in the antibody-positive group (4.27 years) than in the antibody-negative (2.4 years), though the difference was not significant.

Among the important findings of the present investigation was the fact that HIV-positive patients are more liable to develop antibodies to drugs, a fact which should be taken into consideration during dose adjustment. This also means that the production of antibodies to methadone is related to the impairment of immune function in HIV infection. This conclusion is consistent with our theory that antibodies to another drug, morphine, appear in those humans and animals who exhibit pronounced depression of T-cell immunity (Gamaleya et al., 1996). HIV infection increases the probability of appearance of antibodies to drugs, possibly due to the additional impairment of T-cell activity.

### *4.1. Conclusions*

(1) Peroral methadone treatment can result in the production of antibodies to methadone in some of the patients.

(2) At least two types of antibodies to methadone can be induced, antibodies of the IgG class with a rather high affinity to methadone and no cross-reaction with morphine and antibodies of the IgM and IgG class with a rather low affinity that are more specific for morphine than for methadone.

(3) Production of antibodies to methadone detected by the 2nd type of ELISA is associated with higher blood plasma concentrations of the drug.

(4) Antibodies to methadone are detected in 87.5% of HIV- positive patients, which should be taken into consideration during methadone dose adjustment.

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