Endoluminal pharmacologic stimulation of the upper urinary tract

Methodologic aspects, pharmacokinetics, effects and side effects of Isoproterenol in a pig model

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1) **Local and systemic effects of endoluminal pelvic perfusion of Isoproterenol. A dose response investigation in pigs.** Jakobsen JS, Holst U, Jakobsen P, Walter S, Mortensen J Urol. 2007;177(5):1934-8.

2) **Isoproterenol reduces renal pelvic pressure during semi rigid ureterorenoscopy: A porcine model:** Jakobsen JS, Jung HU, Gramsbergen JB, Osther PJ, Walter S. BJU Int. 2010 Jan;105(1):121-4.

3) **Irrigation with isoproterenol diminishes increases in pelvic pressure without side-effects during ureterorenoscopy: A randomized controlled study in a porcine model.** Jung HU, Jakobsen JS, Mortensen J, Osther PJ, Djurhuus JC. Scand J Urol Nephrol. 2008; vol.42 (1) 7-11

1. ENDOSCOPY IN UROLOGY

Brief historical overview

Within the last 4-5 decades, there has been an enormous development in the urologist armamentarium of instrument. The development took its beginning from the early 1800 ranging over centuries and is ongoing. The German general practitioner Philipp Bozzini published his ideas as a monograph in 1807. He had constructed a primitive instrument "lichtleiter"(figure 1.), by means of which rays of light directed from the outside into the body cavity returned back to the eye of the viewer. He thought, it was possible to examine body cavities of living animals, if it did not result in immediate death of the animal.

Endoscopy is derived from the Greek endon: within, and skopein: to view. It was introduced by the French Surgeon Antonin Jean Desormeaux (1815-94)(1). Maximillian Carl Friedrich Nitze (1846-1906) and Joseph Leiter (1830-1892) developed the cystoscope(2). In 1874, Dermatologist Joseph Grünfeld (1840-1912) was the first to successfully and under visual guidance, insert a bougie into the female ureter(3). Joaquín Albarrán (1860-1912) invented in 1897 a small tilting mechanism, which made it much easier to control ureteric catheterization and it became shortly thereafter an everyday procedure in all hospitals(4). He modified a device (by Armand Imbert) for refining the movements of the cystoscope during catheterization of the ureteric orifice, which is still in use today.

Central to the revolution was the development of the rod-lens optical system by Professor Harold H. Hopkins in 1959(5). Almost simultaneously, Karl Storz developed the fiberoptic cold-light system(6). It was a quantum leap in improving image resolution, brightness and width of field. Berci was instrumental in introducing the Hopkins rod-lens system for image transmission into the clinical practise of endoscopy(7). Unfortunately and very little appreciated is the pioneer work of the Danish engineer Holger Møller Hansen. In 1951, he gave an interview to a Danish newspaper that was reported to foreign newspapers such as Los Angeles Times, on a new invention: "an artificial eye which can see round corners" (3). Møller Hansen described the importance of coating the threads as the light can disappear if the threads come in contact with each other. It turned out later that this coating process was the key to success of the fibre optic lighting system.

The introduction of Hopkins optical system led to the construction of a rigid, right angled 15 fr. renoscope(7;8) with a wide visual field. It was initially evaluated in biliary surgery, but its usefulness in visualizing the renal pelvis and calices system was shortly thereafter investigated in cadaver kidneys(8). In the early 1960´s, the Japanese company Olympus entered the scene and dominated the marked. In the late 1960´s, the first nephroscope was constructed. A 15 Fr. right-angle endoscope was used for nephroscopy in one patient undergoing pyeloplasty for ureteropelvic junction stricture and one for solitary renal calculi. The endoscopies were performed through a retroperitoneally approach.

In 1976, Berci introduced a miniature, high intensity, explosion proof xenon arc globe, a light source originally developed by the military(9). This further enhanced the endoscopic image and it is still in use by every manufacture of endoscopic instruments. Berci´s early work brought endoscopic endoluminal surgery from the lower urinary tract to the upper urinary tract.

The fiber optic cable is probably one of the most important optical advances in endoscopy. Development continued and soon a small television camera could be connected to the eye piece allowing the image to be transferred to a monitor, on which everybody was able to see. In the 1980´s, a microchip of few mm.

was placed at the end of the scope and transferred images of significant quality. The development has since focused on improved and digitalized images and smaller diameter and more flexible endoscopes. Even flexible robotic retrograde renoscopy has been described in an animal model(10;11) and may play a significant part in future endourological procedures(12)

Past – present

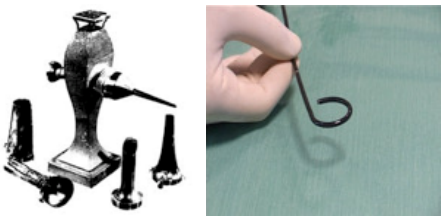

Figure 1
Philipp Bozzini´s "Lichtleiter" (left), Storz Flex-X ureteroscope (right).

Use of endoscopy today

Treatment and diagnosis of neoplasm and of upper urinary tract stone diseases are probably the most frequent causes of performing ureterorenoscopic procedures. Approximately 10% of men and 5% of women will suffer from urinary tract stones disease through a lifetime. 10 % thereof need surgical treatment – retrograde endoscopic/percutaneous. Apart from this some 2000 not-stone related ureteroscopic procedures are performed each year in Denmark.
Before access to instruments and throughout many decades, the clinical courses of lower urinary tract diseases were regarded as very dramatic. Urinary retention was considered a painful, but relatively quick death.

Complications to endoscopic procedures

The urologist's armamentarium of endoscopes has undergone a historic development in shapes and sizes as described previously and ureterorenoscopy today is considered a minimal invasive procedure, although it may be challenging. Extravasation of large amounts of hypotonic irrigant during transurethral resection of the prostate (TURP), leads to a spectrum of cardiovascular and neurologic derangements collectively known as the (TUR-syndrome). Although the morbidity and mortality were significantly reduced after the introduction of nonhemolytic irrigant solutions, it is believed extravasation is more common during endoscopic stone surgery than during TURP(13). This brings into focus, the significance of irrigant solution and irrigant pressure as discussed later. Severe complications are few. The most feared are ureteric rupture and septic complications. Ureteric rupture and mucosa lesions occurs in 3.3 to 5.7 % of upper urinary tract diagnostic and therapeutic procedure(14-17), but the general complication rates, including - among others - postoperative infections and septicaemias sums up to 6.7 – 26.1 % (table 1.). However, complication rates have decreased in the past 2 decades(18;19), attributed to decreased diameter of the endoscopes and increased surgeon experience.

Table 1

Complication rates (%)

	Geavlete et al. *Jour. Of Endourol. 2006 (20) 3 p.179-85* (2735 procedures)	Hollenbeck et al. *Urology 2001, 58 p.351-56* (195 procedures)	Schuster et al. *Jour. Of Urol. 2001 vol.166 p. 538-40* (322 procedures)	El-Nahas et al. *Jour. Of Urol. 2009 vol 181, p. 1158-62* (908 procedures)
Reference nb:	*(14)*	*(15)*	*(16)*	*(17)*
Intraoperataive rate	**15.5**	**5.6**	**8.1**	**5.7**
Perforation/ mucosa laesion	3.3	4.6	4.7	5.7
Instr. faillure/access dificulty	5.9	1	3.4	Not registered
Other	6.3	x	x	Not registered
Post operative rate	**10.6**	**8.7**	**13.3**	
Pain	2.2	3.6	10.3	Not registered
Infection/septicaemia	1.1	4.6	1.6	Not registered
Other	7.3	0.5	1.4	1
Overall	**26.1**	**14.3**	**22.4**	**6.7**

Considerations concerning risk factors for complications

Although complication rates have decreased they still occur. Several studies have focused on the elevated pelvic pressure during upper urinary tract endoscopy as a causative explanation(20-26). The normal baseline renal pelvic pressure in pigs is in the range 5 – 15 mm Hg, which is comparable to pressures observed in man(27).

During upper urinary tract endoscopy, it is essential for complete visualization to use irrigation of fluid through the endoscope. However, this flow significantly increases the renal pelvic pressure. In a pig model, renal pelvic pressures during ureterorenoscopy ranged from 28 – 128 mmHg depending on the height of irrigation bag (25 - 90cm) and bladder filling, reaching 439 mm Hg using uromat (mechanical) infusion(28). Using manual irrigation, renal pelvic pressures up to 410 mm Hg have been recorded during flexible ureteroscopy in man(24). Using a roller pump device (continuous flow), pressures were kept below 110 mmHg due to a pop-off mechanism preventing pelvic pressure to exceed 110mm Hg.

During increased pelvic pressure, the fornix is susceptible to rupture leading to extravasation into the sinus, the cavity between the renal parenchyma and the calyx wall (pyelosinous backflow (PSB). Further intrusion can lead to intrarenal backflow (IRB), pyelovenous backflow (PVB), pyelolymphatic backflow (PLB) or even subcapsular extravasation (SCE) (29). Animal investigations have revealed that IRB may occur, when renal pelvic pressure rises above 30 – 40 mmHg in multipapillary kidneys(29-32).

Whenever urine escapes its normal pathway, it may cause fibrosis(33;34). In the case of ascending infection, microorganisms may reach the systemic circulation and give rise to bacteraemia and endotoxic shock. Even migration of transitional carcinomas cells to the peirenal lymphatic tissue have been reported, suggesting that high intrarenal pressure during ureterorenoscopy can course spread of cancer cells by a backflow mechanism(35).

Schwalb et al(28) showed that a acutely high renal pelvic pressure caused diffuse denudation and flattening of the caliceal urothelium, oedema and congestion not seen at low renal pelvic pressures, when minipigs were subjected to hydraulic dilation and ureterorenoscopy at 90 mm Hg and 150mmHg respectively. In the later case causing a high incidence of focal scarring in the group subjected to high pressures 4-6 weeks after surgery.

The normal filtration pressure is about 30 – 35 mm Hg, thus raised pelvic and tubular pressure may compromise glomerular filtration. However during acute obstruction or acute elevated pelvic pressure glomerular filtration may be uncompromised due to PSB, allowing urine to escape into the sinus thereby reducing pelvic pressure.

Presumably IRB increases the interstitial pressure causing a decrease in total renal perfusion(30) leading to focal ischemia probably causing parenchymal damage. Studies have indicated there may be an important relation between IRB and ischemia in producing a "viscous circle of deleterious effect"(29) Figure 2.

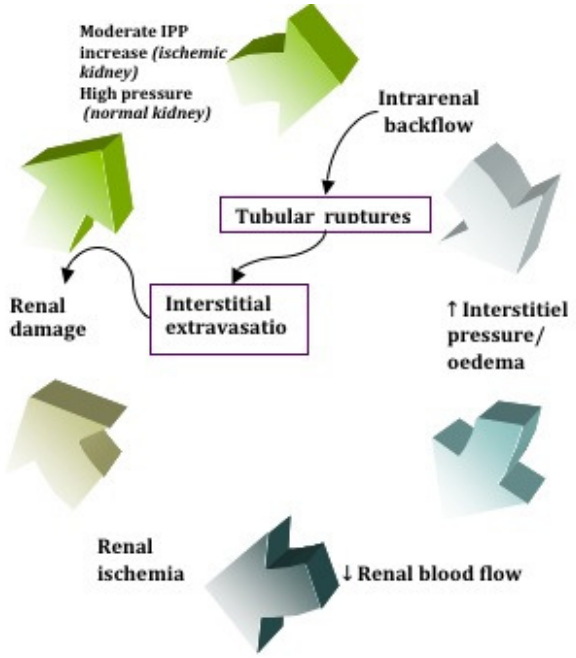

Figure 2

Modified; Thomsen HS. (1984): Danish medical Bulletin; Pyelorenal Backflow. Thesis.(29). (Increased pelvic pressure resulting in intrarenal reflux may cause an increase in interstitial pressure and subsequently a transient reduction in blood flow and renal ischemia, which in turn promotes intrarenal backflow to produce further renal damage etc….)

Viscous circle of deleterious effect:

This model suggests a plausible explanation for the development of renal scars - in areas with intrarenal backflow (reflux) – with the subsequent development of reflux nephropathy. However, exposure time and threshold values in humans have not yet been determined. During vesicoureteric reflux (VUR), Ransley et al.(36) indicated in a pig model, that urinary tract infection (UTI) was of primary importance in the development of renal scars and never occurred in its absence. However, Hodson et al.(32) produced renal scars in a pig model without UTI, when a ring was placed around the urethra to produce high pressure VUR. That lead Ransley et al to a similar study(37) in piglets aged between 2 and 5 weeks. Sterile VUR with raised peak voiding pressures in the range of 30 – 131 mmHg (41 – 177 cm h2o) caused parenchymal damage. In comparison, the lesions tended to be less extensive than in the infected kidneys and distributed more uniformly (polar and midzone). Thomsen et al.(38) demonstrated that the level of intrapelvic pressure at which IRB took place was different for the two types of papillae. IRB was rarely seen in simple papillae at moderate intrapelvic pressure, but frequently at high intrapelvic pressure, suggesting higher pressures were reached in Hodson et al's later study since compound and simple papillae had been affected. Identification of Tamm Horsfellt Protein (THP) in the interstitial space suggests caliceal ruptures had occurred predisposing PSB and IRB probably inducing parenchymal damage.

The fact, that a single hydraulic dilation and ureterorenoscopy in minipigs(28) during high pelvic pressure caused a significant renal scarring suggest that this model also might explain the pathogenesis of renal damage associated with upper urinary tract endoscopy. Furthermore, it seems likely that surgical endoscopic procedures may promote the development of renal damage when the kidney, during these procedures is, subjected to high renal pelvic pressure.

This emphasizes the importance of developing a method to reduce pelvic pressure during endoscopic procedures. The addition of a smooth muscle relaxant drug to the irrigation fluid as in our study (se method) may prove to favour a safer endoscopic procedure.

Purposes of the thesis:

The purposes of this thesis were to investigate:

The effect of endoluminal perfusion of Isoproterenol on the normal pressure-flow relation on the upper urinary tract in a porcine model

The relation between effect and side effect on the cardiovascular system.

The effect of endoluminal perfusion of Isoproterenol on increases in pelvic pressure and cardiovascular function during flexible ureterorenoscopy in pigs.

The effects on the pressure-flow relation of renal pelvic pressure during semirigid ureterorenoscopy and endoluminal perfusion of Isoproterenol 0.1 µg/mL, with emphasis on local effects and cardiovascular side effects.

2. PHYSIOLOGY OF THE UPPER URINARY TRACT

The ureter is located retroperitoneally transporting the urine from the kidney to the bladder. Historically, it was regarded a simple structure leading urine from the kidney to the bladder solely by means of gravity. However, decades of investigation have revealed this indeed is not true. The ureteric wall consists of 2 muscle layers fused by connective tissue - an inner longitudinal and an outer circular layer. Near the bladder, there is a third layer, which emerge from the bladder wall into the ureter – the muscular layer of Waldeyer(39). The lumen is coated with urothelium (figure 3). Reflux of urine during micturation is prevented because the ureter passes diagonally through the bladder creating an "antireflux mechanism". The majority of cell types in the ureter are smooth muscle cells. However, proximal ureter and renal pelvis reveal two other cell types in the muscle layer; Atypical and ICC-like cell. Atypical cells forms a structural network and was proposed by Gosling and Dixon(40) to have pacemaker function.

The basic process regulating ureteric peristalsis in humans and animal is partly myogenic, initiated by spontaneously active pacemaker cells in the renal pelvis located in the proximal portion of the collecting system. Anatomic evidence for a pacemaker system in the multicalyceal system has been provided by Gosling

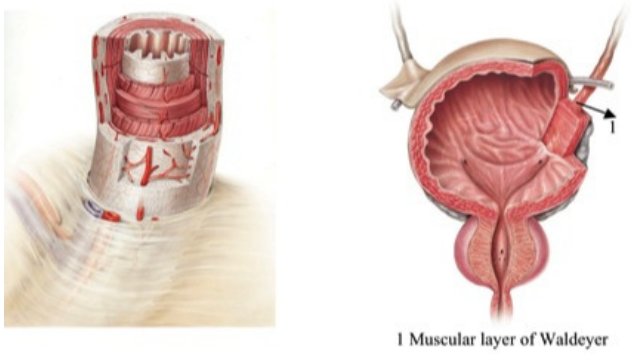

Figure 3
Fröber, BJU int. 2007; 110:949-65, modified(39)

and Dixon(40) and the renal pelvis shows spontaneous contractility(41). Transportation of urine from the kidney to the bladder is a function of the interplay between pelvic and ureteric peristalsis. (27;42-47). However, the process is partly regulated by the autonomic nervous system as adrenergic pathways involving α- and β-adrenoceptors can influence ureteric function(48).

Ureteric peristalsis is under control by the renal pelvis that initiates the contractions propagating down through the ureter at 3cm min-1. During normal urine output every pacemaker contraction of the renal pelvis does not always propagate to the ureter, suggesting some stretching forces are necessary for coupling of incoming pacemaker signal to pass to the ureter. At low urine flow, renal pelvic pressure remains low and pelvis accumulates urine until a triggering mechanism releases a bolus. Constantinou and Djurhuus(49) supports this theory since they observed the frequency of pelvic peristaltic activity became constant during increased urine flow and reaching that of the "pacemaker site". They suggested a hierarchically organized system of multiple coupled pacemakers with the highest peristaltic frequency of 6 min-1(49;50) located at the most proximally region of the renal pelvis. Any further increase in urine output is believed to be due to increasing sizes of boli – accommodation – with a maximum capacity of 1200% in the healthy kidney(51). Once initiated, ureteric peristalsis propellers the urine bolus to the bladder.

Adrenergic receptors

Ahlquist(52) showed in 1948 in different musle types the existence of two types of adrenergic receptors. The receptors primarily affected by norepinephrine and phenylephrine were defined as alpha-adrenergic receptors and the ones primarily affected by Isoproterenol were defined as beta-adrenergic receptors.

In 1966, Boyarsky et al(53) provided evidence of sympathetic modulation/control of ureteric peristalsis. Intra aortic infusion of noradrenaline significantly stimulated ureteric activity. Adrenalin and noradrenalin was detected in the ureter of dogs by flourometric assays and uptake of noradrenaline-H3 after depletion of amines by reserpine supported the evidence for the presence of sympathetic nervous system in the tissue and in turn the neurogenic theory of ureteric peristalsis. In another study, Boyarsky et al(54) showed that adrenal unilateral compression in dogs increased ureteric peristalsis bilaterally. This phenomenon could be reverted by α–adrenergic blockage adding to the perception that α – adrenoceptors existed and to some extent modulated peristalsis in the urinary tract. Furthermore, a β-adrenergic blocker dichloroisoproterenol (DCI) given intravenous reduced ureteric peristalsis and urine output in dogs illustrating that α – and β – adrenoceptors to some extent can modulate ureteric peristalsis(55). Neville Kaplan et al(56) showed that activation of α – adrenoceptors stimulated ureteric peristalsis, whereas β – adrenoceptors only caused minor depressions in the resting pressure of the ureter in dogs, indicating α- and β-adrenoceptors existed in the upper urinary tract.

Using histochemistry techniques, Duarte-Escalante et al(57) provided evidence of adrenergic and cholinergic components innervating the ureter. Both component were found in the nerve plexus in the laminae adventitia and muscularis(58). Malin et al(59) demonstrated in 25 human ureter strips the presence of α – adrenoceptors which seemed to predominate as only β-adrenoceptors were present in the lower third of these specimens. However, it is debatable whether the effect of catecholamines are brought about by direct drug effect on the smooth muscles in the ureter or by reflex mechanisms, since normal ureteric activity has been demonstrated after transplantation(60) and persistence of normal antegrad propagation of the ureteric impulse were seen after reversal of a segment of the ureter in situ(61).

Sigala et al(62) presented evidence for the presence of 3 α-adrenergic subtypes; α1a , α1b and α1d in the human ureter. By semi quantitative reverse transcriptase PCR technique, gene expression of the 3 α-adrenoceptor subtypes was shown in each ureteric region (distal, medial and proximal), although with differences in the amount expressed at the different levels. The density was found to be significantly higher in the distal ureter than in the middle and proximal ureter. Receptor binding experiments showed that the expressed mRNA of each subtype was translated into proteins with the highest density in the distal ureter. By immunohistochemistry using subtype specific antibodies, Park et al(63) confirmed the results of Sigala et al.(62). The highest density of α-adrenoceptors was found in the distal ureter without statistical significant differences in the subtypes expressed.

In the year 2000 β-adrenoceptor subtypes mediating smooth muscle relaxation in human ureter was determined by Park et al(64). They studied the effects of β-adrenoceptor agonist on spontaneous or KCl induced contractions of the human ureter in vitro and the antagonism by β-adrenoceptor antagonist on ISOproterenol induced effects. Selective β2 and β3 agonists suppressed ureteric activity whereas the β1 stimulator dobutamine had little relaxing effect on the ureter. β1-, β2- and β3- adrenoceptors mRNA´s were expressed in the human ureter as determined by RT-PCR assay.

The β2- and β3-adrenoceptor seemed to mediate the relaxing effect of β-adrenergic stimulation in the human ureter since 1) Metoprolol (selective β1 antagonist) was less effective in antagonizing the Isoprenalin induced relaxation. 2) The selective β2-adrenoceptor antagonist ICI-118,551 partially antagonized Isoproterenol induced relaxation. 3) Propanolol (non selective β antagonist) and ICI-118,551 concentration dependently displaced [3H]-dihydroalprenolol binding to the membrane.

A number of investigations have revealed the effect of adrenergic drugs on the ureteric peristalsis and motility. However, comparISOn of these studies regarding drug efficacy, is extremely difficult. Results are obtained over decades with very different and not comparable experimental setup and different sites of drug administration (intravenous, distal-, middle part of- ureter, renal pelvis). The use of different animal species does not make the comparison any easier, as it is known that there are signifi-

cant variations in β-adrenoceptors between species as mainly β-1 predominates in rats, whereas in dogs mainly β-3 and in rabbits β-2-adrenoceptors are present (65). It is known that especially adrenoceptors of subtypes β-2 and β-3 are found in the human ureter (64) and in the porcine ureter(66). However, there seems to be agreement on, that activation of α – adrenoceptors mediates increased ureteric peristalsis and tonus (56;59;67-71), whereas stimulation of β-adrenergic receptors mediates relaxation(59;64-66;68;71-75).

Other receptor types

There are a great number of different receptor types localized in the pelvis/ureter and activation of these modulates ureteric peristalsis/tonus. A thorough review of these receptors would be too comprehensive for this thesis and is not the scope of the present paper. A few reviews have summarized the effect of various pharmacologic agents on pyeloureteric dynamics(42;48). Table 2, summarizes possible drug groups that may cause relaxation of the ureter.

Table 2

Canda et al(48) Urol int 2007; 78: 289-98 (modified)

1.	Selective α-receptor blockers
2.	Selective β-receptor agonist
3.	Purinergic antagonist
4.	Drugs targeting nitric oxide pathway (NO donors)
5.	Serotonin antagonist
6.	Non-steroidal anti-inflammatory drugs
7.	Selective COX-2 inhibitors
8.	Phosphodiesterases (PDE IV inhibitors)
9.	K+ channel openers
10.	Calcium channel blockers
11.	Antimuscarinic agents
12.	Rho-kinase inhibitors

The highlighted drugs seem most promising. However, investigations regarding to what extent they contribute to ureteric activity is still too preliminary. Further studies using human or pig ureteric tissue is mandatory to understand the mechanism of action and possible use of drugs in the clinical settings.

Although a meta analysis by Hollingsworths et al(76) suggests that patients given calcium-channel blockers or α-blockers had a greater likelihood of stone passage than those not given such treatment, knowledge is sparse regarding drug efficacy in the proximal ureter or pelvis.

The joint knowledge of distal and proximal ureteric relaxation may contribute to the discovery of new drugs that might relief ureteric colics and facilitate stone passage and ease endourological procedures.

The pressure - flow relation

Transport of urine in the upper urinary tract depends on peristalsis which is regulated through a complex pacemaker activity in the pelvicalyceal region(47;49;50). Both mechanical and biochemical stimulation regulates this pacemaker activity. Filling of the renal pelvis initiates a co-ordinated pelvic contraction to form a bolus in the ureteropelvic junction. The peristaltic contraction wave continues down through the ureter pushing the bolus to the bladder. This mechanism works especially at a urinary flow rate of 0-2 ml/min. Maximal peristaltic activity in a multicalyceal system is 6/min(50), and beyond this rate increased urine flow is managed by increasing size of boli, until flow ultimately becomes continuous. Peristaltic activity in the upper urinary tract is important for the pressure flow relationship which exhibits four phases (77-80). In the range 0-2 ml/min, pelvic pressure is low because peristalsis manages transport. In the range 2-4 ml/min, pelvic pressure increases significantly because the preceding contraction impedes transport. Between 4-6 ml/min, peristalsis becomes insufficient involving leakage between boli, which causes a decreasing pressure increase. In the fourth phase at higher flow rates the ureter functions as a tube with continuous flow and linearity between flow and pressure.

Methods to reduce pelvic pressure during endoscopy

The normal baseline renal pelvic pressure in pigs is in the range 5 – 15 mm Hg and comparable to pressures observed in man(27). The high intrarenal pressures generated during upper urinary tract endoscopy may potentially induce renal backflow - as described above - and thereby potentially lead to renal damage and septic complications(28-31;81). In order to reduce immediate and long term complications associated with ureteroscopic procedures, a method to reduce pelvic pressure is desirable.

Ureteric access sheaths has been shown to significantly reduce renal pelvic pressure during endoscopy(22;23;82;83), - even below the threshold value of pyelosinous backflow. However, some results were obtained using cadaveric kidneys(23;82;83). In a small series of five patients who underwent stone fragmentation during ureteroscopy due to obstructing calculi, renal pelvic pressure were significantly reduced at all locations (distal, mid, proximal ureter and pelvis) when using ureteric access sheaths. Max pressure using sheath was 40,6 mmHg compared to 94,4 mmHg without a sheath(22). However, in this study renal pelvic pressures were measured after the surgical procedure and pressures obtained using access sheath were recorded after dilation during stone fragmentation and a subsequent ureterorenoscopy for pressure measurement, thus these measurements were performed in "a pre-dilated system" using manual irrigation. This potentially may be in favour of the sheath.

It is well documented that a ureteric access sheath provides protection against elevated pelvic pressure during upper urinary tract endoscopy. Sheaths potentially avoids ureteric trauma associated with repeated reinsertion of the ureteroscope during stone fragmentation procedures. However, the extent of pressure decrease in relation to protection against pyelosinous backflow remains debateable, as the study series are small and uncontrolled. Furthermore, placements of an access sheath require additional instrumentation with potential risk of further surgical trauma.

Sakhadeo et. al.(84) published "A new system of irrigation for ureteroscopy" in BJU 1996. The so-called "Pneupac system" – a constant pressure infusion system - which could ensure a pre-set constant pressure applied during ureterorenoscopy.

3. Material and methods: Considerations .

Animal experimental model

The present thesis is based on experimental studies in young female Yorkshire pigs – Danish land race breed. The included

experiments are not possible to perform in humans due to ethical arguments. When evaluated as or considered a possible human treatment, there is a giant leap from in vitro experiments in a Petri dish to human treatment in the operation theatre. Thus, in vivo animal experiments are necessary to address the questions asked.

There are significant species variations in the functional β-receptor subtypes mediating smooth muscle relaxation. Mainly β-1 in rats, β-2 in rabbits, mainly β-3 in dogs and β-1, β-2 and β-3 in porcine and humans(64;65). Bearing this in mind, the pig act as, a good model for human evaluation of Isoproterenols effect on the upper urinary tract. The upper urinary tract in pigs is multicalyceal and very similar to humans and the pig has been established as a good endourological model for research and surgical training(85).

In order to evaluate a new drug and its possible use in clinical settings several factors need to be considered regarding the experimental setup. The considerations regarding the animals used have been described earlier. Furthermore a well-established and proven method is desirable in order to reduce variations, ensure optimal and valid collection of data. Comparability of data is essential and, hence, a reproducible model is preferred. A chart of the model we have used for the primary evaluation of ISO´s potential use is shown in figure 4.

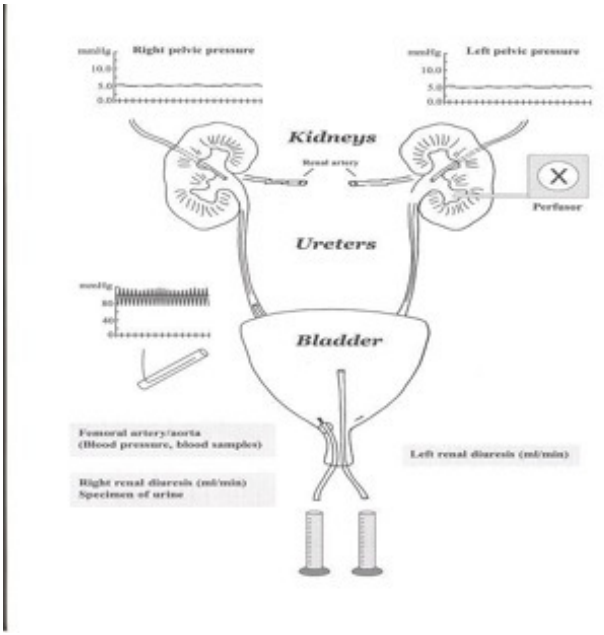

Figure 4
Animal experimental model.

Pressure catheters are placed in the renal pelvis as demonstrated. Via a Cystotomy, the pressure and perfusion catheters are placed retrograde to the renal pelvis and through the parenchyma. The distal tip lies in the renal pelvis for perfusion and pressure measurement. Due to instrumentation of the ureter, oedema could develop and theoretically this could compromise urine output due to increased ureteric resistance, thus leading to false increased pelvic pressure. However, urine output is among other things controlled by propulsive peristalsis. This active mechanism would exceed possible development of oedema, since the normal pelvic pressure obtained using this model is comparable to pressure obtained in humans using antegrad placed pressure catheters(27).

The normal pelvic pressure is influenced by several factors: the intra abdominal pressure, pressure from the surrounding tissue, pelvic wall tension, urine production, the resistance at the ureteropelvic junction and the urine flow down the ureter. In our setup, we identified the kidney via a subcostal incision in order to place the catheters. In order to maintain the pelvic pressure to be affected by surrounding tissue, all incisions were hereafter approximated. IV administration of saline (Sodium chloride, 9 g/l, 37 0C) 10 ml/kg/h through an ear vein maintained the animal well hydrated to ensure renal perfusion for urine output to have its influence on the pelvic pressure. In our first studies, we macroscopically identified the kidney and renal pelvis to identify abnormalities i.e. ureteropelvic stenosis causing increased resistance, thus increased pelvic pressure.

The normal pressure flow relation in pigs is well documented and has been shown to be a reproducible(25) model to examine pharmacologic impact on this relation during endoluminal perfusion of pharmacologic agents(25;77-80;86-88). Thus in this model it has previously been shown that, the pressures obtained are directly related to the drug effect and not caused by continuous dilation or exhausting of the system. Another advantage of this model is that the application of drugs is endoluminal. This simulates the clinical situation during endoscopy, where irrigation is necessary to maintain sufficient view. Thus, this experimental model in a multicalyceal system using endoluminal perfusion is in many ways quite similar to the surgical procedure in humans, ensuring optimal and reproducible conditions in resemblance to the actual settings in the operating theatre.

This is of special interest, when examining drugs of potential clinical usage. The anatomically considerations is self explanatory considering the surgical procedure itself. The pig kidney has been established as “a good endourological model”, due to very similar anatomy(85).

In our studies performing ureterorenoscopy, we used flexible and semirigid endoscopes (Storz 7.8 Fr). One would expect higher renal pelvic pressure obtained during semirigid ureterorenoscopy solely by inducing increased tension to the upper urinary tract. We chose to use flexible and semirigid scopes in order to reach as high pressures as possible to evaluate whether ISO could reach systemic circulation under these conditions. The endoscopes are identical to the scopes used in humans in order to simulate the clinical situation.

The methods of pressure measurements in the upper urinary tract in the present studies need some comment. In our study using flexible ureterorenoscope, perfusion was done through the scope and pressure was measured via a 5Fr Selectip catheter in the renal pelvis, placed retrogradly. The advantage of this method is no leakage occurs through the renal pelvis, unless by mechanism of rupture, pyelovenous or intrarenal reflux. Furthermore, this method ensures that the renal pelvic pressures obtained are, a function of perfusion rate, urine production, pressure from surrounding tissue, abdominal pressure and resistance in the ureter. The resistance in the ureter is increased due to the ureterorenoscope leading to higher renal pelvic pressure. This is to be expected and desired, as we aim to simulate a clinical situation. However, a 5 Fr Selectip catheter is not placed in the ureter during ureterorenoscopy in humans, but a guide wire (safety wire). The increased calibre of Selectip catheter compared to a safety wire leads to increased resistance in the ureter, thus, higher renal pelvic pressure could be expected. Whether this is clinically important in relation to the level of pressures obtained,

we do not know, since such studies have not yet have been carried out. On the contrary, if higher pressures would by reached, this in turn could facilitate pyelosinous backflow and cause ISO to reach systemic circulation resulting in cardiovascular side effects. Thus, it could be argued that this is in favour of the safety profile of ISO prior to clinical trials. As described earlier, only few studies concerning renal pelvic pressure during endoscopic procedures have been performed. However, in these studies pressure was measured via a percutaneous nephrostomy(24;28).

During semi rigid ureterorenoscopy pressure measurement was performed through a nefrostomy tube. Via a sub costal incision, the left kidney was identified retroperitoneally. Through a needle puncture in the renal pelvis, a guide wire was pushed through the renal parenchyma, serving as a guide for a 6 fr. nefrostomy catheter used for renal pelvic pressure measurements. A small purse string suture closed the site of the needle puncture. Hereafter the incision was approximated. Perfusion was not initiated until a pyelography confirmed correct position of the ureteroscope and pressure catheter. This setup ensured that resistance in the ureter was very similar to a clinical setting since only a safety wire along with the ureterorenoscope was placed in the ureter. However it would not be possible to localize the origin of any leakage through the subcostal incision. Pyelography performed at the end of the experiment would detect leakage, but if this were due to pelvic rupture, or leakage along the nephrostomy tube would not be possible to determine.

The three R´s

One of the principles that always need to be considered during Laboratory Animal Science is "the 3 R's" proposed by Russell and Burch, Reduction, Refinement and Replacement. Replacement of the pig (animal-free alternative) or animal of a lower species was not possible, as described earlier. Reduction has been seriously considered during the entire study. We sought statistical knowledge to ensure the number of animals used, was kept at a necessary minimum. Refinement (refined methods that cause animal less suffering). The experiments performed were acute experiments, meaning the animals were euthanized at the end of the experiments. One could argue, this in fact causes suffering. However, the extent of surgical procedures fare exceeds this consideration and euthanasia would be the ethical correct outcome. All experiments were performed on anaesthetized pigs and at the end of the study the pigs were sacrificed during anaesthesia with an overdose of pentobarbital.

Considerations regarding drug selection

It has been shown that endoluminal administrated norepinephrine (NE) significantly and dose dependently diminishes the pressure increase during perfusion of the upper urinary tract in the pig without concomitant systemic side effects(25;88), suggesting that NE has clinical potentials. This is in contrast to the general perception of the effects on activation of adrenoceptors as discussed previously. We have proposed that the mechanism of action during endoluminal perfusion is mediated by stimulation of β – adrenoceptors as Sotalol inhibited the effect of norepinephrine increasing EC50 by about a factor 10(87). However, to obtain ureteric relaxation it seems appropriate to use a pure β-adrenoceptor agonist to avoid possible stimulation of α-adrenoceptors, which might mediate a more unpredictable response for reasons discussed previously (Adrenergic receptors).

Furthermore, ureteric resistance to flow is composed by two elements, ureteric peristalsis and ureteric wall tonus(71). Ureteric peristalsis is important for urine bolus transport. Ureteric wall tonus is an important part in the linear flow range observed at high flow rates, where the ureter does not coapt its wall. Morita et al(71) showed in mongrel dogs, that the resistance to flow was significantly increased at low- (≤2.16ml/min) and high- (≥5.40ml/min) flow rates after intravenous administration of Noradrenaline 25µ g./kg. Isoproterenol on the contrary decreased the resistance at all flow rates.

Stimulation of β-adrenoceptors in swine and human ureter mediates relaxation of the ureteric smooth muscle (64;66;73). Our animal experimental model involves endoluminal pelvic perfusion of the selected drug within the perfusion range of linear pressure flow relation. Taking into consideration that the density of α – adrenoceptors is lowest in the proximal ureter(62;63) and considering ISO´s effect on ureteric resistance to flow(71) – as mentioned - and the rank order of relaxing potency for catecholamine's has been established as Isoproterenol> Adrenaline> Noradrenaline (64), - convinced us, that Isoproterenol should be the drug for further investigation.

Pharmacology of Isoproterenol

Isoproterenol is a non-specific and potent β-adrenergic agonist, which acts directly on β-adrenergic receptors. In therapeutic doses, the drug has little or no effect on α-adrenergic receptors. It is believed that β-adrenergic effects result from stimulation of the production of cyclic adenosine-3',5'-monophosphate (AMP) by activation of the enzyme adenyl cyclase. The main effects of therapeutic doses of Isoproterenol are relaxation of smooth muscle of the bronchial tree, cardiac stimulation, and peripheral vasodilation

The pharmacologic actions of Isoproterenol appear to be terminated principally by tissue uptake. The drug is metabolized by conjugation in the gastrointestinal tract and by the enzyme catechol-O-methyltransferase in the liver, lungs, and other tissue. T½ in fase I is 2.5 – 5 min and in fase II is 3 – 7 hours. 50 – 75 % is eliminated non-metabolised through the kidneys. For a complete profile of the drug see ref. (89)

Approval of the animal experiments

The study was approved by the National Ethical Committee for Animal Experimentation, Copenhagen, Denmark and all participating researchers had the necessary legal license to perform animal experiments.

Specific experimental setup

For specific experimental setup in the enclosed studies 1 through 3 see article descriptions under material and method respectively.

4. RESULTS AND DISCUSSION.

Isoproterenol´s effect on the normal pressure flow relation (Article 1);

The courses of pelvic pressure increase during perfusion (varied rate) with saline and ISO at increasing doses are shown in figure 5. Perfusion with saline caused a characteristic increase in pelvic pressure to 10 mmHg. In group 1 with varied perfusion average left baseline pelvic pressure was 4.0 (±0.3) mm Hg. At a flow rate of 8 ml/min, all solutions of ISO but 0,001µg/ml, caused a significant decrease in the pelvic pressure increase due to perfusion ($p<0.03$).

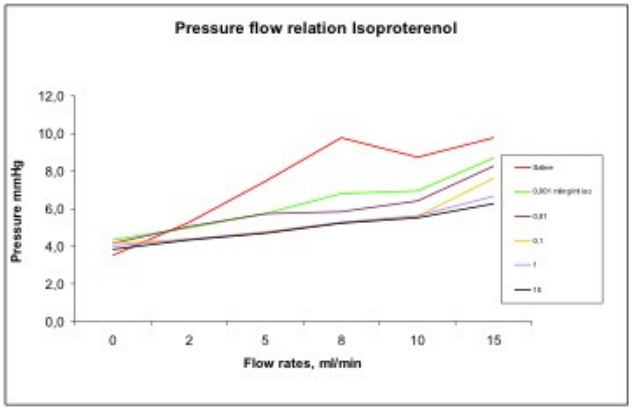

Figure 5

From article 1.): Pressure flow relation during endoluminal pelvic perfusion. Maximal relaxation was seen at a perfusion rate of 8ml/min at ISO 10-, 1- and 0,1 µg/ml.

Heart rate was stable during perfusion with all ISO solutions except 10µg/ml, for which a significant increase in heart rate of almost 90% was seen after 1 min of perfusion ($p<0.001$). Accordingly, a large increase in plasma levels of ISO were found, during the perfusion of 10 µg/ml ISO, and a smaller increase was seen at 1µg/ml ISO, that is 511.7 ρg/ml and 99.8ρg/ml, respectively. Plasma levels of ISO were not detected at perfusions of ISO below 1µg/ml (Figure 6).

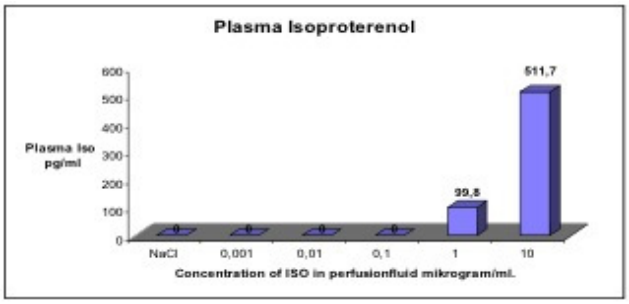

Figure 6

Article 1.): Levels of plasma Isoproterenol during endoluminal pelvic perfusion.

Dose response action of Isoproterenol

Figure 7 shows the pelvic pressure increase due to perfusion at increasing ISO solutions at a constant flow rate of 8ml/min. All ISO solutions significantly and dose dependently decreased the pelvic pressure increase due to perfusion compared with the saline perfusion (each $p<0.001$). ISO at 0.1 and 1µg/ml decreased the pressure increase to saline perfusion by 64% and 81%, respectively. Baseline left pelvic pressure was 4.1 (± 0.4) mm Hg and it was stable between perfusions.

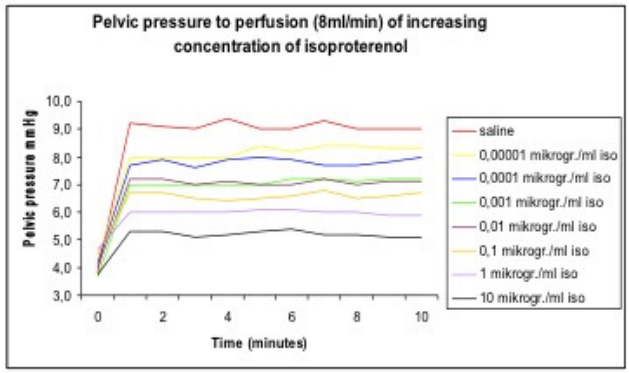

Figure 7

(article 1): Dose response action of Isoproterenol perfusion

We observed, that endoluminal perfusion of ISO effectively inhibits the pelvic pressure increase due to perfusion at increasing flow rates and it dose dependently inhibits the pelvic pressure increase due to perfusion at a single flow rate. The maximal relaxing effect of about 78 % was achieved at concentrations of ISO that did not cause cardiovascular side effects or detectable levels in plasma.

In contrast, in vivo studies by Danuser et al(73) showed that topical application of 0.1 to 200µg/ml ISO effectively caused relaxation of the mid portion of porcine ureter and, in addition relaxation of the contra lateral ureter. Due to severe cardiovascular side effects, the investigators excluded the drug from any potential clinical trials. Our data show that ISO 0.1µg/ml effectively and dose dependently causes relaxation of the renal pelvic smooth muscle without causing cardiovascular side effects or relaxation of the non perfused ureter. However, at 10µg/ml ISO our findings of severe cardiovascular side effects were in accordance with the findings by Danuser et al, indicating that the lack of side effects in our setting was due to lower ISO concentrations.

While Danuser et al. used a constant infusion rate of 2 ml/min, we used an increasing infusion rate of 2, 5, 8, 10 and 15 ml per minute in group 1, and a single flow rate of 8 ml per minute in group 2. A flow rate of 2 ml per minute is a physiological rate and results obtained during this rate are an excellent marker of baseline ureteric function. During ureterorenoscopy, the pyeloureter is put under stress and tension. Hence, we believe that a flow rate of 8ml per minute is more identical to the clinical situation and thus, our results suggest that ISO has the potential for clinical use in the proposed settings.

Furthermore, our findings suggest that this level of side effects during endoluminal infusion occurs at ISO concentrations of about 1 µg/ml, as we achieved measurable plasma levels of ISO during this perfusion. Pressures attained during perfusion were well out of the range of potential intrarenal reflux. Hence, plasma levels of ISO seen at the perfusion of 1 and 10µg/ml ISO were most likely caused by absorption of the drug.

In conclusion, ISO 0.1µg/ml added to the irrigation fluid in this porcine model caused a significant reduction of the pelvic pressure due to perfusion without concomitant cardiovascular side effects or measurable levels of plasma ISO. The pressure decrease was most pronounced at a flow rate of 8ml/min, thus ISO 0.1µg/ml at a flow rate of 8 ml/min seems to be a safe relaxant drug in the proposed setting. Hence, we decided to study ISOs effect on renal pelvic pressures during ureterorenoscopy.

ISO reduces renal pelvic pressure during flexible ureterorenoscopy; article 2.

We studied renal pelvic pressure during 60 min perfusion of 0.1µg/ml ISO compared to pure saline perfusion at a flow rate of 8ml/min during flexible ureterorenoscopy in a porcine model. According to randomization, ISO and saline perfusion was performed in 12 and 10 pelves, respectively. Pelvic and bladder pressures during the ureterorenoscopies are illustrated in figure 8. Mean baseline pressure in group 1 was 12 (± 2.3) mm Hg and 14 (± 3.6) mm Hg in group 2 with no significant difference (p= 0.26). Mean pelvic pressure increase due to perfusion in the ISO group was reduced by 42% compared to saline perfusion.

Mean pelvic pressure reached a maximum of 30 - and 46 mm Hg in the ISO and saline group, respectively. Bladder pressure was independent to renal pelvic pressure, being mean 11.3 (± 0.25) mm Hg during ISO perfusion and 11.9 (± 0.25) mm Hg during saline perfusion (p= 0.067).

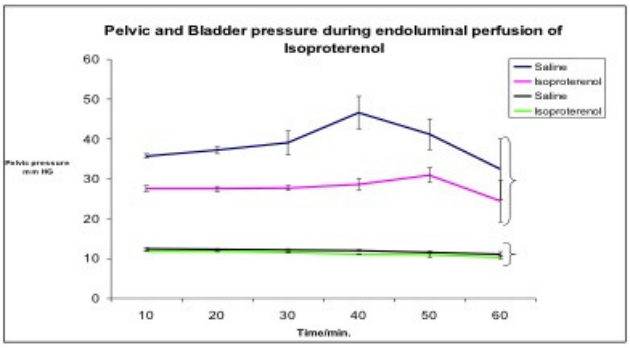

Figure 8

Renal pelvic and bladder pressure during endoluminal perfusion (8ml/min) of Isoproterenol or saline.

Mean blood pressure in group 1 was 83±1.5 mm Hg and 82±1.9 mm Hg in group 2 (p=0.425). Mean heart rate was 80±2.2 in group 1 and 78±1.6 in group 2 (p=0.166).

Thus, we have demonstrated ISO 0.1µg/ml added the irrigation fluid significantly reduces renal pelvic pressure due to perfusion during flexible ureterorenoscopy in this porcine model, without concomitant cardiovascular side effects.

Pelvic pressure increase was reduced 42% from 46 to 30mm Hg during ISO perfusion. Thus, pelvic pressure decreased below the critical pressure value for pyelosinous backflow (PSB) during ISO perfusion. As discussed previously, renal pelvic pressure can be reduced by other means. Ureteric access sheaths has been shown to significantly reduce renal pelvic pressure during endoscopy(22;23;82;83). Even below the threshold value of pyelosinous backflow. However, some results were obtained using cadaveric kidneys(23;82;83), hence pressure levels are not comparable as cadaveric kidneys are denervated without normal ureteric wall tonus or influenced by compression from the surrounding tissue and not subjected to physiological perfusion producing urine output.

Uses of access sheath require further instrumentation and thus beares the potential of surgical trauma as discussed in chapter 2 (Methods to reduce pelvic pressure during endoscopy. Hence endoluminal application of a relaxant drug may prove to be favourable. Although we were able to keep renal pelvic pressure below the critical pressure for intrarenal backflow during ISO perfusion, this critical pressure level is probably reached in the majority of ureteroscopic procedures, as a relatively low irrigation rate of 8 ml/ min caused pressure increases of about 40 mmHg in the control group in this study. It may be beneficial to use a combination of selected relaxant drugs to keep pelvic pressure as low as possible during upper urinary tract endoscopy as permanent renal damage may be the result of repeated increases in pelvic pressure (see: Considerations concerning risk factors for complications; chapter 1).

A-adrenergic antagonist and calcium antagonists are among others potent modulators of pyeloureteric dynamics and thus potential additives to irrigation fluid. In clinically randomized studies(90;91), a1-adrenoceptor blockers and nifidepine, administered orally, effectively reduced the expulsion time and the number of pain epISOdes in patients suffering from distal ureteric stones. Ames et al.(21) found, intraluminally administered verapamil significantly increased proximal ureteric diameter in pigs. The combination of a ß-adrenergic agonist with either a α1-adrenergic antagonist or a calcium-channel blocker may therefore elicit an even better pressure reducing effect. The side effect profile of such a regime has, however, not yet been explored.

Finally, endoluminal drug application versus use of access sheath during endoscopic procedures should not be regarded as opponents. On the contrary, the pressure reducing effect during endoscopic procedures might be synergistic and application of drugs endoluminal may facilitate the placement of access sheath. However, these possibilities have to our knowledge not yet been investigated in vivo.

Isoproterenol 0.1µg/ml added the irrigation fluid does not reach systemic circulation at pelvic pressure below the critical level of PSB. At higher concentration, ISO is suggested to be absorbed across the ureteric wall as described earlier. We have shown that renal pelvic pressure during flexible ureterorenoscopy using pure saline irrigation fluid practically always reaches this critical pressure level, bearing in mind that, we used a relatively low flow rate of 8ml/min. One might suspect higher renal pelvic pressures during semirigid ureterorenoscopy or when using higher flow rates. Thus, ISO 0.1µg/ml could reach systemic circulation by means of renal back-flow which consequently may lead to cardiovascular side effects. This hypothesis was evaluated when studying the pressure flow relation during semi rigid ureterorenoscopy.

The pressure flow relation during semirigid ureterorenoscopy: article 3.

The pressure flow relation during endoluminal pelvic perfusion of saline or saline added ISO 0.1µg/ml at flow rates of 0, 4, 8, 12, 16, 20 and 33ml/min were studied in 12 renal pelves. Figure 9.

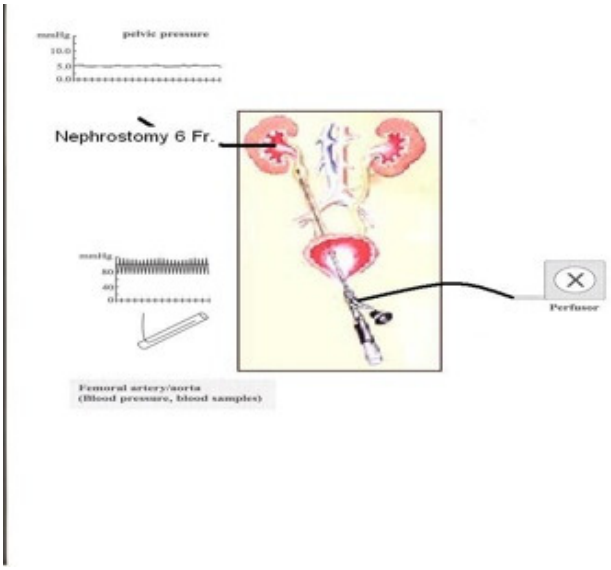

Figure 9
Animal experimental setup.

The mean baseline pelvic pressures in the saline and ISO group were 28 (7.1) and 25 (9.8) mmHg, respectively, with no significant difference (P = 0.079) (See figure 10). The course of renal pelvic pressure during saline and ISO perfusion are shown in Figure 11.

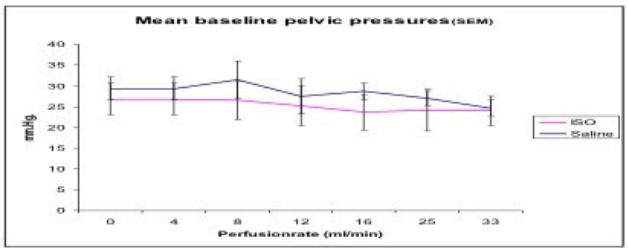

Figure 10
Mean baseline pelvic pressure.

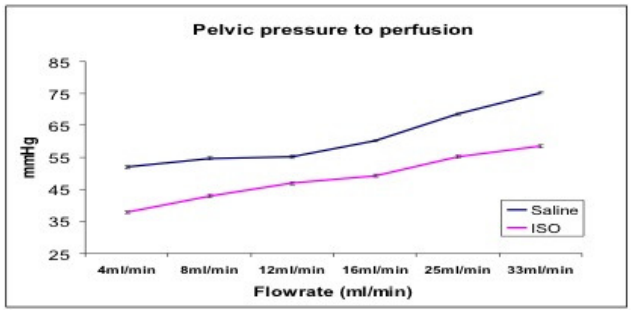

Figure 11
Pressure flow relation during semirigid renoscopy.

At all perfusion rates, ISO caused a significant reduction in the corresponding renal pelvic pressure compared to saline perfusion. Perfusion with saline showed an almost linear relationship between pressure and flow. Renal pelvic pressure increased to a maximum of 75 mmHg during saline perfusion and 58 mmHg during ISO perfusion at a perfusion rate of 33 mL/min., but showed a similar linear relation between pressure and flow. The maximum percentage relaxation (27%, P < 0.001) was obtained at 4 ml/min, from 52 to 38 mmHg, during saline and ISO perfusion, respectively.

This linear relationship is in accordance with previous studies concerning the normal pressure flow relation by Mortensen et al(77;78) who showed a linear pressure-flow relation at flow rates above 6-8 ml/min. However, higher pressures were reached in our study as we performed ureterorenoscopy. During ISO perfusion at 25 and 33 ml/min, the heart rate increased dramatically in one pig; extravasation through the subcostal incision was detected, with correspondingly increased levels of plasma ISO. At a perfusion rate of 12 ml/min, the level of plasma ISO was 341pg/mL, gradually increasing to 881pg/mL at a perfusion rate of 33 ml/min. Apart from this pig, plasma ISO was not detected in any other perfusions during the study, except on three occasions. There were no concomitant systemic side effects in these three cases.

We have shown that renal pelvic pressure during semirigid ureterorenoscopy increased above the critical level for intra renal reflux at flow rates of 4ml/min and above during pure saline perfusion. ISO 0.1µg/ml added to the irrigation fluid significantly decreased renal pelvic pressure at all perfusion rates, but not below the critical level for intra renal reflux. This suggests, as stated earlier; that this critical pressure level probably is reached in the majority of ureteroscopic procedures.

Furthermore, plasma levels of ISO were measured in a few perfusions as mentioned, with significant cardiovascular side effects in one case. ISO reached the systemic circulation, but the mechanism of leakage can only be speculated on. Leakage alongside the nefrostomy tube at high flow rates and secondary retroperitoneal absorption is one possibility. However, it cannot be ruled out that ISO at high pelvic pressure reached the systemic circulation due to a reflux mechanism. This experimental setup does not allow for us to distinguish the mechanism of action, but it implies that caution is needed, if ISO is used in clinical trials using semirigid ureterorenoscopy, until further investigations on the safety of the drug has been conducted.

Although ISO were measured in plasma as noted, mean systolic blood pressure was 111 (1.3) and 110 (2.0) mmHg in the saline and ISO group, respectively, and was very stable (P = 0.330). The mean heart rate in the saline group of 109 (±4.5) beats/min., was significantly higher than in the ISO group, of 97 (±2.1) beats/min (P < 0.001). Surprisingly, the mean heart rate was higher in the saline group. It can be speculated that the difference was due to higher renal pelvic pressures in the saline group and this finding confirms that ISO did not systematically reach systemic circulation.

5. CONCLUSIONS .

Isoproterenol 0.1µg/ml significantly and dose dependently decreased the normal renal pelvic pressure in response to perfusion without concomitant cardiovascular side effects in a porcine model.

Isoproterenol added to the irrigation fluid at concentration above 0.1µg/ml (1 and 10 µg/ml) reached the systemic circulation, causing significant cardiovascular side effects in the latter case.

Isoproterenol 0.1µg/ml at a flow rate of 8ml/min significantly reduced renal pelvic pressure during flexible ureterorenoscopy in

pigs - even below the critical pressure for renal backflow, when compared to pure saline perfusion. No cardiovascular side effects were observed.

The pressure flow relation during semirigid ureterorenoscopy was linear and renal pelvic pressure during saline perfusion was above the critical pressure for renal backflow at all flow rates in this porcine model.

Isoproterenol 0.1µg/ml significantly reduced the pressure flow relationship at flow rates 4, 8, 12, 16 20 and 33ml/min during semirigid ureterorenoscopy without concomitant cardiovascular side effects, however, not below the critical pressure of renal backflow.

Caution is needed if Isoproterenol is used in clinical trials using semirigid ureterorenoscopy as Isoproterenol reaches systemic circulation during high renal pelvic pressures.

6. PERSPECTIVES .

Patients with upper urinary tract stones disease may benefit from receiving medication that causes smooth muscle relaxation in order to promote spontaneous stone passage, reduce time to stone expulsion and reduce pain medication. However, it remains to be documented, whether reducing renal pelvic pressure during endoscopic procedures facilitate the procedure, reduces complication rates or promotes stone passage. Randomized clinical trials should be conducted in order to evaluate the potential benefit of endoluminal administration of a relaxant drug. We have identified and tested a drug (ISO), which may be of potential benefit in a clinical setting, although cautions with regard to cardiovascular side effects should be taken at very high-pressure levels. (See conclusions).

This research area is still in the exploratory phase and further animal experimental studies concerning the potential damage which high renal pelvic pressures cause during upper urinary tract endoscopy is mandatory.

As discussed previously, the level of renal pelvic pressure during upper urinary tract endoscopy appears to play a role in the development of per-/postoperative complications such as pain, fever, septicaemia or ureteric damage (see chapter one: Complication to endoscopic procedures). It seems preferable to keep pelvic pressure as low as possible during these procedures. For this purpose ISO 0.1 µg/ml seems promising and we have confirmed ISO´s relaxing potency in the clinical setting without concomitant cardiovascular side effects or measurable levels of plasma ISO during flexible ureterorenoscopy in humans(92). However, the findings of plasma levels of ISO during semirigid ureterorenoscopy in the present study and considering β2 and β3 adrenergic receptor mediates smooth muscle relaxation(64) in human and pigs it might be preferable to use a more selective β-agonist in order to reduce cardiovascular side effects mediated by β1 adrenergic receptors.

A number of drugs have proven to modulate pyeloureteric dynamics as discussed in the present paper and it may be speculated that a combination of selected drugs (ex. selective β-agonist, α-blockers, NO- donors, Calcium channel blockers etc…) might be preferable in order to further decrease renal pelvic pressure. This should be evaluated in future studies

On the basis of our experiments, endoluminal administration of a relaxant drug seems advantageous regarding side effect profile compared to intravenous application and may prove to favour upper urinary tract surgery in the future.

7. SUMMARY

The experiments performed in this PhD thesis was conducted at the Institute of experimental surgery, Skejby Hospital Århus, Denmark and at the Laboratory of Animal science, Odense University Hospital, Denmark. The thesis is based on 3 peer review articles published in international journals and a review.

Diagnostic or therapeutic endoscopic upper urinary tract procedures are usually characterised as minimal invasive procedures and associated with a low complication rate. Most often fever or pain are seen and sometimes septicaemia. However, mucosa lesion or even ureteric ruptures are known complications. Research has suggested that high renal pelvic pressures generated during these procedures, might contribute to per-/postoperative complications seen, and even possible renal parenchymal damage. Nevertheless, local administration (endoluminal) of a relaxant drug has not previously been tried in order to lower renal pelvic pressure.

The purposes of this thesis were to examine the effect of local administration (endoluminal) of the nonspecific β-adrenergic agonist ISOproterenol (ISO) on: 1) The normal pressure flow relation in porcine ureter, 2) The effect of endoluminal ISO perfusion during flexible ureterorenoscopy, 3) The pressure flow relation during semirigid ureterorenoscopy and 4) The cardiovascular system.

Among other receptor-types β-adrenergic receptor are located in the upper urinary tract and the activation thereof mediates smooth muscle relaxation. We have shown – in an animal experimental model – that ISO added to the irrigation fluid had significant impact on the renal pelvic pressures generated during upper urinary tract endoscopy. ISO significantly and dose dependently reduced the normal pressure flow relations by approximately 80% without concomitant cardiovascular side effects or measurable plasma levels of ISO.

During flexible ureterorenoscopy 0.1µg/ml ISO added to the irrigation fluid significantly reduced renal pelvic pressure during perfusion compared to saline perfusion alone. Pressures obtained during ISO perfusion were kept below the critical pressure for intra renal reflux. The pressure flow relation during semirigid ureterorenoscopy was linear and ISO reduced pelvic pressure significantly, but not below the critical level for intra renal refluxs. In conclusion, ISO 0.1 µg/ml added to the irrigation fluid during endoscopic procedures was safe in this porcine model.

Along side this thesis, we have demonstrated the relaxing potency of 0.1µg/ml ISO added to the irrigation fluid in a human trial and found it safe(92).

Future research in this area, especially randomized clinical trials, regarding the relaxing potency, complication rates, pain episodes etc. should be evaluated. The addition of a relaxant drug to the irrigation fluid may prove to favour therapeutic or diagnostic endoscopic procedures in the upper urinary tract in the future.

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