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The Effects of Intravesical and Intradermal Application of a New B.C.G. on the Dog Bladder

A. P. M. v. d. Meijden[1], P. A. Steerenberg[3], W. H. de Jong[3,*], M. J. J. T. Bogman[2], W. F. J. Feitz[1], B. T. Hendriks[1], F. M. J. Debruyne[1] and E. J. Ruitenberg[3]

Departments of [1]Urology and [2]Anatomic Pathology, St. Radboud Hospital of the Catholic University, Nijmegen, The Netherlands
[3] Dutch National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands

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Summary. Intravesical and intradermal application of B.C.G. (Bacillus Calmette Guérin) has proven to be effective in the prophylaxis of recurrence of superficial bladder carcinoma after transurethral resection and in the treatment of carcinoma in situ (C.I.S.) in man. Different strains of B.C.G. have been used for this purpose. In this article a new strain of B.C.G. (B.C.G.-R.I.V.M.) has been tested to assess its toxicity. The effects of intravesical and intradermal application of B.C.G.-R.I.V.M. were studied on normal and on coagulated canine urothelium. In this study no general side effects of B.C.G.-R.I.V.M. were seen. Only minor local changes occurred in the bladder wall. Small granulomas were found in the suburothelial tissue. No granulomas or signs of active inflammation were observed in the pelvic lymphnodes, spleen and liver. Because B.C.G.-R.I.V.M. seemed to be a safe agent in the dog we have started to use it for prophylaxis in superficial bladder cancer in man.

Key words: Bladder carcinoma, Intravesical B.C.G.-R.I.V.M. toxicity study.

Since 1976 an increasing experience has been obtained with intravesical nonspecific immunotherapy for superficial bladder cancer. Morales et al. (1976) described the beneficial effects of intravesical B.C.G. administration. Since then, several reports have demonstrated that intravesical application together with intradermal administration of B.C.G. reduces the recurrence rate of superficial bladder tumors [2, 3, 9].

It has been shown that intravesical B.C.G. therapy is effective in the treatment of carcinoma in situ (C.I.S.) [2, 4, 12] and of residual papillary tumors [12].

In most studies Pasteur, Connaught or Tice B.C.G. were used. These daughter strains seem to be equally effective in the management of superficial bladder cancer. A considerable variation in the content of viable bacilli in each vial occurs and may be responsible for differences in toxicity. High concentrations might account for increased toxicity observed after some instillations. Severe toxicity was seen after prolonged intravesical instillations [2]. In some clinical cases prolonged episodes of fever, chills and malaise were also observed. Nearly all investigators reported mild toxicity manifest by hematuria, nausea and frequency of micturition [4, 10, 15].

Recently Morales showed that B.C.G. instillations were safe [13].

The Dutch National Institute of Public Health and Environmental Hygiene (R.I.V.M. – Bilthoven, The Netherlands) produces a B.C.G.-preparation with a stable content and a relatively high ratio viable/dead organisms. Most studies have been performed with B.C.G. grown as a surface culture and homogenized in a ball mill. However, B.C.G.-R.I.V.M. orginally obtained from a seed lot of the Institute Pasteur, Paris, France, is cultured and dispersed homogeneously. This culture method results in a high ratio of viable/dead organisms [5, 8, 14].

The aim of the present study was to evaluate the toxicity of B.C.G.-R.I.V.M., used intravesically and intradermally in the dog.

Materials and Methods

Twelve female beagle dogs (average weight 15–20 kg) were used in this study. First a skin test with 0.1 ml of P.P.D. (purified protein derivate of mycobacterium tuberculosis) was performed and evaluated after 48 and 72 h. Cystoscopy was performed under general anaesthesia and three biopsies were taken from the right wall of the bladder. On the left wall of the bladder a coagulation lesion (diameter 2–3 cm) was made. Ten days after this procedure intravesical instillation was started. The dogs were randomly assigned into three groups. Four dogs received 50 ml of 0.9% saline, four

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Fig. 1. Multipuncture apparatus for intradermal application of B.C.G.

were instilled with 1 x 10^9 B.C.G.-R.I.V.M. in 50 ml 0,9% saline and four dogs received an intradermal injection together with the intravesical instillation of B.C.G. For the intradermal administration a multipuncture apparatus was used (Fig. 1). Each instillation lasted for 2 h and was repeated weekly for six consecutive weeks. All instillations were made under general anaesthesia by means of an 8 French silastic catheter. Before and two days after each instillation a blood sample was obtained and analysed for haemoglobin, thrombocytes, leucocytes, GOT, GPT, γ-GT, AF, creatinine and urea. Urine analyses and bacterial culture were also performed.

The body temperature was measured every day. Two days after the last instillation a cystoscopy was performed and biopsies from the right and left bladder wall were taken. P.P.D.-skin tests were repeated at ten and at thirteen weeks.

On three dogs, one out of each group, an autopsy was performed. The bladder, regional pelvic lymph nodes, liver and spleen were examined histologically.

The tissues obtained by the biopsies before and after the instillations of B.C.G. or saline and those removed at autopsy were fixed in 4% buffered formalin and embedded in paraffin. For microscopic examination sections stained with hematoxylin and eosin (H & E) and Periodic Acid Schiff were studied.

Results

Toxicity

All dogs completed the study, except one from the control group. In this dog a bladder perforation was made by taking biopsies from the bladder wall. Prior to the instillation regimen all skin tests were negative. Afterwards they remained negative. After three months none of the dogs had a positive P.P.D.-test. During the treatment there was no significant rise in body temperature (less than 0.2 °C).

Haematological and biochemical parameters remained within the normal range during the study in all animals.

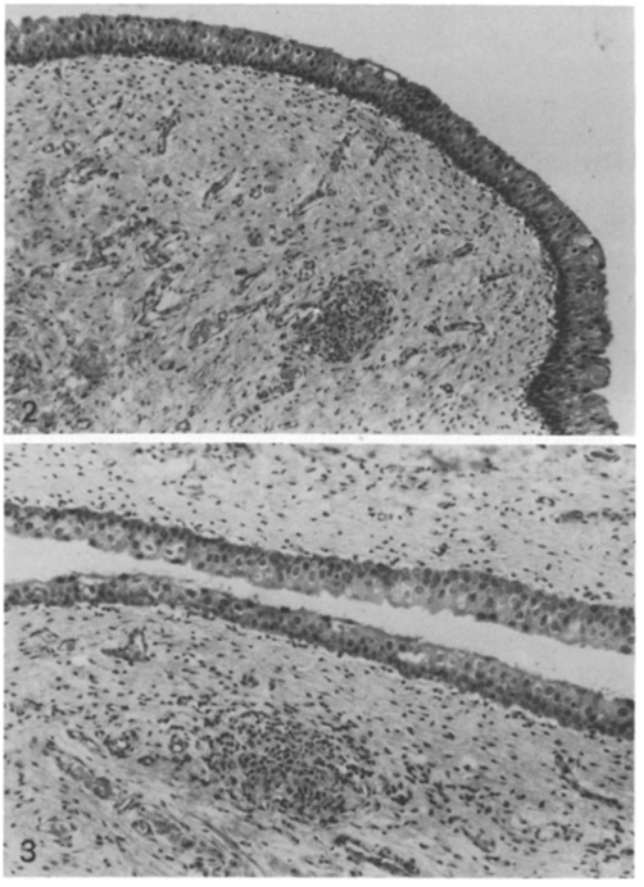

Fig. 2. Histological appearance of the bladder mucosa of a dog after six weeks of intravesical instillations of B.C.G. Apart from a sporadic follicular accumulation of lymphocytes in the suburothelial tissue no pathologic changes are found. H&E, 32x

Fig. 3. A sporadic small granulomatous lesion can be seen in the suburethelial bladder tissue of a dog treated with intravesical instillations and intradermal injection of B.C.G. Otherwise the bladder shows no pathology. H&E, 32x

Only a slight leuococytosis was noted for two or three days after instillation in each dog but there was no significant difference between the three groups.

In none of the dogs was an urinary tract infection observed. Cystoscopy was done after completing the six instillations. A slightly reddish bladder mucosa was seen. All biopsy and coagulation lesions were macroscopically healed.

Histology

Histological examination of bladder biopsies of the 11 dogs taken before instillation of either saline solution or B.C.G. showed no pathological changes, except for some extravasation of erythrocytes and local intravascular accumulation of polymorphonuclear granulocytes, due to the biopsy procedure. In the multiple bladder biopsies taken at one week after 6 instillations of either saline or B.C.G. the

histological picture of the bladder mucosa in both groups was the same. Apart from minimal mechanical lesions the urothelium was intact and showed no abnormalities. In the suburothelial stroma there was some edema or hyperemia, and sporadically a small concentration of lymphocytes (Fig. 2), but no signs of active inflammation. Granulomatous lesions could not be found. However, in the biopsies of the dogs that received BCG both intravesically and intradermally, sporadic small subepithelial accumulations of histocytes resembling early granulomas (Fig. 3), were observed. These histiocytic epitheloid cells were not accompanied by lymphocytes and giant cells were absent in these lesions.

At autopsy, the 3 dogs that were sacrificed, showed no gross pathology. The bladders has a normal appearance but regional pelvic lymphnodes were slightly enlarged. Liver and spleen were of normal weight with no pathological changes. Microscopically the bladder mucosa of the 3 dogs showed the same features as those in the biopsies.

There were no signs of epithelium reaction or active inflammation. In the bladders of the 2 dogs that received B.C.G. instillation few small concentrations of lymphocytes could be found in the suburothelial tissue and also sporadic granulomatous accumulations of histiocytes resembling small granulomas, in one place accompanied with deposition of hemosiderin pigment. The bladder of the dog that received instillation of saline solution showed no granulomas. The muscle tissue of the bladders and the perivesical tissues were normal. The pelvic lympnodes of all 3 dogs showed a slight follicular hyperplasia with perisinusoidal plasmocytosis.

Discussion

Immunostimulation and antitumor activity of B.C.G.-R.I.V.M. were tested in laboratory animals with chemically induced tumors [5] and in naturally occurring tumors in the cow and the horse [6, 7]. In man the B.C.G.-R.I.V.M. is used as an adjuvant therapy in melanoma (EORTC-trial no. 18781). Recently this particular B.C.G. was investigated in a bladder carcinoma phase I study. Thirty patients were treated by intravesical and intradermal administration. In this study no side effects of any significance were seen (unpublished data). In 1975 Bloomberg et al. [1] investigated the side effects of B.C.G. (Tice strain) on the dog bladder. In this study some of the dogs were sensitized for P.P.D. The histological changes of the bladder in sensitized (P.P.D.) and non-sensitized dogs were compared. When B.C.G. was injected directly into the submucosa through an open cystostomy a marked inflammatory reaction was seen in all of the sensitized and some of the non-sensitized dogs. This reaction was characterized by an extensive histiocytic infiltration with polymorphonuclear leucocytes, lymphocytes and plasma cells in varying proportions. There were no granulomas or signs of acute cystitis when B.C.G. was applied as an intravesical instillation regardless of prior sensitization; only a mild vascular congestion and dilatation of the lamina propria was observed.

From the present study, despite the small number of animals, we conclude that intravesical instillation together with intradermal application of B.C.G.-R.I.V.M. is safe and without significant toxicity in dogs. Intradermal administration of B.C.G. seems to enhance the formation of small granulomatous lesions in the suburothelial tissue, but does not sensitize any dog to B.C.G. as was indicated by the negative P.P.D.-skin tests at three months after the start of the B.C.G.-treatment.

We think our results might indicate that B.C.G.-R.I.V.M. is a safe agent in the treatment of superficial bladder cancer after transurethral resection. Therefore a prospective randomised study comparing B.C.G.-R.I.V.M. versus Mitomycin-C in Tis, Ta and T_1 primary and recurrent carcinoma of the bladder has been started.

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A. P. M. v. d. Meijden
Department of Urology
St. Radboud University Hospital
Geert Grooteplein Zuid 16
NL-6525 GA Nijmegen
The Netherlands