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Assay for maprotiline in human serum with improved sensitivity and selectivity

H.-J. Kuss *, S. Sirch, D.Y. Zhao.

Department of Neurochemistry, Psychiatric Hospital, University of Munich, Nussbaumstr. 7, D-80336 Munich, Germany

Abstract

The use of a photoreactor and fluorescence detection enables measurement of the tetracyclic antidepressant drug 3-(9,10-dihydro-9,10-ethanoanthracene-9-yl)-N-methylpropylamine (maprotiline) with a sensitivity of 100 pg/ml serum. This detection system is highly specific and enables the measurement of very low concentrations in the presence of high concentrations of other drugs that are often found in patient samples. The mean free portions of maprotiline and desmethylmaprotiline were found to be 2.2% and 1.5%, respectively.

1. Introduction

One of the first published reports on the antidepressant drug 3-(9,10-dihydro-9,10-ethanoanthracene-9-yl)-N-methylpropylamine (maprotiline) dealt with the correlation of its serum concentrations, as well as those of its desmethyl metabolite, with its therapeutic effects and side effects [1]. Nevertheless no correlation was found. Two papers reported a tendency towards a better therapeutic effect with higher serum concentrations of maprotiline [2,3]. Another paper reported the reverse tendency and a negative correlation for the metabolite [4], while yet another paper reported a correlation only in a subgroup of retarded depressives [5]. Two additional papers finally concluded that no correlation existed [6,7]. One of the reasons for the different correlations mentioned above between clinical effect and serum concentration might be that only the protein-bound portion of the drug could be measured by the existing methods. A four-fold difference was reported in the free concentration of tricyclic drugs and related antidepressants [8]. Since all antidepressant drugs bind to proteins by more than 90%, binding to the receptor sites is dependent on the amount of free drug available. Therefore, small differences in protein binding may dramatically alter the amount of unbound drug, which is not reflected by the total drug concentration in serum. Under chronic therapy of 150 mg maprotiline daily, 20 patients showed a concentration range of 37–208 ng/ml for maprotiline and of 13–111 ng/ml for desmethylmaprotiline [9]. As the free concentration corresponds to only a few percent, it is necessary to measure in the subnanogram range, while published methods report a detection limit of only several nanograms [9].

The determination of tricyclic antidepressants by HPLC can be performed with UV absorbance detection at 200 to 260 nm. Due to its tetracyclic structure maprotiline can only be detected in the lower UV region [9]. Some of the antidepressant

* Corresponding author.

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drugs, maprotiline being one of them, show fluorescent properties. However, fluorescence detection does not afford increased sensitivity, but improved selectivity can be obtained.

Using a photoreactor, the limit of detection for barbiturates with UV absorbance could be increased significantly [10]. The same was reported for chlormezanone [11]. Thus we undertook a comparative study to investigate whether maprotiline shows a similar increase of the UV or fluorescence signal.

2. Experimental

2.1. Chromatographic system

The HPLC system consisted of a LC-9A pump (Shimadzu Europe, Duisburg, Germany), an autoinjector GINA 50 (Gynkotek, Germering, Germany) and a fluorescence detector F-1050 (Merck-Hitachi, Darmstadt, Germany). The photochemical reactor Beam Boost (ICT, Frankfurt, Germany) was equipped with a 20-m reaction coil and connected to a Rheodyne-7110 valve, thus allowing the flow to be switched on-line and off-line. Separations were generally carried out on a 250 × 4 mm I.D. Supersphere Select B 4-μm column (Merck, Darmstadt, Germany). The mobile phase consisted of acetonitrile–phosphate buffer (pH 5.8) (25:75, w/w). For integration, we used a CR3A connected via CDAU software to a PC. Calculations were performed with the UNIPAC software (Shimadzu Europe, Duisburg, Germany). For each sequence of analyses, recovery was verified with spiked serum samples.

2.2. Extraction

To 1 ml serum, 1 ml of 2 *M* $NaHCO_3$ solution was added, and the mixture was extracted with 6 ml hexane and centrifuged at 2000 *g* for 10 min. A 5-ml volume of the hexane layer was mixed with 150 μl of 0.1 *M* phosphoric acid and centrifuged at 2000 *g* for 10 min. The supernatant hexane layer was then carefully aspirated and discarded. A 100-μl sample of the acidic solution was injected onto the HPLC system.

2.3. Ultrafiltration

Ultrafiltration was carried out with a J2-21 centrifuge (Beckman, Munich, Germany) using a Centrifree micropartition system (Amincon, Witten, Germany). The temperature was maintained at 37°C.

2.4. Chemicals and reagents

Water was obtained from a Milli-Q system (Millipore, Eschborn, Germany). Phosphoric acid and acetonitrile (Merck) and triethylamine (Sigma, Deisenhofen, Germany) were of the highest available purity. Maprotiline and desmethylmaprotiline were kind gifts of Ciba-Geigy (Basel, Switzerland).

The phosphate buffer was prepared by adding 2 ml of 85% phosphoric acid and 4 ml of triethylamine to 1 l of water.

3. Results

3.1. Chromatography

The use of a photoreactor led to a 28-fold increase (Fig. 1) of the fluorescence signal (em: 275 nm, ex: 315 nm) for maprotiline (desmethylmaprotiline: 24-fold). Under these detection conditions no other tricyclic antidepressant drugs gave a signal. Fig. 2 shows the height of the fluorescence signal *versus* the irradiation time. This figure may confirm the hypothesis, that the fluorescence signal detected without photoreactor could result from the short irradiation time occurring in the cell of the fluorimeter. In order to separate this degradation process from the fluorescence of the native compound, we measured the relation between the flow and the signal. Table 1 shows the measured areas and the areas calculated at a flow-rate of 1.0 ml/ml for the UV-absorbance at 214 nm and the fluorescence signals. From the results it can be concluded, that maprotiline and desmeth-

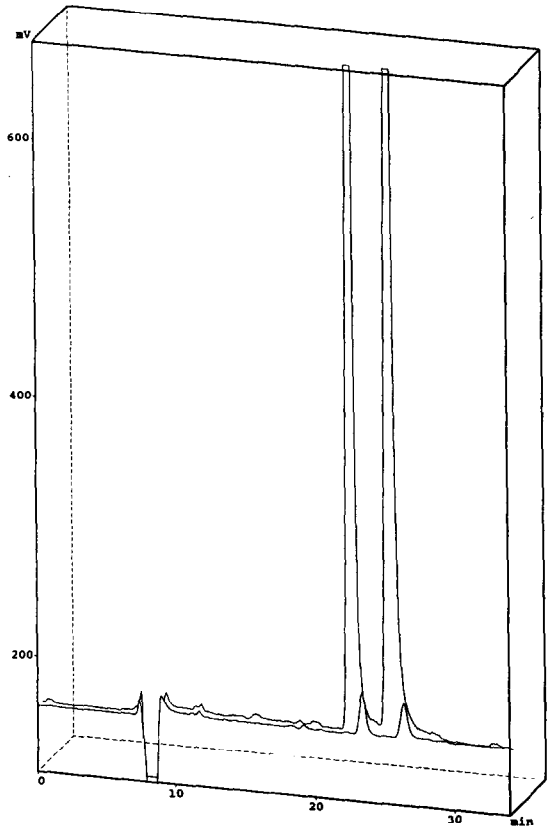

Fig. 1. Enhancement of the fluorescence signal (ex 275 nm, em 315 nm) of maprotiline and desmethylmaprotiline by UV irradiation for 113 s. Front: normal signal, back: enhanced signal.

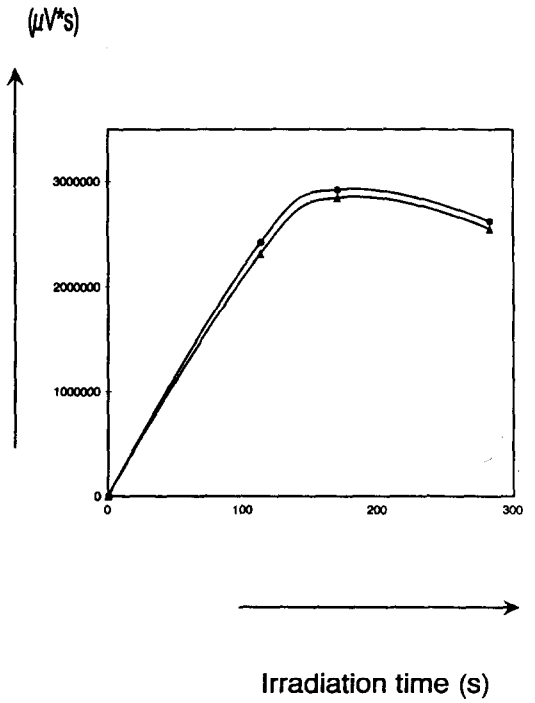

Fig. 2. Influence of the irradiation time on the fluorescence signal (ex 275 nm, em 315 nm) of maprotiline (▲) and desmethylmaprotiline (●).

ylmaprotiline show native fluorescence. That means, that the excitation and emission for maprotiline and desmethylmaprotiline must be optimised separately with and without irradiation.

3.2. Determination of half-life

The half-life values for the tricyclic antidepressants reported in the literature differ considerably. In six healthy volunteers a half-life of 40 ± 15 h was reported [12]. In another single-dose study, a value of 60 h was determined [13]. A mean half-life of 32 h was reported in elderly subjects [14]. Due to the improved sensitivity of the above described method, maprotiline can be detected for some weeks after termination of the drug treatment in long-term treated patients. Therefore, it is possible to measure the concentration during an extended post-dose period. Patient A.R. received, after chronic therapy, a last dose of 75 mg maprotiline at day 0. Blood was drawn after 6 days and the serum concentration of maprotiline was measured (Fig. 3). A concentration of 2.1 ng/ml could be determined at the day the patient left the hospital, which was 23 days after termination of medication. As shown in Fig. 3, the half-life for the drug for this patient was 5 days. Therefore, we assume that even after 4 weeks, the maprotiline concentration would still be slightly above the detection limit. Maprotiline has a unexpectedly long influence even after drug withdrawal. Generally, the half-lives cited in the literature are too short, because it was previously not possible to accurately measure the concentrations at the lower end of the drug concentration–time curve.

Table 1
Detector response *versus* flow-rate (irradiation time)

Flow-rate (ml/min)	UV 214 nm		Fluorescence	
	Area[a] (μV s)	Area[b] (μV s)	Area[a] (μV s)	Area[b] (μV s)
0.75	2 226 845	1 670 134	6 754 521	5 065 891
0.5	3 243 408	1 621 704	9 892 793	4 946 397
0.3	5 793 559	1 738 068	17 061 314	5 118 394

[a]Measured area after injection of 100 ng.
[b]Area calculated for a flow-rate of 1.0 ml/min.

3.3. Free amount in patients

Serum ultrafiltrate from 10 patients under maprotiline therapy with different doses was extracted as described in Experimental. The co-medication in this group of patients consisted of: nortriptyline, perphenazine, chloral hydrate, lorazepame, clomipramine, haloperidol, mianserin, fluonitrazepame, thioridazine, perazine and some drugs used in internal medicine. Table 2 shows the total and the free concentrations of maprotiline and desmethylmaprotiline. The free portion of the total concentration ranges from 1.1% to 3.3% for maprotiline and from 0.9% to 3.3% for the metabolite. This variation may mask the correlation with the therapeutic effect, in spite of the larger variation in the total concentrations.

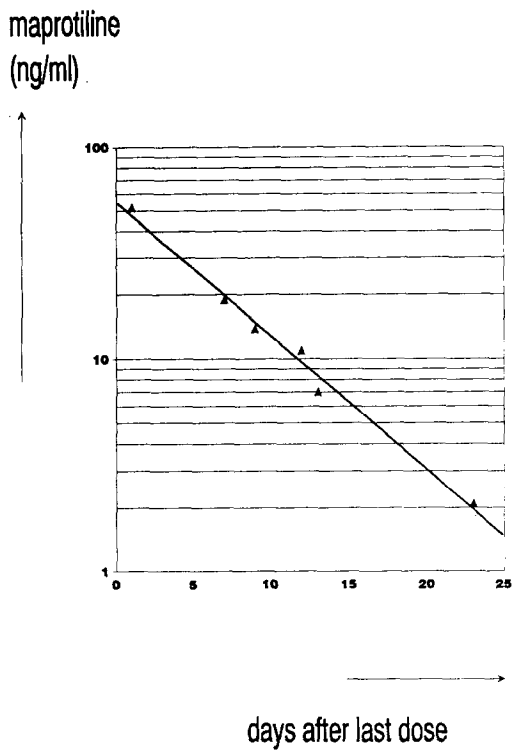

Fig. 3. Decay of maprotiline serum concentration after termination of medication.

Table 2
Total and free concentration of maprotiline and desmethyl maprotiline

Subject	Concentration (ng/ml)			
	Maprotiline		Desmethylmaprotiline	
	Free	Total	Free	Total
C.S.	0.96	86	0.37	43
M.B.	3.62	294	0.25	28
T.R.	2.55	154	0.51	56
A.L.	8.80	255	0.93	57
I.H.	7.56	172	1.18	69
E.F.	1.44	76	1.42	43
E.P.	6.28	268	1.42	91
M.H.	7.34	335	0.89	88
P.E.	6.36	289	1.41	93
M.S.	8.49	484	1.60	94
Mean	5.27	241.30	1.00	66.20
S.D.	2.71	117.26	0.46	23.5
C.V. (%)	51	49	46	35

4. Discussion

Using a photoreactor, it was possible to increase the fluorescence signal of maprotiline and desmethylmaprotiline more than 20 times. Such a great sensitivity can otherwise only be obtained by radioactive methods or by gas chromatography–mass spectrometry. A detection limit of 100 pg/ml (C.V. 11% for injected maprotiline standard and 12% for injected desmethylmaprotiline; $n = 10$) makes it possible to measure the free amount in patient serum with reliable variability [C.V. 8.9% (maprotiline) and 10.8% (desmethylmaprotiline) after extraction of 1 ng/ml]. The C.V. of the standard curve at 1, 3, and 9 ng/ml was 6.7% for maprotiline ($r = 0.9982$; $n = 9$) and 8.6% for desmethylmaprotiline ($r = 0.9971$). The extraction recovery was 55.6% due to volume loss. The 10 patients cited in Table 2 received a lot of comedication, but no interference was observed from these drugs (Fig. 4), thus showing that the detection method used here is also very specific. The method described in this paper presents almost a "maprotiline-detector". However, it seems necessary to perform a study with a fixed dose of maprotiline and to measure its free amount together with the psychopathological effects. Then the question can be answered, whether the free concentration shows a better correlation with the therapeutic effect than the total concentration. The method described here enables such a study to be carried out for the first time.

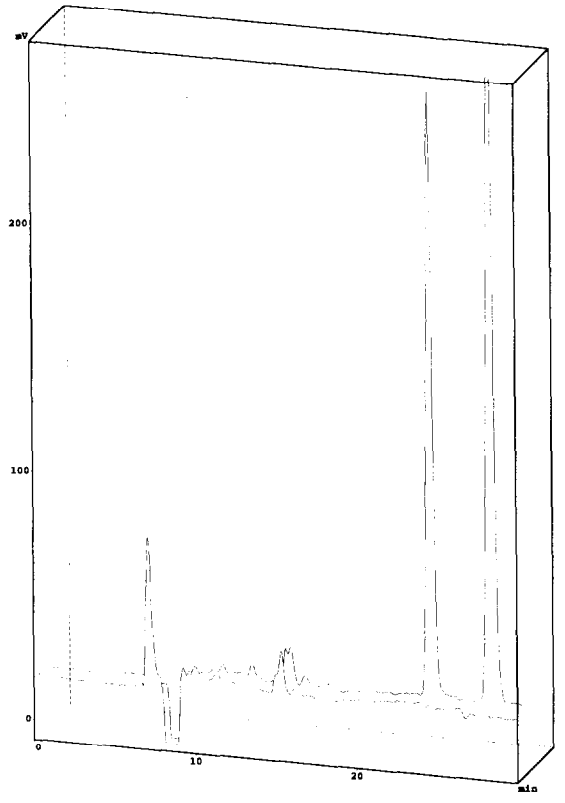

Fig. 4. The foremost chromatogram shows the extracted ultrafiltrate of a blank serum. The hindmost chromatogram shows the extracted serum ultrafiltrate of a control serum (total concentration of maprotiline and desmethylmaprotiline 90 ng/ml). The peak at $t_R = 24.3$ min is desmethylmaprotiline, the peak at $t_R = 28.1$ min is maprotiline.

5. References

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