SERUM, TEAR AND SALIVARY CONCENTRATIONS OF METHOTREXATE IN MAN

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- 1 Methotrexate handling has been studied in four patients with psoriasis and eleven patients with neoplastic disease.
- 2 Methotrexate levels in serum, tears and saliva were measured by radioimmunoassay while protein binding studies were performed by continuous ultrafiltration.
- 3 There was a close correlation between methotrexate concentrations in tears and serum ($P < 0.001$, $r = 0.714$). Parotid salivary and serum methotrexate levels were similarly correlated ($P < 0.001$, $r = 0.557$) but not mixed salivary and serum levels ($P > 0.1$, $r = 0.232$).
- 4 The mean protein binding was $95.11\% \pm 2.26$ (s.d.) while the ratio of methotrexate levels in tears to the free serum methotrexate level was 1:1.04. The corresponding parotid salivary level: free serum level ratio was 1:18.11.
- 5 No relationship could be determined between the methotrexate levels in tears and conjunctivitis observed in some of the patients under study.

Introduction

Methotrexate (4-amino-4-deoxy-N¹⁰-methyl folic acid) is an antimetabolite used to therapeutic advantage in a number of clinical conditions. Since its introduction by Farber, Diamond & Mercer (1948) methotrexate has been established in the chemotherapy of several malignant diseases (Capizzi, de Conti, Marsh & Bertino, 1970; Mitchell, Wawro, de Corti, Kaplan, Papac & Butino, 1968; Gottlieb & Serpick, 1970; Jaffe, 1972; Chabner, Myers, Coleman & Johns, 1975; Vincent, Pickren, Fergus & Takita, 1975). Gubner (1951) first described the effect of methotrexate on epithelial tissue and the drug has subsequently been used to treat some cases of psoriasis (McDonald & Bartino, 1969; Spiro & Dermis, 1969; Van Scott, Auerbach & Weinstein, 1964; Weinstein, 1971; Baker, 1970).

Methotrexate treatment schedules have been modified in an attempt to avoid toxicity by using folinic acid to replenish the intracellular folates depleted by methotrexate. High dose methotrexate with folinic acid rescue, which has the ability to abrogate the significant toxicity of this agent, enabled an improvement in the therapeutic index (Goldin, Martel & Greenhouse, 1953; Djierassi, Roger, Treat & Cavin, 1968). However, oral toxicity and conjunctivitis may still complicate therapy. In the past few years there has been considerable interest in the salivary excretion of drugs and a good correlation has been shown between salivary concentration and un-

bound fraction of drug in plasma (Graham & Rowland, 1972; Koysooko, Ellis & Levy, 1973; Huffman, 1975; Matin, War & Karam, 1974; Paxton, Rowell, Ratcliffe, Lambie, Nanda, Melville & Johnson, 1977; Horning, Brown, Nowlin, Levtratanangkoon, Kellaway & Zion, 1977; Stephen & Speirs, 1976). The development of a specific sensitive radioimmunoassay for methotrexate (Paxton & Rowell, 1977) has allowed us to determine methotrexate levels in saliva and tears. We have related these levels to free serum methotrexate concentrations.

Methods

Four patients with psoriasis and eleven patients with various forms of malignant disease participated in the study. The study had the approval of the local hospital ethical committee, all patients gave informed consent. Each patient was given either low dose (15–50 mg) or high dose (500 or 1000 mg) methotrexate i.v. or i.m. and serum, tear parotid and mixed saliva samples were obtained simultaneously (all sampling was completed within 3–5 min) at various times after initiation of treatment for assay of drug concentrations.

Methotrexate concentrations were measured by a specific double antibody radioimmunoassay (Paxton & Rowell, 1977) with an intra assay precision between

Figure 1 Serum (○), tear (●), parotid (■) and mixed (□) salivary methotrexate (MTX) concentration time curves in patient A.C. after a single 50 mg i.v. injection.

Figure 2 Serum (○), tear (●), parotid (■) and mixed (□) salivary methotrexate (MTX) concentration time curves in patient A.S. following a single 500 mg i.v. infusion.

4–8.5% for the serum and saliva assays and 5–10% for the tear assay. The assay sensitivity was 200 pmol l⁻¹.

Protein binding of methotrexate to serum proteins was studied by continuous ultrafiltration (Campion & Olsen, 1974) using the Amicon Multimicro Concentrator system. Amicon Diaflo M50 ultrafiltration membranes were used at pH 7.4 and 37°C. As drug recovery was consistently near 100% when the reservoir contained phosphate buffered saline (pH 7.4), these membranes did not bind significant amounts of methotrexate. A reservoir concentration of 12 µmol l⁻¹ was employed which enabled the study of binding over the range 1–30 µmol l⁻¹. Adjustments in drug concentration within this range were performed stepwise yielding ten to fourteen estimates of protein binding each pertaining to a different drug concentration. The protein binding of methotrexate was constant over this concentration range the errors on individual estimates ranging from 1.0–4.0%.

Tears were collected on Schirmer test strips on which the sample volume (20–30 µl) was determined

by weighing. Methotrexate was eluted using methanol (2 × 0.5 ml) which was subsequently evaporated to dryness, the methotrexate being redissolved in 400 µl of buffer at 37°C. Standard solutions absorbed on the strips and eluted according to this procedure gave a good recovery (90–95%).

Parotid saliva samples were collected using a parotid cup (Stephen & Speirs, 1976), salivary secretion being stimulated with citric acid (0.5 ml of 1% solution). The pH of the parotid saliva was checked to determine whether any citric acid was contaminating the sample.

Mixed saliva was collected directly by expectoration into plain tubes. Serum, tear, parotid and mixed saliva samples were kept at –20°C until assayed.

Results

Soon after administration, methotrexate was detectable in tears and both parotid and mixed saliva (Figures 1 and 2). Regression analysis showed a close

Figure 3 Relationship between tear and serum levels of methotrexate in eleven patients.
($P < 0.001$, $r = 0.714$)

Figure 4 Relationship between parotid salivary and serum levels of methotrexate in eleven patients.
($P < 0.001$, $r = 0.557$)

correlation ($P < 0.001$, $r = 0.714$, Figure 3) between methotrexate levels in tears and serum. Parotid saliva and serum methotrexate levels also showed a close correlation ($P < 0.001$, $r = 0.557$, Figure 4). No significant correlation was found between methotrexate concentrations in mixed saliva and serum ($P > 0.1$, $r = 0.232$). The ratio of methotrexate level in tears: level in serum was 0.047:1 while the ratio methotrexate level in parotid saliva: level in serum was 0.003:1.

The mean protein binding was $95.11\% \pm 2.26$ (s.d.) and remained constant over the range $1\text{--}30\text{ mol l}^{-1}$. The tear levels of methotrexate would appear to be in equilibrium with the unbound fraction in serum. The parotid saliva levels are much lower, being 5–6% of the unbound drug levels in serum.

No oral toxicity was observed in any of the patients during this study but a number showed signs of conjunctivitis. Table 1 summarizes the dose of drug given, the incidence of ocular toxicity (*viz* conjunctivitis) and the unbound drug levels in serum. Having established that the ratio of methotrexate levels in tears to unbound drug levels in serum is very near unity, in the present study we could not demonstrate any relationship between tear concentrations of methotrexate and the incidence of conjunctivitis.

Table 1 Summary of drug dose, % unbound drug in serum and the incidence of conjunctivitis

Patient	Dose (mg)	Conjunctivitis	% unbound drug
D.H.	1000	+	4.15
R.N.	1000	—	6.37
M.L.	1000	—	5.16
A.S.	500	—	3.73
R.P.	500	—	—
N.N.	50	+	2.77
A.C.	50	+	3.31
R.P.	50	+	5.73
M.G.	50	+	3.92
D.C.	50	+	4.61
B.H.	50	—	3.15
M.B.	20	—	3.77
J.M.J.	15	—	6.03
A.H.	15	—	4.03
A.M.	25	—	11.74

500–1000 mg: i.v. infusion over 12 h

15–50 mg: i.v. injection

25 mg: i.m.

Discussion

This investigation demonstrates in patients receiving methotrexate significant linear correlations between drug concentrations in tears, serum and parotid saliva.

There is little information in the literature on the binding of methotrexate to serum proteins and considerable discrepancies exist in published estimates with values of 50–94% bound being reported (Henderson, Adamson & Oliverio, 1965; Liegler, Henderson & Hahn, 1969; Koch-Weser & Sellers, 1976; Taylor & Halprin, 1977). Methodological differences may account, at least partly, for this variation. We have shown that the method adopted in the present study has negligible membrane effects and is reproducible.

Since the unbound fraction of methotrexate is 4.89%, it is apparent that free drug in serum is in *passive* equilibrium with drug in tears. Indications to study drug concentrations in tears are few so that we would hesitate to extrapolate this finding to other compounds. Furthermore there is a predictable relationship between methotrexate in serum and drug in parotid saliva. However, no advantage would be served by the prediction of serum concentrations from that found in tears and saliva. The collection procedures are time consuming and require special devices and materials which would be quite inappropriate under clinical conditions. Further, the collection of these tissue fluids, particularly tears, would be unsuitable in children, where development of non-invasive and painless techniques for monitoring drug levels is most desirable. Considerably lower drug levels were found in parotid saliva compared with those in tears. A possible explanation for this would be that methotrexate secretion is dependent in the rate of salivary flow, but it seems more likely (in view of the very low concentration in parotid saliva) that methotrexate is actively secluded from the secretion of the parotid gland. Physico-chemical processes may determine this partition of methotrexate between serum and parotid saliva.

Ocular toxicity did not correlate with drug levels in tears. Higher concentrations of methotrexate were present in the tears of those patients receiving the high dose infusion. However, these patients were 'rescued' from toxicity with folinic acid. In the low dose group, conjunctivitis was evident, in all but one patient, at 7–14 days after drug administration. At this time no methotrexate could be detected in tears but persistence of low residual levels of methotrexate could contribute to the observed ocular toxicity when folinic acid is not given.

The work was supported by Cancer Research Campaign Grant No. SP 1429.

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(Received March 10, 1978)