Evaluation Of Long-term Gastrocystoplasty In Rats. A Histopathological Study

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ABSTRACT

Objective: Evaluate the functional outcome of bladder augmentation and complications associated directly to the use of the gastric patch.

Materials and Methods: Gastrocystoplasty was performed on male Wistar rats (n=26) weighing an average of 200 g. These animals were divided into two groups. Group I (13 rats) was observed over a period of 155 days and group II (13 rats) over a period of 360 days. At the end of this period an urodynamic evaluation of group I was carried out without the use of anesthetic. The rats in both groups were then euthanized, and the bladder and a patch of transplanted stomach - along with the vascular pedicles - were removed for histopathological evaluation. Electrolytes and arterial blood gases were studied.

Results: Macroscopic evaluation of rats in group I revealed three bladders with papilloma and stones. Microscopic evaluation verified metaplasia of squamous epithelium in transplanted flaps, hyperplasia and distention of the transplanted gastric mucosa. Group II rats presented hyperchloremia, hypophosphatemia, hypomagnesemia, hyperkalemia, normonatremia, discreetly elevatedcreatinine and urea, normal ionic and serum calcium values and unaltered arterial blood gases. Microscopic evaluation showed squamous metaplasia, metaplasia and glandular dilation of the transplanted gastric mucosa. A light and moderate nonspecific chronic inflammatory process was observed in both groups. No histological sign of neoplasm or alterations of the urothelium was diagnosed.

Conclusions: Rats subjected to gastrocystoplasty showed processes of squamous metaplasia, hyperplasia, glandular dilatation and papillomatosis. The gastric mucosa presented foci of a light or moderate inflammatory process and ulceration foci. Despite all of this, gastrocystoplasty seems to be a good surgical option.

INFORMAÇÕES

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INTRODUCTION

The use of stomach segments has been sugges ted as an alternative to treat some pathologies of the lower urinary tract when other interventions (ileal, caecal, and colonic segments) are contraindicated[1]. Sinaiko was the first to become interested in the use of stomach segments in the treatment of the lower urinary tract[2]. Later on, it was verified that the stomach could be, at least theoretically, a suitable reservoir for individuals with renal failure, and concluded that tract gastrocystoplasty would be a good alternative for bladder augmentation[1,3] since this procedure maintains the acid/base balance in carriers of renal insufficiency.

Frequently, when urinary diversion is indicated as a therapeutic conduct, the dysfunction of the lower urinary tract can be associated with impaired renal function and/or existing metabolic acidosis. Because of these alterations, the employment of the small or large intestine for reconstruction of the bladder can worsen the patient's symptoms because of extra-renal

reabsorption of urinary solute, particularly of ammonium chloride (the principal mechanism leading to the production of acidosis)[2,4,5]. Moreover, subsequent development of chronic acidosis with other implications may be induced[6]. The productions of mucus, the susceptibility to urinary infection and electrolyte disturbances (hyperchloremia, hypercalciuria, hypophosphatemia) have been considered the main problems when intestinal segments are used[6].

To decrease these complications, some investigation has been carried out changing a part of or the entire body of the bladder by a portion of the stomach. On a long-term basis it has been verified that the stomach does not reabsorb chloride and ammonium, and retains bicarbonate to maintain metabolic[7,8,9]. With that verification, it becomes evident that the stomach might offer advantages over segments of the large and small intestine in the reconstruction of the bladder.

A possible solution to decrease unfavorable side-effects in the use of intestinal segments has been the implantation of reconfigured or "detubularized" segments of the ileum or colon in the bladder. This procedure can minimize the segment's own contractile activities, increase the capacity of the bladder and reduce intravesical pressure. However, although this procedure can offer some benefits, nevertheless the risk of serious complications still persists[10,11,12,13].

Thus, the aim of this work is to evaluate the functional outcome of bladder augmentation and the complications associated directly to the use of the so-called gastric patch (antrum) in the ectopic position in direct contact with urine during a long-term follow-up.

MATERIAL AND METHODS

Male Wistar rats (n=26), weighing an average of 200 g, were operated on. The rats were not fed on the eve of surgery so that their stomachs were empty during the procedure. They were anesthetized with pentobarbital (50 mg/kg) intravenously. The dose was increased during surgery, when necessary. The abdomen was shaved and a wide midline incision was performed to allow for satisfactory exposition of the stomach and bladder. As the blood-vessels of the great curvature of that organ are incomplete in this species of rat, it was decided that the most suitablesegment to be removed would be a portion of the antrum. After the suture of the tributary vessels (vasa brevia) up to the 3rd-4th portion of the duodenum, and complete isolation of the right gastroepiploic pedicle artery, a bull-dog clamp was placed in the antrum in elliptic form so as not to compromise or injure the left gastroepiploic artery and a wedge segment of the greater curve mobilized and harvested as a vascularized flap, corresponding to 10 X 7 mm^2. That portion of the stomach was washed with a cold solution of 0.9% saline and kept in the solution for a period of 15-20 minutes. The stomach was sutured with 7-0 monofilament polypropylene. Soon after, the bladder was opened at the dome with an antero-posterior detrusorotomy measuring 8-10 mm in length. The stomach patch was placed over the open part of the bladder, and a continuous suture with 7-0 dexon "S" thread was performed. After that, a careful check of the pedicle of the patch was made, and no tension was observed. At the end of the procedure it was verified that the autoimplanted stomach patch presented a satisfactory blood flow and good coloration. Soon after, the autoaugmentation detrusorrhaphy was finished. The bladder was tested with fluid to make sure that the anastomosis was water tight. The abdominal incision was closed in layers.

The rats were housed in standard cages at 22 ºC, and a 12 h light/dark cycle, and had free access to commercial standard rat food pellets and tap water ad libitum. The animals were maintained without the use of antibiotics or any other drug. During the postoperative period no rat presented hematuria. The animals were randomized into two groups. Group I (n=13) was observed for a period of 155 days and Group II (n=13) for 360 days.

For evaluating the bladder augmentation, a midline abdominal incision was made for withdrawal of the amplified bladder together with the gastric patch and corresponding left gastroepiploic artery in rats of group I. Specimens were immersed in 10% buffered formalin, dehydrated and embedded in paraffin. Representative samples were stained with hematoxylin-eosin.

After 360 days of surgery, the rats in group II were submitted to biochemical evaluation of sodium, potassium, chloride, serum calcium, ionic calcium, phosphorus and magnesium electrolytes, creatinine, blood urea nitrogen (BUN), urine culture as well as a study of acid-base balance. In the same manner as in the group I, specimens were formalin fixed and paraffin embedded, and stained with hematoxylin-eosin.

RESULTS

In the period following the implantation surgery, no sign of urinary incontinence, hematuria or any other occurrence

was verified in the animals, that is, the abdominal hair of the rats was found to be always dry, which is indicative that they suffered no alterations in urinary control after the surgical procedure. No alterations were observed in the external urethral meatus of those animals as a result of acid urine or the presence of mucus. It was verified that the size of the gastric patch used in the experiment seems to have been significant enough to increase the bladder capacity (unpublished observation).

Urine culture of seven rats from group II (360 days post surgery) was performed, and no growth of bacteria was observed in five rats, but two rats showed positive urine cultures: one for the bacteria Escherichia coli and the other for Pseudomonas aeruginosa. The urine pH was 7.3±0.2 (medium±sem). Rats of group II presented hyperchloremia (108.7±1.8 mEq/L), normonatremia (141.1±1.7 mEq/L), normocalcemia (9.9±0.2 mg/dL), hypomagnesemia (1.9±0.1 mEq/L) and hypophosphatemia (4.7±0.3 mg/dL). The creatinine level was 1.9±0.2 mg/dL (normal 0.7 to 1.3), the BUN level was 60.0±3.0 mg/dL and the potassium level was 5.9±0.3 mEq/L (Table-1). The ionic calcium level was 1.34±0.01 mg/dL, with a pH of 7.43±0.01 in arterial blood gases. The acid -base balance of these rats (n=11) was normal (Table-2). None of the animals exhibited any type of disturbance during the observation period.

TABLE 1 - Values of electrolytes of normal (n=5) and operated (n=13) rats after 360 days of gastrocystoplasty (medium ± S.E.M).

	NORMAL	OPERATED
Na+	137.2 ± 2.7	141.1 ± 1.7 #
K+	5.7 ± 0.2	5.9 ± 0.3 #
Cl-	103.7 ± 1.5	108.7 ± 1.8 #
Ca++*	10.9 ± 0.1	9.9 ± 0.2 ‡
Mg++	1.7 ± 0.1	0.3 ± 0.8#
P+++	7.4 ± 0.5	4.7 ± 0.3 ‡
Creatinine	1.1 ± 0.1	1.9 ± 0.2 ‡
BUN	76.2 ± 3.9	60.0 ± 0.3 ‡
Ionic Ca	1.4 ± 0.02	1.3 ± 0.01†

= mEq/L; ‡ = mg/dL;† = mmol/L; * = serum calcium

TABLE 2 – Values of the acid-base balance of operated rats (n=11) after 360 days of a gastrocystoplasty (medium ± sem).

pH	7.4 ± 0.02
PO2	63.0 ± 4.2
PCO2	32.0 ± 23
HCO3-	23.6 ± 0.8
BE	0.3 ± 0.8
Sat. O2	91.7 ± 3.9

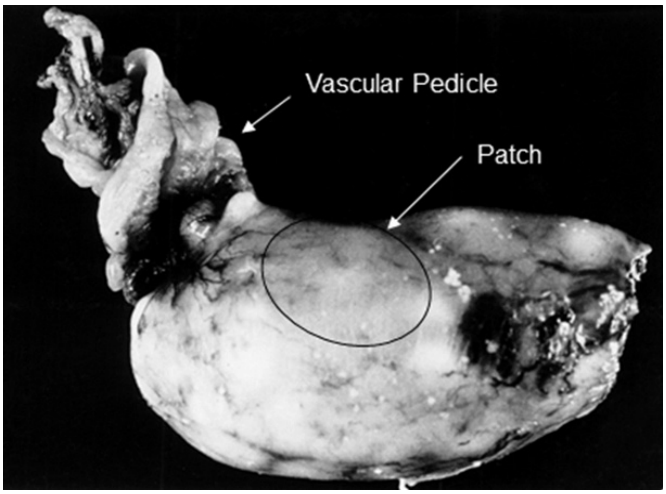
After longitudinal opening of the bladder of rats in group I, no suggestive inflammatory change was observed in the bladder mucosa, even in the proximity of the ureteral meatus that could have resulted from urine with sufficiently low pH. Mucus was not found inside the neobladders. The ureters had an unchanged size, without signs of dilation upon inspection. With the neobladders full of fluid, the patch and the bladder wall were on the same plane, showing adequate structural architecture, and suggesting that the patch implantation had not produced pseudodiverticulum (Figure-1).

Evaluation of the mucosa of the autograft showed a brown-gray coloration. Three neobladders (23%) presented papillomatosis (Figure-2) of brown-yellowish color (Table-3); three other neobladders (23%) had multiple calculi; the two largest measuring 3.0 mm approximately in diameter and were whitish in color and of brittle consistency.

The autografts of both groups were embedded in paraffin, cut to 5 μm in thickness, and stained with hematoxylin-eosin. Through histology it was noted that the tissue vitality in the transition of gastric and bladder mucosa was preserved (Figure-3). In the mucosa of the stomach patch from rats in group I, a light and moderate nonspecific chronic inflammatory process, squamous metaplasia with several degrees of maturity (23%), hyperplasia (31%) and papillomatosis tissue (23%) were observed in three neobladders. In the rats of group II, under the optical microscope, the neobladders presented squamous metaplasia (46.2%), metaplasia in the gastric patch (15.4%) and glandular dilation of the mucosa (38.5%). Ulceration and infection foci were found in the bladder wall of three rats (23%). A light inflammatory process was observed in both groups.No change was found in the area of transition between gastric and bladder mucosa (Figure-3). In macroscopic evaluation of neobladders of rats in group II, the aspect

FIGURE 1

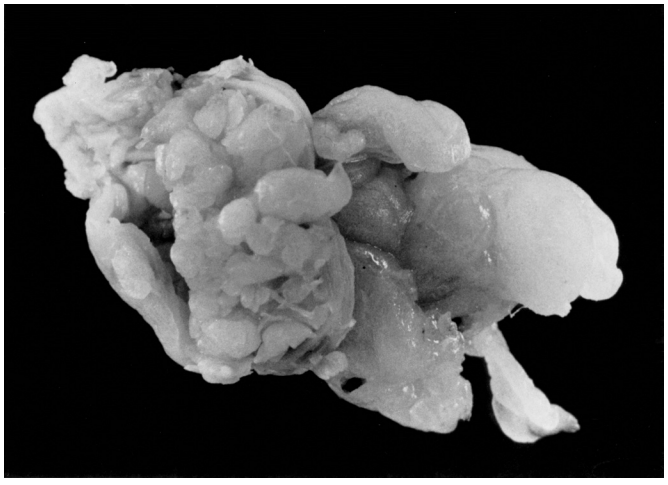
Stretched rat neobladder with fluid after 155 days of gastric patch implantation. Vascular integrity, defining the limits of the graft and vascular pedicle (arrows), can be observed in the corresponding gastric patch.



Fonte: Arquivo Pessoal.

FIGURE 2

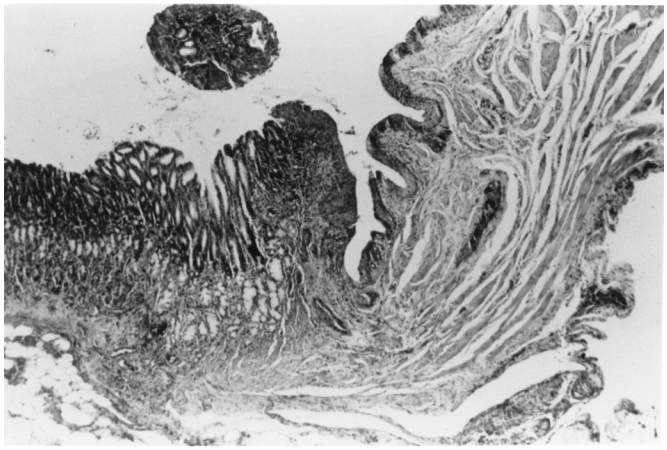
Neobladder of open rat in longitudinal sense after 155 days of gastric patch implantation, showing papilloma (arrows).



Fonte: Arquivo Pessoal.

FIGURE 3

Histology of rat neobladder after 155 days of gastric patch implantation. The area of transition of gastric and bladder mucosa (arrow) is noted to be normal (HE 50X).



Fonte: Arquivo Pessoal.

was similar to that observed in group I, *i.e.*, a whitish-gray appearance in the bladder mucosa, however, without calculi. Upon macroscopic evaluation of the kidneys and ureters, no significant alteration was found in either group.

DISCUSSION

Gastrocystoplasty has been widely used as an alternative to intestinal augmentation, despite possible complications. Metabolic alterations are uncommon because the gastric mucosa is relatively impermeable; it inhibits the ammonium chloride absorption and prevents hypochloremic acidosis[1,6]. Reports indicate that the complication rate has been low; there were no problems related to mucus production, availability of the tissue, absorption of electrolytes, production of calculi, possibly due to the bactericide effect of acid secretions, which could result in reduced nitrosamine production, reducing the long-term risk of carcinogenesis[12].

TABLE 3 - Histological appearance in bladder augmentation of rats in the group I (155 days) and in the group II (360 days).

GROUP I	Papillomatosis	23,0%
	Squamous Metaplasia + Hyperplasia	31,0%
	Squamous Metaplasia	23,0%
GROUP II	Ulceration Foci	23.0%
	Squamous Metaplasia	64.2%
	Metaplasia in the Gastric Patch	15.4%
	Glandular Dilatation	38.5%

The use of stomach segment implantation for bladder treatment seems to surpass some of the difficulties in surgical procedures in urinary diversions. Experimental research has suggested that the stomach secretes H^+, Cl^-, $NH4^+$, and that it does not reabsorb those elements and their elimination avoids acidosis and the loss of buffers[12], even when the stomach is used in diversions in which the urine is stored for a long-term[8]. This procedure contrasts with those findings using segments of the small or large bowel[7,14,15].

The rats evaluated in this research presented light hyperchloremia when compared with normal rats, and such data conflicts with data published elsewhere[1,7]. By some means, surprisingly, the values found for phosphorus and magnesium were reduced in operated rats. Hypomagnesemia is, as a rule, related to a drop in serum calcium while hypophosphatemia is associated with hypocalcemia, which was not noted in this study. It is possible that the reduction of the serum phosphorus is due to its secretion by the transplanted flap[7]. The acid-base balance of the animals in this experiment and the normal values of serum calcium and ionic calcium are indicative that hyperchloremia was not high enough to provoke any significant clinical abnormalities.

Urea and creatinine levels in both normal and treated rats were found to have slightly altered values, when compared with younger rats. These results raised the possibility that control and treated rats showed decreased renal function, probably caused by their age. It may be that the clearance of creatinine in these animals could possibly improve the interpretation of data.

According to Rudick et al, the urine pH in animals with gastrocystoplasty is 5 - 6, and that level can decrease significantly after meals, as was verified in studies performed on dogs[8]. However, the medium level of pH observed in the urine of rats with gastrocystoplasty was higher than that verified by those authors in their study of dogs, but similar to the values noted by other researches[16].

Through cystomanometry graphs, a significant increase in bladder capacity was noted. The volume collected in the neobladder was larger than the volume collected in the rats of the control group (interval of the micturition contraction) without graphic signals of uninhibited bladder contractions, *i.e.*, good storage with increased compliance occurred. In this case, treated rats presented larger micturition intervals and reduced micturition pressure amplitude when compared with untreated rats (unpublished data).

The opening of the bladder in the antero-posterior sense and the interposition of the stomach flap (10X7 mm) does not seem to alter the bladder physiology. As there was nomodification in the geometric configuration of the neobladder with a possible formation of a pseudodiverticulum in the organ because of the implantation of the gastric patch (tested by filling the bladder with fluid to provoke considerable distention), this observation suggests that the tension of the stomach patch and of the detrusor is similar in these animals. This fact indicates that the gastric patch is easily adaptable to the geometrical form of the bladder, and that format should facilitate the bladder emptying as occurs in the bladder of untreated animals. Moreover, no contractions of the neobladder were recorded during the intervals of micturition, *i.e.*, cystomanometric studies in these animals showed no compromise in bladder function[17].

Through macroscopic evaluation it was observed that the gastric patch transplanted to the bladder presented a smaller degree of alteration. Through histological study those structures corresponded to the metaplasia reactions and hyperplasia of the squamous epithelium.Three neobladders had papillomatosis in the graft sites. It is possible that metaplasia and hyperplasia processes facilitated papilloma formation[18]. Upon further investigation, there was no histological sign suggesting premalignant or malignant alterations in the gastric segment or in the native bladder. Although our experimental observation took place in a shorter time limit than that of Klee et al, the histological discoveries were similar to those found by these authors[14]. A review of published research up to date has revealed no clinical report of neoplasic processes in gastrocystoplasty, except for two cases noted by Vemulakonda et al[19], unlike what has been verified in

other enterocystoplasties[12,20,21]. In studies in rats with urinary diversion, in which the bladder mucosa was in direct contact with feces, it was possible to determine that alterations such as metaplasia, dysplasia and adenocarcinoma occurred[14,22,23,24,25]. It has also been reported that the occurrence of this neoplasm is more frequent at the location of the implantation of the ureter, the site of suture of the urothelium and the intestinal mucosa, even if the tissue of the urinary tract is free from fecal substances[12]. In relation to gastrocystoplasty, no changes were noted in the present investigation.

Abnormalities found in the gastrocystoplasty, such as papillomatosis, ulceration foci, hyperplasia and squamous cell metaplasia in the bladder tissue, metaplasia in the gastric patch cells and glandular dilation can be interpreted as degenerative and/or adaptable alterations of the gastric and bladder mucosa.

Although some authors have claimed that ulceration or erosion was not present in the gastric mucosa[3,7] microscopic evaluation showed that all the neobladders in our experiments presented a light or moderate inflammatory process, and that slight ulceration foci were present in 23% of them. These data are similar to those observed by Muraishi et al[15]. The hypothesis that those alterations were provoked by local acidity[2] is not consistent with our data, since the urine pH of those animals was almost neutral. Other factors probably exist, although not yet identified, which could be responsible for these alterations. These could be associated to clinical cases of hematuria-disuria syndrome found in individuals submitted to gastrocystoplasty who subsequently presented hypergastrinemia[16] and low urinary pH[15].

In the present experiment, no mucus was noted in the urine of the animals during the period of observation, and this was also not verified inside the neobladders at the time of sacrifice. According to the report of Bogaert et al, secretion exists, however it is in a much smaller quantity than that produced by the large or small intestine when isolated and connected to the urinary tract[26]. It is possible that a small amount of secretion was enough to cause the formation of stones found in three neobladders. On the other hand, it has been observed that in enterocystoplasties the levels of serum sulfate are increased[27], and the high renal excretion of this element can facilitate hypercalciuria and osteopenia, favorable factors in the formation of stones[5,28,29]. The hypothesis that excessive acidification of the urinary tract can facilitate the precipitation of salts and consequent formation of calculi, especially uric acid as commented by Rudick et al is controversial since the urine of the animals in this research was alkaline[8].

In conclusion, it was observed that pH values of the rats subjected to gastrocystoplasty were neutral; and that the patch for the gastrocystoplasty in rats showed processes of squamous metaplasia, hyperplasia, glandular dilatation and papillomatosis. The gastric mucosa presented foci of a light or moderate inflammatory process and small ulceration foci. Despite some of the complications, the bladder mucosa and the area of anastomosis of the gastrocystoplasty presented no histological alteration. Furthermore, we verified that the physiological bladder function was preserved. This means that our results indicate that this type of procedure might be a viable alternative, as the benefits might outweigh the problems.

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