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M. Sorber · E. A. De Bruijn · M. Kockx · J. Bultinck
A. T. Van Oosterom · L. Denis

Effects and systemic uptake of the new mitomycin C analogue KW-2149 in beagle dogs after intravesical administration

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Abstract The present study was designed to evaluate the local effects of the new mitomycin C analogue KW-2149 after intravesical instillation, together with its penetration into the systemic circulation in healthy beagle dogs. Two reference dogs were treated with two instillations of mitomycin C (30 mg in 30 ml phosphate buffer). Four dogs were given two, three, four and six instillations, respectively, of KW-2149 (60 mg in 30 ml phosphate buffer). KW-2149 concentrations measured in the systemic circulation were very low and were frequently found to be below the limit of determination. The number of instillations had no influence on the KW-2149 concentrations measured in the systemic circulation. Blood analysis showed no systemic toxicity. The histopathological findings in the bladder were comparable in both groups. The number of instillations had no influence on the severity of the lesions found in the bladder wall. On the basis of its in vitro activity KW-2149 can be regarded as a promising agent for intravesical treatment of superficial bladder cancer.

Key words Mitomycins · Bladder · Systemic uptake · Toxicity · Urothelium · Dogs

Superficial transitional cell carcinoma of the bladder is easily accessible with intravesical treatment. Tumor recurrence is common and can be accompanied by stage and/or grade progression. The efficacy of chemoprophylaxis of mitomycin C (MMC), epirubicin and BCG in the reduction of tumor recurrence rates has been demonstrated [1, 2, 9]. KW-2149, a new MMC analogue with enhanced antitumor activity and reduced toxicity, has recently been synthesized. It is a water-soluble MMC analogue which has a side chain at the 7-*N* position (Fig. 1). The compound forms DNA-DNA and DNA-protein cross-links 20-fold more effectively (on a weight basis) than MMC [5]. The IC_{50} of KW-2149 was markedly lower *in vitro* than that of MMC in the human bladder cancer line T24 [7]. Preclinical toxicology studies have shown that systemic administration of KW-2149 induces pathological changes in the kidneys, lungs and heart comparable to those of MMC. However, KW-2149 appears to be less myelotoxic than the parent drug in animal studies [6]. In the present study, the effects of KW-2149 (60 mg in 30 ml phosphate buffer) intravesical instillations on the normal dog bladder together with systemic uptake are described and compared with the effect of MMC (30 mg in 30 ml phosphate buffer).

M. Sorber (✉) · L. Denis
Department of Urology, A.Z. Middelheim Hospital, Antwerp, Belgium

E. A. Bruijn · A. T. Van Oosterom
Laboratory of Cancer and Clinical Oncology, Antwerp University, Wilrijk, Belgium

M. Kockx · J. Bultinck
Department of Pathology, A.Z. Middelheim Hospital, Antwerp, Belgium

Materials and methods

Chemicals and experimental animals

KW-2149 and MMC (for structural formulas see Figs. 1, 2) were provided by Kyowa Hakko Kyoto Ltd. The animals were supplied by Janssen Pharmaceutica, Belgium, and were purebred female beagle dogs weighing 11.5 ± 2 kg. They were maintained on a diet of pelleted dog food (Hendrix, Belgium) and tap water. Bladder instillations were performed with the animals under general anesthesia: fentanyl 0.1 mg, droperidol 5 mg, nembutal (Abbott) 20 mg/kg, N_2O, Ethrane and O_2. A control dog received one bladder instillation with 30 ml phosphate buffer for blood controls only. Two dogs (A, B) were given two intravesical instillations of MMC (30 mg in 30 ml phosphate buffer). Four dogs (C, D, E, F) were given, respectively, two, three, four and six intravesical instillations of KW-2149 (60 mg in 30 ml phosphate buffer). The instillation time was 1 h and the dogs were given one instillation/week. During the instillations, the animals were rotated 90° clockwise every 15 min. Blood for drug concentration analysis was collected at 0, 30, 60, 90 and 120 min

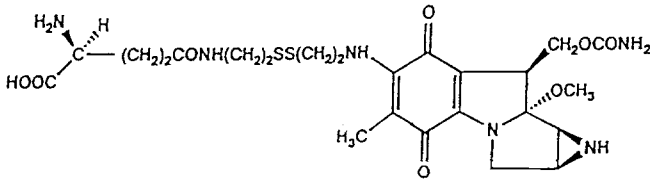

Fig. 1 Structural formula of KW-2149 7-*N*-(2-((2-(gamma-L-glutamylamino)ethyl)dithio)ethyl)mitomycin C. Its molecular formula is C24HB4N60852 and its molecular weight is 598.70

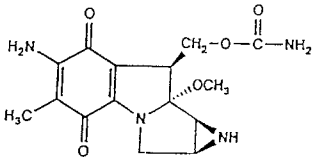

Fig. 2 Structural formula of MMC (4.1 cm on the left side of figure)

after the start of intravesical instillation. Before ($t = 0$) and after ($t = 60$ min) instillation, blood samples were taken for analysis of glucose, sodium, potassium, chloride, HCO_3, proteins, bilirubin, urea, creatinine, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactate dehydrogenase (LDH), γ-glutamyltransferase (GGT), alkaline phosphatase (AF), red blood cell count (RBC), white blood cell count (WBC) + formula, platelets, hemoglobin, hematocrit and sedimentation rate. The bladder was emptied before instillation. After instillation ($t = 60$ min) the urine was collected for drug concentration analysis. A 14-French catheter was used. One week after the last instillation, the bladder was resected and biopsies (± 1 cm^3) of kidney, liver, spleen and bone marrow were taken.

Bioanalysis

Following the methods described for MMC [9], octylsilica (Merck Lichrospher 100 RP-8, 5 µm, 125 × 4.0 mm ID) and octadecylsilica (Beckman, Ultrasphere 5 µm, 250 × 4.6 mm ID) columns were used [8]. The mobile phase applied was either methanol-water or acetonitrile-water. UV detection was performed at 362 nm, 368 nm and 375 nm. Solid-phase extraction of plasma was carried out with XAD-2. The recovery of KW-2149 on XAD-2 was 50%; that of MMC was ca. 80%. The MMC analogue porfiromycin can be used as an internal standard; the compound is eluted between MMC and KW-2149 [8].

Histopathology

The bladder was fixated in and filled with 30 ml 4% formalin. After fixation for at least 24 h, fragments of the fundus, corpus and neck were processed for histopathological examination. The other biopsies were also fixed in 4% formalin. For microscopic examination, sections stained with hematoxylin-eosin (H&E) were studied.

Results

Drug monitoring

Drug concentrations were not measured in the control dog. MMC concentrations in blood and urine are shown in Table 1. The maximum concentrations of systemic plasma measured during instillation were in the range of 14–231 ng/ml. In three instillations, MMC concentrations could still be measured at $t = 120$ min, and were between 57 and 79 ng/ml. MMC recovered from urine was between 83% and 92% at $t = 60$ min. KW-2149 concentrations in blood and urine are shown in Table 2. The concentrations of KW-2149 were frequently found to be below the limit of determination. Only in dog C was the metabolite M-16 detectable over the whole period; the concentrations ranged between 47 and 11 ng/ml. In several cases, M-16 could still be measured at low concentrations after $t = 60$ min, whereas KW-2149 could no longer be detected. The KW-2149 recovered from urine was between 82.8% and 98.5%.

Table 1 MMC concentrations in blood (ng/ml) and urine (µg/ml)

Dog	Instillation No.	Blood					Urine 60 min	Recovery (%)
		0 min	30 min	60 min	90 min	120 min		
A	1	–	222	231	109	79	657	92
	2	–	145	185	66	61	607	83
B	1	–	12	14	10	–	921	92
	2	–	192	180	102	57	910	91

Blood analysis

The results were within normal ranges in the buffer, MMC- and KW-2149-instilled dogs, except for the LDH values, probably as a result of red blood cell lysis. There was also an isolated high GPT value (93 U/l) before autopsy in dog F.

Histological results

The significant microscopic findings of the bladder are described below and summarized in Table 3. Focal lesions of the bladder mucosa were present in both the MMC- and KW-2149-treated animals (Figs. 3, 4).

MMC group. The animals showed urothelial flattening and desquamation of the bladder mucosa, although the picture varied considerably. There were no differences in histopathological changes between the slides of the bladder fundus, corpus and neck.

KW-2149 group. The animals demonstrated a mild to moderate chronic inflammation. In one dog, there was a follicular cystitis. Foci of urothelial hyperplasia in up to 12 cell layers were found in 3 animals. Eosinophilic change and vacuolization of the cytoplasm, flattening and desquamation of the urothelium were variable.

Table 2 KW-2149/M-16 concentrations in blood (ng/ml) and urine (μg/ml)

Dog	Instillation No.	Blood					Urine 60 min	Recovery (%)
		0 min	30 min	60 min	90 min	120 min		
C	1	–/–	141/29	19/25	–/14	–/11	1659/–	94.0
	2	–/–	199/39	22/47	–/23	–/18	1496/ –	92.3
D	1	–/–	11/–	–/–	–/–	–/–	1753/–	87.7
	2	–/–	–/–	11/–	–/–	–/–	1563/–	93.8
	3	–/–	24/6	11/7	–/6	–/–	1762/–	96.9
E	1	–/–	24/4	20/–	–/–	–/–	1804/–	90.2
	2	–/–	12/7	10/5	–/–	–/–	1906/–	98.5
	3	–/–	11/7	12/8	–/6	–/–	1611/–	96.7
	4	–/–	46/7	37/8	–/6	–/–	1304	91.3
F	1	–/–	50/–	30/6	–/10	–/–	1993/–	96.3
	2	–/–	17/–	–/–	–/–	–/–	1656/–	82.8
	3	–/–	–/–	–/–	–/–	–/–	1038/–	83.0
	4	–/–	–/–	–/–	–/–	–/–	1109/–	98.0
	5	–/–	–/–	–/–	–/–	–/–	1514/–	98.4
	6	–/–	–/–	–/–	–/–	–/–	1341/–	96.1

Table 3 Significant microscopic findings in the bladder

Dog	Inflammation	Urothelial hyperplasia	Eosinophil coloring	Vacuolization	Flattening	Desquamation	Urothelial atypia	Fibroblastic atypia
A	+ + +	+	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
B	+	–	–	–	+ + +	+ + +	+	+ +
C	+ +	+ +	+ +	+ +	+	+ + +	+ +	+ +
D	+ + +	–	+	+	+	–	+	–
E	+ + +	+	+ +	+ +	+ +	+ +	+ + +	+ + + +
F	+ + +	+	+	+ + +	+ + +	+ +	+ +	+ +

– absence, + minimal, + + mild, + + + moderate, + + + + severe, *Inflammation* presence of mononuclear leukocytes within the bladder wall, *Urothelial hyperplasia*, the urothelium was considered hyperplastic if it exceeded six cell layers in thickness, *Eosinophilic change*, increased eosinophilic staining often accompanied by vacuolization of the cytoplasm of the urothelial cells, *Loss of urothelial cells*: *Flattening*, between one and three urothelial cell layers, *Desquamation*, total absence of urothelial cells, combined with the presence of fibroblastic cells in the lamina propria, *Urothelial atypia*, nuclear changes of urothelial cells such as marked enlargement and vesiculation of the nuclei and disturbance of the normal maturation pattern, *Fibroblastic atypia*, enlargement, nuclear hyperchromasia and pleiomorphism of subepithelial spindle cells assumed to be fibroblasts

Urothelial atypia was mild to moderate in three animals and minimal in one animal. Fibroblastic atypia was mostly found in the lamina propria of complete desquamated foci. A diffuse increase in fibroblastic cells within the lamina propria was found in two animals. There was no difference in histopathological findings between the slides of the bladder fundus, corpus and neck, although in some of the dogs there were iatrogenic lesions at the bladder neck (necrosis and fibrin deposition). The number of instillations had no effect on the histopathological findings within the bladder mucosa.

Kidney, liver, spleen, bone marrow. The histopathological findings showed no specific abnormalities either in the MMC group or in the KW-2149 group.

Discussion

Taking into account at least comparable activity of KW-2149 versus that of MMC, the higher molecular weight of KW-2149 than that of MMC [3] and therefore the lower expected absorption and reduced toxicity of KW-2149 [6], we decided to use a concentration dose of KW-2149 (60 mg in 30 ml phosphate buffer) double that of MMC (30 mg in 30 ml phosphate buffer).

The uptake of MMC in the blood circulation after intravesical instillation is higher in dogs than observed in humans. Even though the dog urinary bladder is morphologically similar to the human bladder, this can be explained by a difference in bladder wall thickness (note that in dogs the thickness is about one-half that of

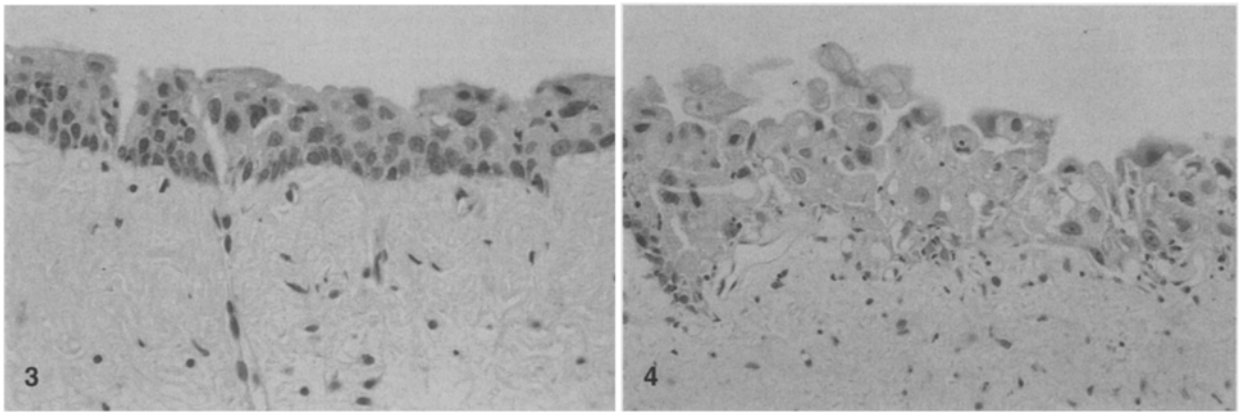

Fig. 3 Normal-appearing bladder mucosa of a treated animal.

Fig. 4 Focal lesion of the bladder mucosa of a treated animal. The urothelium demonstrates eosinophilic changes, vacuolization and cytological atypia. These changes occurred in both the MMC- and KW-2149-treated animals

humans). Other factors responsible for this difference may be the degree of bladder distension, the condition of the urothelium and the blood perfusion rate of the urinary bladder [11].

The data for MMC plasma concentration are in agreement with those published previously [11]. The concentrations are high and it cannot be excluded that systemic toxicity develops upon repeated administration. In contrast, the data for KW-2149 resorption (though used in a dosage double that for MMC) indicate an extremely low transfer from the bladder to the systemic compartment, independent of the number of instillations. Also, concentrations of the first metabolite of the metabolic pathway of KW-2149, i.e., M-16, were found to be low, and therefore it is assumed that the role of the metabolites in possible development of systemic toxicity is negligible. Moreover, plasma concentration-time profiles obtained in a clinical phase I study are not indicative of development of toxicity at minimum concentrations up to 10 μg/ml of KW-2149 [3]. As KW-2149 exhibits markedly higher activity against a wide spread of tumors, it is concluded that KW-2149 is a promising drug in the intravesical treatment of superficial bladder cancer.

No biochemical or hematological changes were observed in the treated animals, which is in concordance with the data of low systemic availability of MMC and KW-2149. Also in the kidney, liver, spleen and bone marrow, no histopathological changes were observed.

The main finding in the KW-2149-treated animals was that large areas of the bladder mucosa were covered by an abnormal epithelium, which was composed of one cell layer of enlarged cells (± 100 μm in diameter), demonstrating an increased eosinophilic staining of the cytoplasm with vacuolization. These histopathological findings are qualitatively comparable with the bladder mucosal changes of the MMC-treated animals in our study and in those reported by other authors [4]. These changes are compatible with toxic damage to the epithelium. Since the abnormal epithelium contained only one cell layer, this toxic damage is associated with a significant cell loss. Moreover, there were foci of complete desquamation, beneath which numerous atypical fibroblasts and capillaries were present. These changes were present in both the KW-2149- and the MMC-treated dogs, though to a lesser degree in the KW-2149-treated group. The lesions were equally distributed over the bladder mucosa. This was to be expected since the dogs were rotated 90° every 15 min during the instillation to ensure a homogeneous distribution of the active product on the bladder mucosa. In the KW-2149 group, the number of instillations did not influence the severity of lesions within the bladder mucosa. Even though KW-2149 was used in a double dosage compared with MMC, the histopathological findings were comparable to the lesions found in the MMC-treated group.

In conclusion this study has shown that KW-2149 (60 mg in 30 ml phosphate buffer) is a safe agent for treatment of superficial bladder cancer by intravesical instillation.

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