Methaqualone in human serum and cerebrospinal fluid after oral intake

Methaqualone has been used as a hypnotic since 1958. To determine methaqualone in human serum and cerebrospinal fluid we needed a sensitive method for its quantitative analysis. The methods of Berry (1969); Mitchard & Williams (1972) and Douglas & Shahinian (1973) were sufficiently reliable to measure therapeutic serum concentrations of the drug.

On the supposition that only the unbound fraction of a drug of this type is therapeutically active, we have examined the serum protein-binding of methaqualone in man and compared the unbound fraction with the concentration of methaqualone in cerebrospinal fluid. The mechanism of transportation of methaqualone into the cerebrospinal fluid is discussed.

Methaqualone in serum and cerebrospinal fluid was analysed using a modification of the method of Douglas & Shahinian (1973). To a sample (1 ml) was added 2 N NaOH (200 ml) and hexane (8 ml) containing the internal standard (n-tetracosane) (18 nmol). The sample was gently shaken for 10 min and after centrifugation at 3000 rev min^{-1} for 5 min, 5 ml of the organic phase was removed. The extraction procedure was repeated using hexane (5 ml) containing internal standard (11 nmol). Hexane extracts were pooled in a centrifuge tube and evaporated at about 30°. The dry residue was re-dissolved in hexane (50 μl) and 2 μl was analysed by gas-chromatography.

A Perkin-Elmer model 900 gas chromatograph equipped with a hydrogen flame ionization detector and a Hitachi 156 recorder were used. The stationary phase was 2·5%, E 301 (J. J.'s Chromatography Ltd., King's Lynn, Norfolk) on 80–100 mesh diatomite M AW-DMCS treated, packed into a 2 m glass column (i.d. 2 mm). The instrument settings were: column temperature 220°; injection port temperature 250°; detector temperature 300°; nitrogen carrier gas 30 ml min^{-1}. Standard curves were prepared by using serum solutions of methaqualone containing 1·6–160 μmol $litre^{-1}$. The ratio of the peak height of methaqualone to tetracosane was linear with respect to the concentration of methaqualone.

Binding studies were made *in vitro* by the equilibrium dialysis technique of Goldstein (1949) in the range 1–10 μmol $litre^{-1}$. Serum from healthy volunteers, each taking 200 mg methaqualone, was collected and dialysed against Tyrode solution (Paul, 1965), using Visking membrane. The dialysing was at 37° and the content of methaqualone in the two chambers was quantitatively analysed. The binding of methaqualone to protein in serum was calculated by means of the equation: $P\% = (1 - C_t/C_s)\ 100\%$, where C_t is the concentration of methaqualone in Tyrode solution and C_s its concentration in serum.

The protein bound fraction of methaqualone in serum was about 80% in the whole therapeutic range. Further it was established that the equilibrium between the two dialysis chambers was established within 24 h. Serum protein-binding of methaqualone was linear over the therapeutic concentration range. This relationship differs from that found by Brown & Smart (1970) who, using an ultracentrifugation method, found that the bound fraction of methaqualone was 67–90%, and was dependent on concentration.

Methaqualone was measured in the cerebrospinal fluid of neurosurgical patients 11 of whom were given the drug (200 mg) about 3 h before a lumbar puncture, and 9 of whom received the drug about 12 h before lumbar puncture. Blood samples were taken 1 and 2 h before and 1, 2, 3, 4 and 5 h after lumbar puncture.

The serum concentrations of drug were plotted against time on semilogarithmic

Table 1. *Simultaneous concentrations (μmol litre^{-1}) of methaqualone in serum and in cerebrospinal fluid.* (Samples were obtained at the time of lumbar puncture).

Patient	Concentration (total) in serum (C_s)	Concentration in cerebrospinal fluid (C_L)	Concentration of unbound (free) methaqualone in serum (C_f)	Ratio $\frac{C_L}{C_f}$
A	6·80	1·33	1·29	1·03
B	5·75	0·97	1·10	0·89
C	4·85	0·95	0·92	1·03
D	4·75	0·85	0·90	0·95
E	2·98	0·50	0·56	0·90
F	2·05	0·37	0·39	0·95
G	1·85	0·37	0·35	1·06

paper and by interpolation the serum concentration of methaqualone at the time of the lumbar puncture was determined for each patient. Table 1 shows the methaqualone concentration in the serum at the time of lumbar puncture, the calculated concentration of free methaqualone, the measured concentration in cerebrospinal fluid and finally the ratios between the concentration in cerebrospinal fluid and the concentration of the free fraction of methaqualone in serum.

The concentration of methaqualone in the cerebrospinal fluid after several days *in vitro* remained unchanged.

Investigations of the cerebrospinal fluid (which can be considered to be practically free of protein) should elucidate the mechanism of transfer from blood to cerebrospinal fluid. The ratios in Table 1 illustrate that the transfer into the cerebrospinal fluid is most likely controlled by simple physico-chemical factors, i.e. a passive transfer; on the other hand, there is no inhibition of the transfer, which is dependent only upon the difference between the concentration of the free fraction of methaqualone in serum and the concentration of methaqualone in the cerebrospinal fluid. The fact that methaqualone is protein-bound to a high degree, suggests that an increase in the rate of transfer to cerebrospinal fluid could be affected by drugs which interact by competition at the level of protein-drug-binding sites. This type of drug interaction should be further investigated for a drug like methaqualone.

We thank Bendt Broager, M. D., Department of Neurosurgery G and Erik Skinhøj, M.D., Department of Neurology N, Bispebjerg Hospital, Copenhagen, for permission to study patients in their care.

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January 17, 1975

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