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THE EFFECT OF POVIDONE-IODINE ON THE URINARY BLADDER MUCOSA OF EXPERIMENTAL ANIMALS

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Abstract

Objective: **To determine the effect of povidone-iodine on the bladder mucosa of experimental animals and to compare the urinalysis and urine culture between the NSS and povidone iodine group.**

Design: **Descriptive experimental study.**

Setting: **Animal laboratory at the Veterinary Research Department, Research Insitute for Tropical Medicine.**

Patients/Participants: **Ten rabbits randomly assigned to 2 groups: Control/NSS (N=3) and Test/povidone-iodine group (N=6). One rabbit served as a baseline and was not treated with any substance.**

Intervention: **Each rabbit of both groups was catherized and instilled with NSS or povidon-iodine for 15 minutes three times daily for 10 days. One control and two test animals were sacrificed on days 3, 7 and 10. Histopathologic sections of the urinary bladders were read by a pathologist blinded as to the agent used for the bladder instillation.**

Results: **Majority of the animals in the test group demonstrated moderated to severe inflammation, mucosal erosion, submucosal edema and congestion in their bladder mucosa. Only in this group were tissue necrosis and microabscess seen. One rabbit died from the povidone-iodine group. Pyuria was seen more in the povidone iodine group with no pathogen isolated from the culture.**

Conclusion: **Bladder instillation with povidone-iodine appears to cause tissue damage in the bladder mucosa of rabbits.**

INTRODUCTION

Iodine (I_2) is a valuable agent that has survived despite the present wide choices of antiseptics. This is because of its efficacy, economy and low toxicity to tissues. Moreover, iodine is bactericidal, sporicidal, fungicidal, protozoacidal, cysticidal and virucidal. In order to increase the iodine content, as well as to augment dispersibility and penetrance, iodophors (I_3-reservoirs from which I_2 can be continuously released) are included in solutions and tinctures.

Povidone-iodine is an iodophor where iodine is complexed with polyvinyl-pyrollidone. Its principal use has been in the prevention of postoperative infection, mainly as a preoperative hand scrub or as a local skin disinfectant. It is also widely used for office and emergency chemosterilization. Novel applications have included its use as a lavage in patients with small-bowel perforation,[2] as an irrigant in children with empyema thoracis,[3] in the management of patients with non-specific vaginitis,[4] as well as in the treatment of aortic graft infections.[5]

Several studies have been performed utilizing povidone-iodine as a bladder antiseptic, particularly in catheter-related urinary tract infections in hospitals and chronic care facilities. Schneeberger and colleagues used a single dose of povidone-iodine as a bladder irrigant prior to catheter removal and studied its effect on subsequent bacteriuria[6]. A similar study was performed by Richter et al on patients scheduled for prostatectomy[7]. In contrast to these studies where only a single instillation of the solution was used, Giannoni et al performed continuous bladder irrigation for three to seven days on patients with an indwelling catheter placed after transurethral prostatectomy[8], while Sharpe and associates compared the effect of twice-daily instillations of povidone-iodine solution against a single-dose regimen[9]. These and other studies concentrated on the efficacy of povidone-iodine solution in the prevention of bacteriuria[10,11]. However, no mention was made ot its adverse effects, if any, on the bladder mucosa. Although several studies have documented its toxicity on the peritoneum of animal models when used as a lavage solution,[12,13,14] there are conflicting reports regarding its effect on the bladder epithelium[8,9,15,16,17,18].

In the local setting, bladder instillation with povidone-iodine is used by some physicians as an option for treatment of chronic urinary tract infection especially in those with long-standing indwelling catheters. To date no local studies have been done on the effect of this agent on the urinary bladder mucosa on treated subjects.

Data from previous studies have documented the efficacy of a 2% povidone-iodine solution in eradicating bladder infections[19,20,21] since it has also been reported that the free iodine concentrations (available for immediate antimicrobial effect) might actually be higher at concentrations below 5%, there seems to be

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no benefit in using higher strengths for bladder irrigation[22]. Although this concentration appears to be well-tolerated, painful irritation or bladder contraction have sometimes been reported[10].

This study aims to use an animal model (1) to determine the effects of exposure to 2% concentration of povidone-iodine on the bladder mucosa and (2) to compare urinalysis and urine culture results between the povidone iodine and saline treated groups. Data from this study may potentially be useful for patients requiring either intermittent or indwelling catheterization.

LIMITATION OF THE STUDY

The sample size was small, with only ten experimental animals included (three served as controls given normal saline solution (NSS), six served as test animals treated with povidone-iodine and one animal served as a baseline not treated with any substance). Limitation of the sample size was due to the principle of reduction based on the code of practice in the care and use of laboratory animals[23],[24].

METHODOLOGY

The Veterinary Research Department of the Research Institute for Tropical Medicine was the venue for the procedure.

Ten rabbits, all male with mean weight of 2.1kg, 2-3 months old and apparently healthy, were selected and quarantined for three weeks at the animal laboratory. During this time, the rabbits were held in separate cages and acclimatized to the air-conditioned animal laboratory maintained at an optimal temperature of 19°C. Each rabbit was dewormed and fed ad libitum. The animals were weighed on days 0,3, 7 and 10.

The urinary bladder capacity was estimated by infusing sterile saline (at increments of 1 ml) through a catheter into the bladder until the rabbit urinates. The mean bladder capacity was 7.5 ml.

The rabbits were divided randomly into two groups. Three rabbits were categorized under the control group while the remaining six rabbits were categorized under the test group. One rabbit served as a baseline and was not treated with any substance. This rabbit was sacrificed on Day 0 and the urinary bladder was submitted for histopathologic examination.

All the rabbits underwent straight catheterization using a French 5 infant feeding tube for the baseline (Day 0) urinalysis and urine culture. Prior to each catheterization, the urethal meatus was cleaned with 10% povidone-iodine (Betadine) solution. A new catheter was used for every collection or drainage of urine and for every instillation of specific bladder wash. 2% xylocaine jelly was used as lubricant during all catheterizations.

Bladder instillation, bladder irrigation and bladder wash are used interchangeably in this study to mean introduction of a sterile fluid into the bladder through a catheter, leaving it there for a variable holding time and draining it afterwards.

Starting on Day 1 of the actual experiment and daily thereafter, the urinary bladder of each rabbit was emptied of urine then instilled with 5-10 ml of either normal saline (NSS) or 2% povidone-iodine solutions. 2% povidone-iodine was prepared in the pharmacy using 1.5 dilution of 10% povidone iodine with sterile water.

The bladder of the test group was instilled with povidone-iodine three times daily through a French 5 catheter. The catheter was clamped for 15 minutes, after which it was allowed to drain and the catheter was then removed. The same procedure was done on the control groups, using NSS instead of povidone-iodine. This procedure was done for 10 days. Urine samples for urinalysis and culture were obtained on days 3,7 and 10 prior to instillation of povidone-iodine or NSS to document possible infection. Cultures of NSS and povidone-iodine used in this experiment were likewise done on the same period to check for possible contamination of fluids instilled in the bladder.

One control and two test rabbits were sacrificed on days 3,7 and 10 and their urinary bladders were submitted for histopathologic examination. The rabbits were sacrificed by ketamine overdose and exsanguination.

Histopathologic examination was done by a pathologist blinded as to the substance instilled in the urinary bladder. Sections were obtained from the uninjured portion of the bladder mucosa as determined grossly by the pathologist.

DATA ANALYSIS

Descriptive statistics were used for this experimental study since the sample size was too small to be able to compare the significant differences between the two groups.

The bladder mucosal changes were graded according to the presence or absence of a given histologic characteristic, i.e., from "negative" to "severe" (Table 1). The histologic findings used to describe the mucosal changes include the following: inflammation, intramucosal edema, mucosal erosion, submucosal

Table 4. Urinary bladder mucosal changes (histopathology) in the rabbits on specific days of sacrifice. D, day; NSS, normal saline solution; B, baseline; C, control

	D0	D3			D7			D10		
	Baseline	NSS	Povidone-iodine		NSS	Povidone-iodine		NSS	Povidone-iodine	
	B1	C1	T1	T2	C2	T3	T4	C3	T5*	T6
Inflammation	-	+	+++ §	+++	+	++++	-	-	++++ ¶	++++
Intramucosal Edema	-	+	+	++++	+++	+	++++	++++	ND	++
Mucosal Erosion	-	+++	+++ §	+++	++	++++ §	-	-	++++ §	+++
Submucosal Congestion	-	+	+++	+	++	++	+	++	+	++
Submucosal Edema	-	+	++++	++++	+	+++	-	++	++++	+++
Squamous Metaplasia	-	-	-	-	-	+	-	+	-	+
Fibrosis	-	-	-	-	-	-	-	-	-	-
Hemorrhage	-	-	+++	-	++++	-	-	-	++	-

* rabbit died on Day 8
\- normal/negative
\+ trace
++ mild
+++ moderate
++++ severe
§ necrosis
¶ microabscess
ND no data

Intramucosal edema

The control group showed increasing severity (from trace to severe) of edema from Days 3-10. The test group showed severe intramucosal edema in one rabbit as early as Days 3 and 7.

Figure 2. Histologic section depicting severe intramucosal edema of the urinary bladder of one of the test rabbits (Day 7). High-power magnification.

Mucosal erosion

Moderate degrees of mucosal erosion were noted on Day 3 of infusion of NSS decreasing in severity till Day 10. The test group showed 83% of the rabbits having moderate to severe mucosal erosion with necrosis on Days 3-10.

Figure 3. Histologic section showing moderate mucosal erosion (arrow) of the urinary bladder of one of the test rabbits. Low-power magnification.

Submucosal congestion

The control group showed trace to mild submucosal congestion. The test group showed mild to moderate degrees of submucosal congestion.

Submucosal edema

Trace to mild submucosal edema was seen in the control group. 83% of the test group showed moderate to severe submucosal edema.

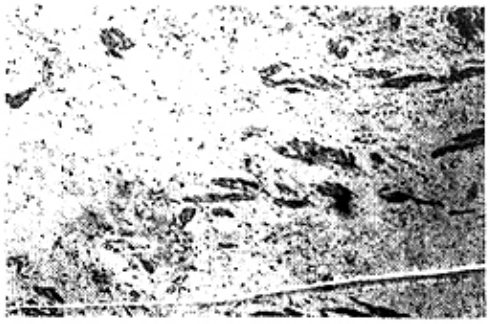

Figure 4. Histologic section showing submucosal edema of the urinary bladder of one of the test rabbits. Low-power magnification.

Squamous metaplasia

A trace of squamous metaplasia was noted on Day 10 for the control group and one rabbit each on day 7 and 10 for the test group.

congestion, submucosal edema, squamous metaplasia, fibrosis and hemorrhage.

RESULTS

All the rabbits belonging to the control group tolerated 15 minutes, thrice daily exposure to normal saline solution. In contrast, the mean dwelling time for the test group (where 2% povidone iodine solution was instilled) was only 9.2 minutes (Table 2). Intolerance was manifested by spontaneous urethral expulsion of the catheter and povidone-iodine solution after a few minutes of dwelling time. Initially (Days 1-6), it was observed that the test group had low tolerance but starting on Day 7, the tolerance to povidone-iodine was better. This was shown by the increase in dwelling time of more than 12 minutes, reaching the highest average of 13.5 minutes on Day 10 of instillation.

Table 1. Grading of urinary bladder mucosal changes.

Symbol	Description
-	Absent/negative
+	Trace
++	Mild
+++	Moderate
++++	Severe

Table 2. Comparison of mean dwelling time per day (in minutes) between the control and test groups during the daily bladder instillation of normal saline solution (NSS) and Povidone-Iodine

Day Group	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
NSS	15	15	15	15	15	15	15	15	15	15
Povidone-Iodine	5.9	6.6	4.8	7.6	10.6	9.4	12.5	13.3	13.3	13.5

Urinalysis

The yellow color, turbidity and alkaline reaction of urine were similar for both groups. Specific gravity ranged from 1.000-1.023. Both groups had varying amounts of protein. There was no glucosuria noted in all the rabbits. Hematuria of>5 RBCs and pyuria of>10 WBCs were seen more in the test (povidone-iodine) group. Pus cast was noted in one rabbit of the test group on day 3 and was not seen in the control group. Bacteriuria and abundance of amorphous phosphates were similar in both groups while urinary crystals like cystine, uric acid and calcium carbonate were seen more in the control group up to day 7.

Urine Culture

Baseline urine cultures on Day 0 showed no growth. On Day 3 of bladder instillation, one rabbit from the control group had *E. coli* and another one had *Diphtheroids*. Both of these cultures did not show significant pyuria (>10 WBC) in their respective urinalysis. However, one of them had few bacteria. Subsequent cultures on Day 7 of the same rabbits showed no growth. None of the rabbits from the test group had a positive urine culture despite significant pyuria in most of their urinalysis results. (Table 3)

Table 3. Comparison of urine culture between NSS and Povidone Iodine group on specified days. N = number of rabbits (%).

	NSS				Povidone-Iodine			
	D0	D3	D7	D10	D0	D3	D7	D10
	N=3	N=3	N=2	N=1	N=6	N=6	N=3	N=1
(-) growth	3(100)	1(33)	2(100)	1(100)	6(100)	6(100)	3(100	1(100)
(+) growth	No growth	2*(66)	No growth	No growth	No growth	No growth	No growth	No growth

*>100,000 *E. coli* and *Diptheroids*

NSS/Povidone-iodine Culture

Cultures of the agents instilled in the urinary bladder did not grow any microorganism.

Hispathology Results

The urinary bladder of the rabbit not treated with any substance (baseline) on Day 0 showed normal mucosa.

Inflammation

Compared with the control group, the test group showed more inflammation with necrosis as early as the third day of treatment with povidone-iodine. This became more severe on Days 7 and 10.

Figure 1. Histologic section depicting moderate mucosal inflammation (blue arrow) and submucosal congestion (green arrow) of the urinary bladder of one of the test rabbits (Day 3). Low-power magnification.

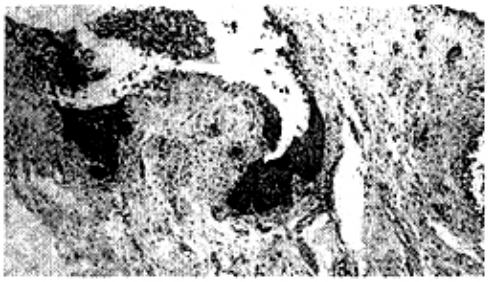

Figure 5. Histologic section revealing trace of squamous metaplasia (arrow) of the urinary bladder mucosa of one of the test rabbits. Low-power magnification.

Fibrosis

No fibrosis was noted in both groups

Hemorrhage

Severe hemorrhage was seen on Day 7 of control group. Mild and moderate hemorrhages were noted in 2 rabbits of the test goup.

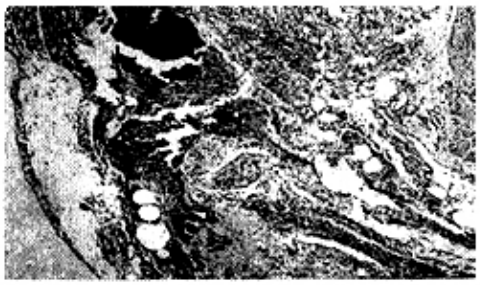

Figure 6. Histologic section depicting hemorrhage in the urinary bladder mucosa of one of the control rabbits. Low-power magnification.

In summary, moderate to severe inflammatory changes, mucosal erosion, submucosal congestion and submucosal edema were observed more in the povidone-iodine group (Table 6). Milder changes were seen in the NSS group except for intramucosal edema and hemorrhage. Squamous metaplasia was observed in both groups. Evidence of fibrosis was not seen in all of the specimen.

Table 7. Summary of histopathologic changes of the test and control groups according to the degree of change.

Degree of change Histologic Characteristics	Trace-Mild		Moderate-Severe	
	NSS (N=3)	Povidone-Iodine (N=6)	NSS (N=3)	Povidone-Iodine (N=6)
Inflammation	2 (66%)	---	---	5 (83%)
Intramucosal edema	1 (33%)	3 (50%)	2 (66%)	2 (33%)
Mucosal erosion	1 (33%)	---	1 (33%)	5 (83%)
Submucosal congestion	3 (100%)	5 (83%)	---	1 (16%)
Submucosal edema	3 (100%)	---	---	5 (83%)
Squamous metaplasia	1 (33%)	2 (33%)	---	---
Fibrosis	---	---	---	---
Hemorrhage	---	1 (16%)	1 (33%)	1 (16%)

Postmortem results

The rabbit that died on day 8 of the experiment belonged to the group given povidone-iodine. Postmortem findings of the urinary bladder showed severe inflammation and mucosal erosion with necrosis, submucosal edema and mild hemorrhage. Microabscess was also seen in the bladder mucosa, this was not seen in the control group. Pulmonary congestion with pulmonary hemorrhage and mild congestion of the liver were also seen. The rest of the organs were normal.

DISCUSSION

The urine of rabbits normally varies in color from turbid-yellow to reddish brown, depending on the diet and is not clear due to the mucous and crystals it contains[25]. The low specific gravity, alkalinity of urine and some amounts of proteinuria observed in this experiment are representative of the typical urinalysis of normal rabbits. Urine crystals like phosphates, calcium carbonate and cystine crystals are also normally seen. Casts, bacteruria, pyuria and hematuria may be rare or occasionally seen[26].

Hematuria was observed in both groups and may be related to trauma secondary to repeated catheterization. The increased degree of hematuria in the test group may be due to povidone iodine solution. In an article by Getliffe, it was mentioned that solutions used in bladder instillation may increase the presence of red blood cells in the urine[27]. The same article also observed significant reduction of urinary crystals in bladders treated with acidic solution though it did not mention specifically povidone-iodine.

Pyuria was observed more commonly in the test group. This could be attributed to inflammation brought about by the irritant effect of povidone-iodine, catheterization or presence of an infection. Even with the presence of pyuria and some bacteria, no urinary pathogen was isolated in the culture of the test group. Introduction of bacteria into the bladder was possible during catheterization since majority of the rabbits had some form of resistance during the procedure. The absence of growth in the urine culture may be due to intermittent sterilization brought about by the antiseptic effect of povidone-iodine as proven by previous studies[6,11]. Two rabbits of the saline group had growth of microorganisms. One had a true urinary pathogen, *E. coli* and the other had *Diphtheroids*, usually considered a contaminant. One study stated that normal saline does not inhibit bacterial growth compared to other urologic irrigation fluids[28].

The initial short dwelling time of the test group most probably was secondary to the irritant effect of povidone-iodine on the bladder mucosa. Irrigating solutions can be associated with mucosal irritation especially so with repeated catheterization[15]. Subclinical mucosal changes may develop under these circumstances[15]. In most experiments, saline-treated subjects had the least of these irritative effects[15,18]. In relation to the histopathology of the urinary bladder, as early as Day 3 of bladder instillation with povidone-iodine, moderate degree of inflammation and mucosal erosion were observed even if the mean dwelling time during this period was still low. Inflammation is characterized by edema and predominance of neutrophils. Another noticeable observation was the presence of necrosis seen only in the test group. Necrosis is the major morphologic manifestation of an irreversible cell injury that follows cell death in a living tissue or organ. It is usually the result of an acute inflammatory reaction if the stimulus is not removed in response to an injury[29]. In our experiment, some of these inflammatory changes progressed in severity during subsequent days of treatment. Varying degress of intramucosal edema, submucosal congestion and edema observed in both groups were part of the acute inflammatory process which could be brought about by any injury. In this instance, chemical injury was caused by exposure to NSS and povidone-iodine, more severe in the latter.

In a study of Elliot et al (1989), exfoliation rates of urothelial cells following bladder irrigation with NSS, chlorhexidine and noxythioline showed an increased shedding of urothelial cells suggesting that bladder irrigation further damages the already disrupted urothelium brought about by catheterization[27,30]. The risk of tissue damage was observed to be less in the saline group. Bladder irrigation using povidone-iodine in rat bladders likewise showed erosive reactions compared to saline[18].

Some degree of metaplasia was noted in the latter part of the experiment, Day 7 and Day 10 (1 rabbit in the control group, 2 rabbits in the test group). Metaplasia is the substitution of cells more sensitive to stress by other cell types better to withstand the adverse environment[29]. It is a form of adaptation of cells to injury especially seen in chronic inflammation.

Microscopic hemorrhage seen in both groups may be produced in loose tissues with marked vascular congestion.

One test rabbit (T5) died before being sacrificed. The cause of death by autopsy showed pulmonary hemorrhage, the etiology of which could have been due to urosepsis since microabscesses were seen in the urinary bladder. There were significant pyuria and hematuria by urinalysis though no specific pathogen was isolated by culture.

If at all possible, pain and distress to the rabbits were minimized in conformity with animal welfare regulations (as investigators were guided by a senior laboratory technician). However, we could never really determine how they responded to the repeated catheterizations. It may be assumed that T5 has the lowest threshold to pain and distress and this could be expressed in terms of its decrease food consumption, loss of weight and decrease activity during the latter part of the experiment. This may be contributory to the early demise of the rabbit in addition to the infection.

It could have been ideal if instead of sacrificing the animals, chronologic bladder biopsies (Days 0, 3, 7 and 10) were done on the same rabbit to accurately compare the degree of urinary bladder changes treated with povidone-iodine. However, this was technically difficult to do hence we opted to sacrifice different rabbits on specified days to compare the bladder changes. This could explain the variable results on histology of the bladders treated consecutively with povidone-iodine or NSS.

Our experiment is in agreement with some studies that showed that povidone-iodine causes tissue damage to the bladder mucosa [15,16,18].

CONCLUSION

Although it was difficult to derive a definite conclusion from this experiment due to the limitations in the study, it was observed that those treated with povidone-iodine had more pyuria with no pathogen isolated and more severe bladder mucosal changes. This should lead us to reassess the use of this agent as a form of bladder wash.

RECOMMENDATION

It is recommended that a larger sample size be used for further studies so that the significant difference between the two groups can be computed statistically. Urinary bladder changes treated consecutively with povidone-iodine should be compared using the same rabbit. It is also recommended that electron microscopy should be used to examine ultrastructural changes in the bladder epithelium.

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