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Influence of high-dose methotrexate on the distribution of body fluid volumes in the dog*

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Summary. We studied the influence of high-dose methotrexate (HDMTX) on body fluid volumes in the dog, using indicator dilution techniques. In six healthy mongrel dogs total body water volume (TBW), extracellular water volume (ECW), body mass, and plasma osmolality were measured before and after infusion of both saline and HDMTX. TBW and ECW were determined simultaneously, using a double-indicator (D_2O/ferrocyanide), single injection technique. In vitro experiments confirmed the reliability of ferrocyanide as an indicator for ECW, also in the presence of methotrexate. Results showed an increase in ECW after HDMTX ($P=0.029$, paired Student's t-test), while TBW remained constant. Infusion of the same volume of isotonic saline in the control experiments did not result in any demonstrable change in either TBW or ECW. Therefore, infusion of HDMTX appears to cause a water shift from the intracellular to the extracellular compartment. Such a change in body water volumes may have implications for estimates of body composition and for pharmacokinetic studies in cancer patients receiving HDMTX.

Introduction

It has been well established that cancer may have an impact on body composition and nutrition [9]. Considerable data on changes in lean body mass and in the extracellular compartment have been obtained by measuring body fluid volumes using indicator dilution methods [7, 8]. At present, an increasing number of cancer patients are receiving chemotherapy as part of their treatment. Cytostatic treatment may put patients at a high risk of malnutrition, as the drugs may cause aversion to food, nausea, emesis, and stomatitis. Therefore, cancer patients treated with chemotherapy are pre-eminently eligible for studies on their nutritional status [4]. Estimates of total body water volume (TBW) and extracellular water volume (ECW) may be particularly helpful in such studies [7].

However, before using data on body fluid volumes for nutritional studies in patients treated with chemotherapy, the possibility has to be considered that anticancer agents themselves might have a direct effect on water volumes. That drugs may cause an unexpected change in water distribution has been demonstrated for pentobarbital. Anesthesia with pentobarbital may be associated with a 10% reduction in ECW in the dog, due to net water entry into the cells [12].

We studied the effect of high-dose methotrexate (HDMTX) on TBW and ECW in the dog. We elected to use healthy animals that did not receive any other drug, to avoid any confounding factor.

Materials and methods

Animals. Six healthy mongrel dogs of both sexes, weighing 17–35 kg, were housed and fed under standard conditions for at least 4 weeks. The animals were maintained on Canex pellets (Hope Farms B. V., Woerden, the Netherlands) and water ad libitum. After an acclimatation period, permanent catheters were implanted in the descending aorta and the pulmonary artery [10]. The free ends of the catheters were tunneled subcutaneously to the dorsum of the neck, exteriorized, and fitted with matching connectors. The catheters allowed venous and arterial sampling as well as infusions in the conscious dog without disturbing the animal. At least 2 weeks elapsed between the implantation and the beginning of the experiment.

Experimental design. In each dog the experiment consisted of four measurements of TBW and ECW, body mass, and plasma osmolality. On the first day two measurements were made. The experiment was started with a first indicator injection for the determination of TBW and ECW. Following a 3-h sampling period, isotonic saline was infused through the catheter in the pulmonary artery. The volume given was 10 ml per kg body weight, and this was administered in 1-h period. At 1 h after completion of the infusion, the second measurement was started with the second indicator injection. One week later the third and fourth measurements were carried out in the same way, except that instead of saline, 250 mg/kg methotrexate in isotonic saline was given. Again, a volume of 10 ml/kg was infused in 1 h. Immediately after the methotrexate infusion a blood sample was taken to determine the peak concentration of methotrexate in plasma. During the experiments the dogs were not anesthetized or otherwise sedated: They were awake and lying quietly in a basket. This allowed the dogs to be observed for overt behavioral changes. Before each measurement of TBW and ECW the dogs were weighed and the plasma osmolality was measured using a Knauer Halbmikro Osmometer.

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Measurement of body fluid volumes. TBW and ECW were measured simultaneously using a double-indicator (D_2O/ferrocyanide), single-injection dilution technique. This technique allows the calculation of the distribution volume of a tracer substance at time zero from the disappearance curve of the substance [11, 12]. Through the catheter in the descending aorta, a solution containing 50 mg ferrocyanide per ml D_2O was administered to each dog in a dose of 1 ml/kg body weight. Blood samples of 2 ml were collected through the aortic catheter just before administration of the tracers (time zero) and subsequently at 3, 6, 9, 12, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, 135, 150, 165, and 180 min after administration.

TBW was determined from the distribution volume of deuterium oxide (D_2O) [13]. About 0.5 ml red cells was vacuum-sublimated at about 0.7 Pa to near-total dryness, and the condensate was collected in traps immersed in liquid nitrogen. The absorbance of the condensate was measured at a wave number $\lambda^{-1} = 2486\ cm^{-1}$ by means of a Perkin Elmer 177 infrared spectrophotometer. A sealed liquid cell with CaF_2 windows and a light path length of 0.01 cm was used. The frame of the cell was equipped with a sensor for measuring the sample temperature. A similar cell filled with distilled water, or a piece of ordinary window glass having the same absorption as the water-filled cell at the wavelength used, was used in the reference beam. The measured absorbances were converted into D_2O concentrations using a calibration line specially made for each series of determinations.

ECW was determined from the distribution volume of ferrocyanide (hexacyanoferrate II, $Fe(CN)_6^{4-}$). To a 0.5-ml plasma sample, 4.5 ml of a solution containing trichloric acetic acid and perchloric acid was added and the mixture was ahken and allowed to stand for 10 min. Next, it was centrifuged for 10 min at 7000 *g*, and to 4.0 ml supernatant, 1 ml 5 g/l $FeSO_4$ in 90 mmol/l H_2SO_4 was added. Once the blue color had developed, which took 30–45 min, the absorbance was measured at $\lambda = 700$ nm against a reagent blank by means of an Optica CF4 grating spectrophotometer. A low-volume cuvette with a light path length of 4.00 cm was used. The measured absorbances were converted into ferrocyanide concentrations using a calibration line specially made for each series of determinations [5, 11].

Experiments in vitro. To test the reliability of ferrocyanide as an indicator for ECW in the presence of methotrexate, the following experiment was carried out. To 30 ml heparinized blood were added 3.3 ml methotrexate solution in water, containing 10 mg methotrexate per ml. The methotrexate level in plasma was measured. Isotonic saline, 3.3 ml, was added to another 30 ml blood of the same dog. Ferrocyanide, 4 μmol, was added to the blood samples and incubation at 37 °C for 1 h followed. Then the two samples were each divided into 15 portions of 2 ml each. In each portion the hematocrit, as well as the ferrocyanide concentration in plasma, was measured.

Statistical analysis. Data from both saline and methotrexate experiments were analyzed by means of paired *t*-tests.

Results

During the experiments no infectious complications from the indwelling catheters occurred. The dogs behaved quietly during the whole procedure; no change in overt behavior was observed. The implanted catheters could easily be used for infusions and blood sampling during the whole experiment.

As is shown in Table 1, after saline infusion no significant changes were detected in TBW, ECW, body mass, and plasma osmolality. Data from the methotrexate experiments are given in Table 2. Mean body mass and TBW remained the same, but 1 h after HDMTX infusion an 8.9% increase in mean ECW was observed, which was statistically significant ($P = 0.029$). Plasma osmolality showed a slight decrease ($P = 0.038$).

Methotrexate levels in plasma samples, obtained from the dogs immediately after the HDMTX infusion, were 2.2 ± 0.2 mmol/l (SD).

Table 1. Body mass, water volumes and plasma osmolality before and 1 h after infusion of isotonic saline (10 ml/kg) in the dog ($n = 6$)

		Before saline	After saline	Difference	*P*-value[a]
Body mass	(kg)	27.5 (±2.9)[b]	27.2 (±2.8)	−1.1%	0.131
Total body water	(ml)	16257 (±1476)	16258 (±1459)	0	0.997
Extracellular water	(ml)	6356 (± 899)	6299 (± 679)	−0.9%	0.851
Plasma osmolality	(mmol/kg)	293 (± 1)	290 (± 1)	−1.0%	0.064

[a] Student's paired *t*-test
[b] Mean values ± standard error

Table 2. Body mass, water volumes and plasma osmolality before and 1 h after infusion of high-dose methotrexate in the dog ($n = 6$)

		Before MTX	After MTX	Difference	*P*-value[a]
Body mass	(kg)	26.5 (±2.5)[b]	26.5 (±2.5)	0	0.854
Total body water	(ml)	15336 (±1230)	15266 (±1124)	−0.5%	0.660
Extracellular water	(ml)	5943 (± 545)	6470 (± 692)	+8.9%	0.029
Plasma osmolality	(mmol/kg)	295 (± 3)	292 (± 3)	−1.0%	0.038

[a] Student's paired *t*-test
[b] Mean values ± standard error

In the experiment in vitro with heparinized blood, a methotrexate level of 2.3 mmol/l was produced by the addition of methotrexate to one of the blood samples. Following addition of the ferrocyanide indicator and incubation, the measured ferrocyanide plasma concentration in this sample was $161 \pm 6\ \mu M$ (SD); the hematocrit was 0.28 ± 0.01 (SD). In the other blood sample, to which saline was added instead of methotrexate, a ferrocyanide plasma concentration of $162 \pm 5\ \mu M$ (SD) was measured; the hematocrit in this sample was 0.27 ± 0.01 (SD). Thus, both plasma ferrocyanide concentration and hematocrit were the same, whether or not a high level of methotrexate was present in the sample.

Discussion

In each dog four measurements of the body water volumes were performed. The first two measurements served as controls and were intended to assess whether isotonic saline in a volume of 10 ml/kg body mass results in a detectable change in body water volumes 1 h after infusion. After a week the third and fourth measurements were carried out to determine any possible effects of HDMTX on the water volumes in the dogs. By this design, the effect of methotrexate per se could be studied. The dose of methotrexate used in the dog experiment lies in the same range as is administered to cancer patients in clinical practice when they are treated with HDMTX. The experimental peak levels of methotrexate in plasma were also similar to those reported for patients after high-dose methotrexate infusion [1].

The suitability of the indicator dilution methods used, with D_2O and ferrocyanide as indicators for TBW and ECW, respectively, has been confirmed in previous work in our laboratory [11, 13]. It has been shown that ferrocyanide is an appropriate indicator for ECW; the ferrocyanide ion does not enter the cells [2, 6, 11]. However, no data were available on the behavior of this indicator in the presence of a high level of methotrexate. Da Costa and Iqbal [3] found some increase in free hemoglobin when red cells were exposed to a high concentration of methotrexate. Therefore, a possible alteration in permeability of the cell membrane by methotrexate had to be considered, resulting in permeability for the ferrocyanide indicator. In that case, the distribution volume of ferrocyanide would be larger than the extracellular space. In the experiments in vitro, the same amount of ferrocyanide was added to two identical blood samples. The same ferrocyanide concentration and hematocrit were found, irrespective of whether the sample had been incubated with methotrexate or with saline. The methotrexate concentration in the blood sample was similar to the peak levels reached in the dog experiments. From these data we conclude that methotrexate at the concentration studied does not change the permeability of the erythrocyte membrane for the ferrocyanide ion. This confirms the suitability of ferrocyanide as an indicator for ECW, even in the presence of a high methotrexate level.

The results of our measurements in dogs show an 8.9% increase in extracellular water volume, one hour after infusion of HDMTX. The increase in ECW is not likely to be merely the result of the volume load of the HDMTX infusion. First of all, in the control experiment the same volume of isotonic saline did not produce a detectable change in ECW. Secondly, the increase in ECW was almost twice the infused fluid volume. Thirdly, we observed a fluid shift between the intracellular and extracellular compartments rather than a mere volume increase due to exogeneous fluid administration. A simple osmotic mechanism is not a satisfactory explanation for the observed water displacement, as plasma osmolality showed an 1% decrease. In case of a solely osmotic fluid shift from the cells to the extracellular space, plasma osmolality would have been increased. Therefore, we conclude that the observed water shift from the intracellular to the extracellular compartment is due to an effect of HDMTX. The mechanism of this effect is unknown.

Studies of body composition in cancer patients have demonstrated an increase in TBW [9] and a low body cell mass [8]. These reports concern patients who were not treated with chemotherapy. Our experiments were performed in healthy dogs with a normal body composition. The results suggest that cytostatics may influence the distribution of water over the fluid compartments of the body, apart from the effect exerted by the malignancy. Although at present we do not know how long the observed fluid shift persists, this could make the nutritional assessment in these patients even more complex. Changes in the usual distribution of body fluid volumes should also be considered in pharmacokinetic studies in patients receiving HDMTX. Indicator dilution techniques as used in our dog experiments are also suitable for application in patients and may be helpful in addressing this problem.

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References

1. Bleyer WA (1978) The clinical pharmacology of methotrexate. Cancer 41: 36–51
2. Calcagno PL, Husson GS, Rubin MI (1951) Measurement of extracellular fluid space in infants by equilibration technic using inulin and sodium ferrocyanide. Proc Soc Exp Biol 77: 309–311
3. Da Costa M, Iqbal P (1981) The transport and accumulation of methotrexate in human erythrocytes. Cancer 48: 2427–2432
4. Donaldson SS, Wesley MN, DeWijs WD, Suskind RM, Jaffe N, Van Eys J (1981) A study of the nutritional status of pediatric cancer patients. Am J Dis Child 135: 1107–1112
5. Husson GS (1950) A method for the determination of sodium ferrocyanide at low concentrations in body fluids. Proc Soc Exp Biol 74: 230–231
6. Kleeman CR, Epstein FH, Rubini ME, Lamdin E (1955) Initial distribution and fate of ferrocyanide in dogs. Am J Physiol 182: 548–552
7. Shizgal HM (1985) Body composition of patients with malnutrition and cancer. Cancer 55: 250–253
8. Warnold I, Lundholm K, Schersten T (1978) Energy balance and body composition in cancer patients. Cancer Res 38: 1801–1807
9. Watson WS, Sammon AM (1980) Body composition in cachexia resulting from malignant and non-malignant disease. Cancer 46: 2041–2046
10. Zweens J, Schiphof P (1976) Permanent catheterization of aorta and pulmonary artery in the dog. Pflügers Arch 362: 201–202

11. Zweens J, Frankena H, Rispens P, Zijlstra WG (1975) Determination of extracellular fluid volume in the dog with ferrocyanide. Pflügers Arch 357: 275–290
12. Zweens J, Frankena H, Zijlstra WG (1978) The effect of pentobarbital anaesthesia upon the extracellular fluid volume in the dog, studied by continuous infusion and single injection methods. Pflügers Arch 376: 131–138
13. Zweens J, Frankena H, Reicher A, Zijlstra WG (1980) Infrared spectrometric determination of D_2O in biological fluids. Pflügers Arch 385: 71–77

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