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Intratunical versus parenteral administration of methotrexate

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Methotrexate (MXT) distribution in serum, testicle and epididymis after administration into the tunica vaginalis cavity or intravenously was studied in 36 dogs. The dogs were divided into two equal groups: (I) an intratunical group in which 20 mg MXT solution was injected into the tunica vaginalis sac of each dog and (2) a parenteral group in which the same MXT dose was administered intravenously. The MXT concentration in serum, testicle and epididymis was determined 2, 4 and 24 h after MXT administration. Clinical and histologic examination of the tunica vaginalis was performed weekly for four consecutive weeks. The intratunical route, in contrast to the parenteral route, achieves a high MXT level in the testicle and epididymis with a low serum level, resulting in low toxicity and high drug efficacy. This route may therefore be suitable for a more effective treatment of testicular and epididymal lesions, notably malignant tumors.

Key words: Epididymis, methotrexate, testicle, tunica vaginalis.

Introduction

A recent study in our institute has shown that injection of rifampicin into the tunica vaginalis cavity of four tuberculous epididymitis patients resulted in disappearance of the epididymal mass in all treated patients (unpublished results). By contrast, oral antituberculous treatment resulted in partial disappearance of the tuberculous epididymal mass in only one of the four treated patients. This result led to the assumption that medications injected into the tunica vaginalis sac reach the epididymis in high concentrations compared with oral treatment. The study suggested that the administration of cytotoxic drugs through this route might be effective for the treatment of tumors of the epididymis and testicle.

To explore this suggestion, a series of investigations were conducted. The present experimental study deals with the distribution of methotrexate (MXT) in serum, testicle and epididymis after MXT administration into the cavity of the tunica vaginalis.

Anatomical considerations

The tunica vaginalis of the dog is a flask-like serous sac which extends through the inguinal canal to the bottom of the scrotum.[1] It is an evagination of the peritoneum and consists of two layers: parietal and visceral. The parietal layer lines the scrotum and has a narrow tubular part inside the inguinal canal. The visceral layer covers the testicle, epididymis and the spermatic cord. The cavity of the tunica vaginalis contains a small quantity of serous fluid.[1]

Histologically, the parietal and visceral epithelia of the tunica are similar to those of the peritoneum.[2] The tunica vaginalis, being derived from the peritoneum, is a secretory membrane.[3] Fluid is generated by the serous surface of the tunica vaginalis and is resorbed at constant rate through the extensive venous and lymphatic systems of the spermatic cord.[3]

Materials and methods

Thirty-six adult male mongrel dogs were used in the study. Their weights varied from 11 to 18 kg (mean 13.8 ± 2.2 kg). Both the testicles and spermatic cords were examined clinically to ensure the absence of testicular, epididymal or spermatic cord abnormalities. The animals were divided into two equal groups of 18 dogs each. A dose of 20 mg MXT in 5 ml solvent was given in the tunica vaginalis sac in dogs of one group (intratunical group) and intravenously in the other group (parenteral group). The MXT concentration in serum, testicle and epididymis was determined in each group 2, 4 and 24 h after MXT administration.

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Testicular and epididymal specimens were taken by needle biopsy. Multiple biopsies were taken each time from different parts of the testicle and epididimis to determine uniformity of MXT distribution. The MXT level was estimated by an enzyme immunoassay kit (Syva, California).

Intratunical injection technique

With the dog lying on its side and its tail hyperextended, the scrotum was exposed and one testicle pushed down to the scrotal bottom using one hand. With the other hand a 21-gauge needle applied to an empty syringe was inserted through the scrotal skin into the tunica vaginalis cavity. The proper position of the needle was assured by the appearance of tunical fluid on aspiration. Once the proper needle position was ascertained, the syringe was exchanged against another syringe containing the dissolved MXT solution and MXT was injected into the tunical sac. A further guarantee that the injection had been properly placed in the tunical sac was the appearance around the testicle of a homogenously diffuse swelling which represents the MXT-filled tunical sac.

Histologic studies of the tunica vaginalis

Unilateral epididymo-orchidectomy for two animals of each of the intratunical and parenteral groups was done every week for four consecutive weeks to examine the effect of local and systemic MXT administration on tunica vaginalis, testicle and epididymis. The tunical sacs were examined visually and then multiple strips were taken from each. The specimens from tunica vaginalis, testicle and epididymis were preserved in 10% formaldehyde solution, and examined histologically after staining with hematoxylin and eosin, and Verhoeff–van Gieson's stains. Furthermore, the MXT tissue concentration was determined in the epididymis and testicle in both animal groups. Multiple tissue specimens were taken from different parts of the testicle and epididymis for examination.

Statistical analysis

The results of the study were analyzed statistically using the Student's t-test.

Results

MXT intratunical injection was not accompanied by complications at the injection site. None of the dogs died during the period of study.

The results obtained are shown in Table 1 and Figures 1–3. Two hours after MXT administration the mean MXT serum level was higher in dogs of the parenteral group than in dogs of the intratunical group ($p < 0.01$) (Figure 1); however, the MXT level was higher in the epididymal and testicular tissues following intratunical injection (Table 1). The MXT concentration in the testicles of the parenteral group was zero. Four hours after MXT administration, the mean MXT serum level of the parenteral group was still higher than that of the intratunical group ($p < 0.01$) (Table 1). The mean MXT concentration in the epididymis and testicle was 0.32 and 0.33 μmol/g tissue, respectively, in the intratunical group, and zero in the parenteral group (Figures 2 and 3). The 24 h examination (Table 1) showed residual serum MXT in both groups with a higher concentration in the parenteral group ($p < 0.01$) (Figure 1). The epididymal and testicular MXT tissue concentration showed a mean of 0.21 and 0.14 μmol/g tissue, respectively, versus 0 μmol/g tissue in the parenteral group (Figures 2 and 3). The multiple biopsies taken each time from different parts of the testicle and epididymis confirmed the uniformity of MXT distribution.

As regards the epididymo-orchidectomy specimens, the MXT tissue concentration was zero in all the specimens examined in both study groups. Visual (naked eye) examination of the tunical sac in both groups showed no gross morphologic changes. Microscopic examination of the stained tunica vaginalis revealed no histopathologic abnormalities except for mild infiltration of inflammatory cells in the submesothelial layer of the tunica vaginalis in the two dogs of the intratunical group which were orchidectomized at the end of the first post-injection week. Microscopic examination of the testicle and epididymis in the dogs of both groups showed no abnormal findings or changes in all the specimens collected during the 4 weeks following MXT injection.

Discussion

The MXT concentration in the epididymis and testicle was significantly higher in dogs given the drug via the intratunical route than in those given MXT via the parenteral route. By contrast, the

Table 1. MTX serum and tissue (testicle and epididymis) levels 2, 4 and 24 h after intratunical and parenteral administration[a]

	MTX level (μmol)			
	intratunical		parenteral	
	range	mean	range	mean
After 2 h				
serum (μmol/l)	1.12–1.43	1.29 ± 0.13	1.42–1.93	1.72 ± 0.19
epididymis (μmol/g)	0.32–0.46	0.41 ± 0.04	0–0.1	0.03
testicle (μmol/g)	0.29–0.45	0.38 ± 0.07	0	0
After 4 h				
serum (μmol/l)	0.93–1.23	1.13 ± 0.09	1.33–1.84	1.54 ± 0.09
epididymis (μmol/g)	0.29–0.38	0.32 ± 0.03	0	0
testicle (μmol/g)	0.27–0.40	0.33 ± 0.05	0	0
After 24 h				
serum (μmol/l)	0–0.25	0.12 ± 0.03	0.26–0.38	0.30 ± 0.04
epididymis (μmol/g)	0.15–0.24	0.21 ± 0.03	0	0
testicle (μmol/g)	0.12–0.19	0.14 ± 0.02	0	0

[a] Values are given as mean ± standard deviation.

MXT serum level was significantly higher in the latter than in the former group. Intratunical MXT administration did not lead to complications or major histologic changes in the tunica vaginalis, testicle or epididymis.

After parenteral administration, MXT is distributed into the tissues and body fluids, and then undergoes renal clearance. The amount of MXT reaching the testicle and epididymis is small, and accordingly leads to a low MXT concentration in the tissues of these organs. The epididymal MXT concentration was low in the 2 h examination of this group when compared with the intratunical group, and was zero in the 4 and 24 h assays. No MXT could be detected in the testicular tissues in the 2, 4 and 24 h assays.

The high MXT concentration in the testicle and epididymis of the intratunical group is likely being achieved by diffusion of the drug through the tunica vaginalis to the epididymis and testicle. The tunica vaginalis, being a part of the peritoneum, is a semipermeable membrane. Like the peritoneum, it allows the absorption of medication through the submesothelial lymphatics and vessels.

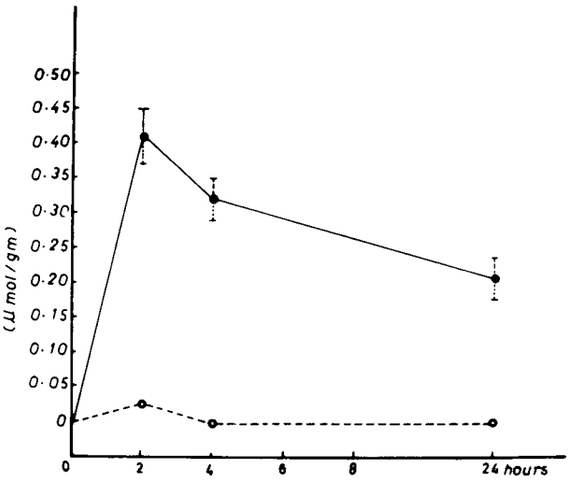

Figure 2. Mean MTX concentration in the epididymis 2, 4 and 24 h after intratunical (——) and parenteral (---) MTX administration.

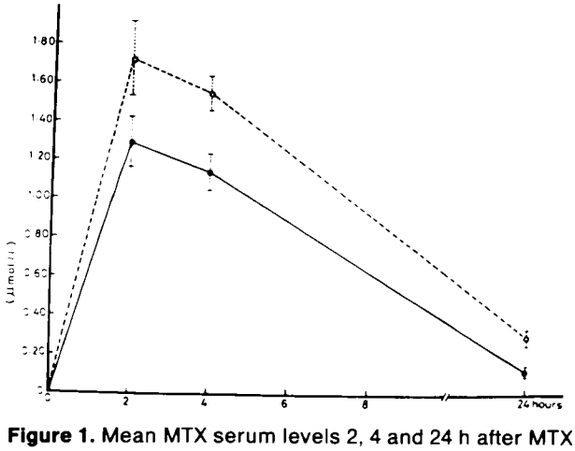

Figure 1. Mean MTX serum levels 2, 4 and 24 h after MTX administration intratunically (——) and parenterally (---).

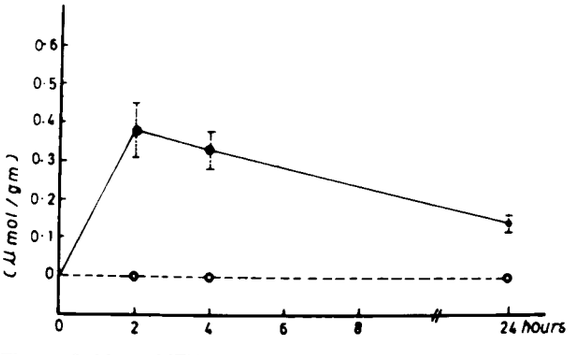

Figure 3. Mean MTX concentration in the testicle 2, 4 and 24 h after intratunical (——) and parenteral (---) MTX administration.

The low serum MXT level in the intratunical group could be due to the local concentration of the drug in the testicle and epididymis. Volume calculations performed have shown that the MXT entrapped in testicle and epididymis could account for the diminished serum values. Thus the intratunical route, in contrast to the parenteral route, achieves a high MXT level in the testicle and epididymis with a low serum level. This would result in low toxic manifestations and a high drug efficacy in testicular or epididymal tumors. Furthermore, this route allows for the administration of higher doses of MXT to increase its efficacy with a minimum of toxic manifestations.

To conclude, MXT intratunical injection leads to high drug concentrations in the testicle and epididymis with low serum levels. This route is simple, easy and without complications. It is currently being applied in the treatment of testicular and epididymal lesions, notably malignant tumors, in a group of patients. The results will be reported in another publication.

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