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MOLECULAR TOXICOLOGY

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Hepatotoxic effects of polidocanol in a model of autologously perfused porcine livers

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Abstract Polidocanol is an effective sclerosing agent that consists of 95% hydroxypolyethoxydodecane and 5% ethyl alcohol and is known to have a low risk of complications. However, since the compound has been proposed for the local treatment of liver diseases, the potential for topical hepatic side effects should be examined. Therefore, the new model of normothermic-hemoperfused isolated porcine slaughterhouse livers was used to examine polidocanol-hepatotoxicity encompassing the advantages of slaughterhouse organs to reduce animal experiments and autologous blood as an optimal perfusate. Polidocanol was administered via the hepatic artery and portal vein and the effects of the sclerosant on organ function parameters were compared with those in an untreated control group. In contrast to the untreated control organs, significant differences were found in the polidocanol group for parameters such as alanine aminotransferase or organ weight after perfusion. The most striking differences were found for hepatic bile flow, which dropped in the polidocanol group to 0.24 ± 0.02 ml/min per 1000 g after administration of the compound compared with 3.80 ± 1.08 ml/min per 1000 g in the control group. In summary, the present observations indicate a risk of hepatotoxic effects of polidocanol. Clinicians should be aware of this problem and the use of polidocanol for intrahepatic sclerosing should be restricted to specialized centers.

Keywords Liver · Toxicology · Polidocanol · In vitro · Alternative testing

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Introduction

Polidocanol is an effective sclerosing agent. It is known to have a low risk of systemic and local complications (Conrad et al. 1995; Feied et al. 1994), and therefore it is commonly used for sclerotherapy, which is a relatively safe and worldwide-used therapeutic option for the treatment of various diseases (Sharara and Rockey 2001). In the field of gastroenterology and hepatology, next to its use in the treatment of esophageal varices and bleeding gastroduodenal ulcers (Guglielmi et al. 2002), use in hepatological indications such as portal vein embolization (Kaneko et al. 2002), venous malformations or hydatid cysts have been reported (Ormeci et al. 2001).

Among the different sclerosing agents, polidocanol is widely used in different fields, as it is an effective, relatively weak sclerosant that has been described as being successfully employed for the treatment of venous malformations for many years (Conrad et al. 1995; Guex 1993). Polidocanol is virtually painless upon

administration and has displayed relatively low incidences of hyperpigmentation and skin necrosis (Guex 1993).

Polidocanol consists of hydroxylpolyethoxydodecane (Conrad et al. 1995). This active component of the compound is a local anesthetic that differs from classic amide and ester anesthetics by the lack of an aromatic ring. Its detergent action leads to a rapid over-hydration of endothelial cells and consecutive vascular injury (Guex 1993).

There has been a series of reports addressing the toxicity of polidocanol. In a first study, the depth of injury caused by submucosal injection of increasing concentrations of polidocanol, and other sclerosing agents such as sodium tetradecyl sulphate, 5% ethanolamine oleate and 5% varicosid were analyzed in rabbit stomach using histological examination (Robertson et al. 1989). Macroscopic ulceration was seen in 14.6% of injection sites and increasing concentrations of polidocanol produced increasingly extensive microscopic inflammation. At 3% polidocanol caused a full thickness inflammation and on the basis of these findings it was suggested that 5% ethanolamine should be the most suitable agent for injection sclerotherapy (Robertson et al. 1989).

A further study assessed the effects of intravenous and intraperivenous injections of polidocanol and sodium tetradecyl sulfate on the rat femoral vein (Morsiani et al. 1987). Intravenous (i.v.) and intravenous plus perivenous (i.v. + p.v.) injections of both sclerosants and physiologic saline were compared as to vein lumen occlusion, fibrosis, and damage to the artery and surrounding nerve and muscle tissues, and a statistically significant number of solid occlusions of the femoral vein resulted after i.v. + p.v. injection of polidocanol at 48 h, and 7 and 30 days ($p < 0.05$; $p < 0.01$) (Morsiani et al. 1987). After i.v. + p.v., a marked inflammation of muscle with signs of focal necrosis was found at 48 h and 7 days. It was concluded that i.v. + p.v. injection of polidocanol may lead to a severe inflammation and necrosis of the tissues surrounding the sclerosed vein and that paravariceal injection of sclerosants is a dangerous procedure, owing to the high risk of iatrogenic ulcers and esophageal perforation caused by muscular and mucosal necrosis (Morsiani et al. 1987).

As polidocanol may get into the hepatic artery or portal vein during interventions, and side-effects may arise from the reaction of polidocanol with the vascular bed of the liver, the present study was carried out to assess the effects of polidocanol on liver function using a previously established perfusion system (Grosse-Siestrup et al. 2001, 2002c, 2003).

Materials and methods

Animals

A total amount of 12 livers from white German Landrace pigs (age 6 months, weight 106 ± 12 kg) were harvested at an abattoir with approval of the official veterinarian institutions. The animals were housed and bred under standard conditions, which were approved by the official veterinarian institutions. Veterinarian inspections were performed on a routine basis and did not reveal any diseases.

Test substance

As test substance, 0.5% polidocanol (8.3×10^{-6} mol/l, Aethoxysklerol, hydroxylpolyethoxydodecane, 0.5%; Kreussler & Co. GmbH, Wiesbaden, Germany) was used. The substance was administered via a single injection of 25 ml into the blood reservoir.

Organ harvesting

All animals were electrically stunned according to the protocol of the abattoir. After blood collection, the abdomen was surgically explored and the organs were cautiously removed. Then, the portal vein and hepatic artery were cannulated and flushed with Eurocollins preservation solution (2000 ml and 3000 ml, respectively, at 4°C) as described previously (Grosse-Siestrup et al. 2002a). The livers were then stored at 4°C in sterile boxes, transferred to the laboratory and reperfused after a cold storage of 3 ± 0.33 h.

Autologous blood collection

The autologous blood was harvested through opening of the cervical vessels. The blood was anticoagulated with heparin (5.000 IU/l, Liquemin N; Roche, Germany) and sodium citrate (18 ml/l), filtered using a transfusion device (Sangofix ES; B. Braun, Melsungen, Germany) and stored in blood bags (Biopack-Compoflex P4162; Biotrans GmbH, Dreieich, Germany).

Organ perfusion

Cannulae were inserted into the suprahepatic and infrahepatic vena cava and common bile duct (the portal vein and hepatic artery were already cannulated) as previously described (Grosse-Siestrup et al. 2001). The organs were then transferred to a water jacket adjusted to 37°C and autologous hemoperfusion was performed in a pressure-controlled manner starting with an initial blood flow of 200 ml/min, which was then increased continuously until pressures of 15 mmHg in the portal vein and 100 mmHg in the hepatic artery were finally reached.

Perfusion setup

The liver perfusion setup was built up by two separated circuits that were linked via a dialysis module (F7 low

flux module; Fresenius AG, Bad Homburg, Germany; Fig. 1). The blood circuit contained 2000 ml heparinized blood that could be diluted with modified Krebs-Henseleit solution to adjust the hemoglobin concentration. The dialysis circuit contained 6000 ml dialysate medium (mmol/l: 142.5 Na^+, 2.9 K^+, 109.9 Cl^-, 37 HCO_3^-, 0.5 Mg^{2+}, 1.5 Ca^{2+}, 3.55 glucose, 2.5 CH_3COO^-),

Autologous blood was transported in the blood circuit via pumps from the blood reservoir through the three parallel fiber dialyisis modules into the portal vein and hepatic artery (Fig. 1).

A roller pump (Duo-head Stöckert 10-20-00; Stöckert Instrumente GmbH, Munich, Germany) delivered the dialysate medium in the dialysis circuit from the reservoir to the dialysis module (flow 1000 ml/min). The dialysis medium was permanently enriched with 97.5% O_2 and 2.5% CO_2. The optimal temperature range of 37°C to 38 °C was generated by a heat exchanger, which was connected to a thermostat-regulator (Stöckert 16-21-00).

Parameters

Besides different hemodynamic parameters, numerous further parameters (Tables 1, 2) of organ viability were examined at the Department of Laboratory Medicine and Pathobiochemistry, Charité School of Medicine, Humboldt University Berlin.

Histology

After termination of the perfusion experiments, the organs were processed for histology. Tissues were processed routinely, as described earlier, and were submitted to routine pathological examination using hematoxylin and eosin (HE) staining (Groneberg et al. 2002c, 2002d; Springer et al. 2004).

Experimental groups

In order to assess the effects of polidocanol, two groups each consisting of six perfused organs were built. After an initial period of 30 min used to establish a steady-state perfusion, a single injection of 25 ml 0.5% polidocanol was administered to the blood reservoir in the polidocanol group. Polidocanol is not able to pass through the dialysis module to the dialysis circuit.

Data presentation and statistical procedures

All assessed data are expressed as mean ± standard deviation (SD). To test the levels of significance between the control and polidocanol groups ($p < 0.05$), the Wilcoxon's Signed Rank Test (StatView 4.5 for Apple Macintosh) was used.

Results

Analysis of a variety of parameters including hemodynamic parameters demonstrated constant perfusion settings in both the control and the polidocanol groups, which guaranteed similar perfusion settings (Tables 1,2). In the polidocanol group, the arterial pressure ranged from 56.2 ± 7.5 mmHg (at 90 min) to 66.8 ± 6.5 mmHg (30 min), while in the control group pressures ranged between 62.4 ± 12.2 mmHg (0 min) and 67.2 ± 5.0 mmHg (60 min). Also, there were no signifi-

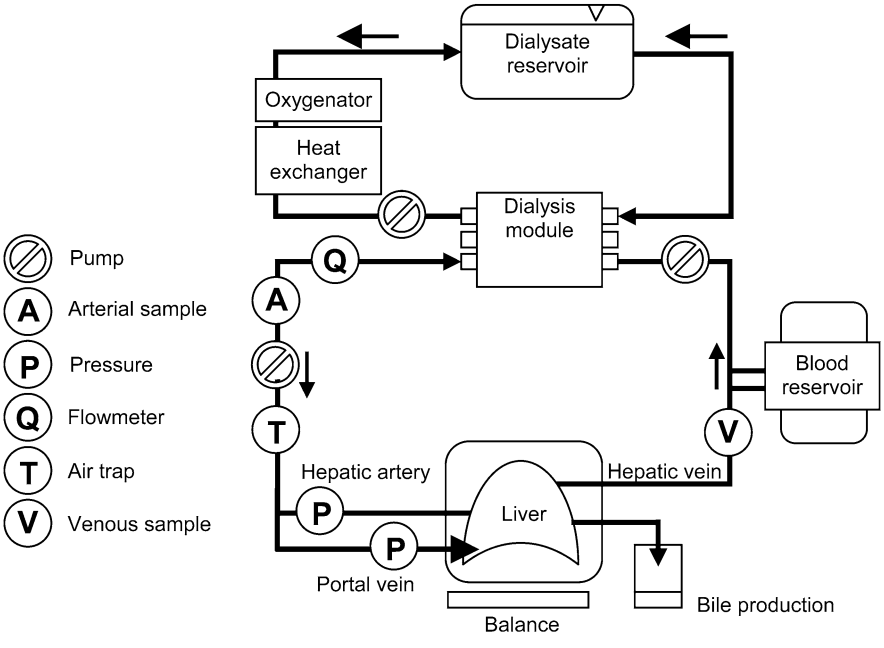

Fig. 1 Liver perfusion system. The liver perfusion model consists of two separate perfusion and dialysis circuits. The circuits are connected conventional by glassfiber dialysis modules with roller pumps, pressure controllers and flow meters controlling the perfusion

Table 1 Blood and hepatic parameters of the hemoperfused liver preparation at different time points in the control and polidocanol groups. Polidocanol (25 ml of a 5% solution) was administered after 30 min. *AST/GOT* aspartate aminotransferase, *ALT/GPT* alanine aminotransferase

Parameter	Units	Time: 0 min				30 min				60 min				90 min			
		Control		Polidocanol		Control		Polidocanol		Control		Polidocanol		Control		Polidocanol	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sodium	mmol/l	140.7	0.8	141	1.4	141	1.5	142	2.7	137	0.4	142	2.5	134	2.1	136	2.2
Potassium	mmol/l	5.26	1.49	5.26	0.90	4.40	0.80	4.48	0.50	4.60	0.62	4.90	0.53	4.80	0.60	5.16	0.49
Urea	mg/dl	10.2	0.8	9.4	0.5	14.8	1.3	11.8	1.3	14.8	1.8	14.2	2.2	17.4	2.9	15.6	1.5
Glucose	mg/dl	171.4	4.6	169.6	5.6	171.0	1.4	169.0	1.9	163.4	2.1	165.6	1.8	159.8	5.4	163.8	1.5
Total protein	g/dl	7.82	0.52	7.83	0.41	3.63	0.20	3.63	0.33	3.26	0.58	4.18	0.46	3.03	0.71	4.53	0.38
Albumin	g/dl	4.50	0.07	4.45	0.13	2.26	0.53	2.24	0.21	2.34	0.48	2.50	0.56	2.66	0.52	2.82	0.54
Creatinine	mg/dl	2.26	0.14	2.34	0.23	0.50	0.12	0.48	0.08	0.56	0.05	0.48	0.16	0.50	0.07	0.58	0.04
Bilirubin	mg/dl	0.46	0.08	0.38	0.17	0.28	0.13	0.27	0.15	0.24	0.08	0.24	0.15	0.26	0.13	0.24	0.15
AST/GOT	U/l	577	194	578	217	864	616	847	141	1395	488	1519	376	1695	519	2197	632
ALT/GPT	U/l	50.4	16.0	53.6	15.1	61.2	12.6	64.8	13.2	78.8	14.8	122.2	30.3	96.2	19.5	146.4	28.7
Hemoglobin	g/dl	15.1	0.7	14.3	1.1	11.1	0.6	10.2	1.4	10.6	0.3	9.0	1.4	9.9	0.3	7.9	1.0
Hematocrit	%	39.0	9.2	40.0	2.2	32.4	6.1	33.2	1.8	27.8	1.1	21.2	2.4	26.5	2.1	19.8	1.6
pH		7.26	0.03	7.25	0.03	7.23	0.03	7.23	0.03	7.39	0.04	7.40	0.03	7.35	0.12	7.37	0.03
Bicarbonate	mmol/l	32.0	0.4	31.2	0.3	23.2	2.3	22.0	0.7	21.8	3.1	21.3	0.9	20.4	3.1	20.0	0.7
Bile flow	ml/min per 1000 g	2.5	0.28	2.5	0.22	3.0	0.45	2,7	0.39	4.1	1.44	0,2	0.24	5.6	3.17	1.7	1.52

Table 2 Development of hemodynamic parameters of the hemoperfused liver preparation during perfusion. Polidocanol was administered after 30 min

Parameter	Units	Time: 0 min				30 min				60 min				90 min			
		Control		Before polidocanol		Control		Before polidocanol		Control		Polidocanol		Control		Polidocanol	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Arterial pressure	mmHg	62.4	12.2	62.0	4.5	66.6	3.1	66.8	6.5	67.2	5.0	56.8	13.2	64.8	12.9	56.2	7.5
Portal pressure	mmHg	11.5	1.22	11	0.89	10.5	2.35	12	0.63	12	2.28	12.17	1.17	12.83	1.17	11.8	1.3

cant differences in the portal pressures ranging from 10.5 ± 2.35 mmHg (30 min) to 12.83 ± 1.17 mmHg (90 min) in the control group, and from 11 ± 0.89 mmHg (0 min) to 12 ± 0.63 mmHg (30 min) in the polidocanol group (Table 2).

The average organ weight was 2212 ± 252 g before conservation, 2269 ± 251 g before perfusion, and 2392 ± 280 g after perfusion in the control group. In the polidocanol group, the values were 2495 ± 49 g before conservation, 2604 ± 46 g before perfusion, and 3441 ± 181 g after perfusion, with a significant difference between before and after polidocanol treatment ($p = 0.009$) (Table 3). Both groups were treated uniformly with regard to warm and cold ischemia, and a number of significantly differences in the biochemical and hematological parameters were obtained after polidocanol was administered (Tables 1,2).

In both groups, sodium concentrations remained constant, with values ranging between 136 ± 2.2 mmol/l (90 min) and 142 ± 2.7 mmol/l (30 min) in the polidocanol group, and between 134 ± 2.1 mmol/l (90 min) and 141 ± 1.5 mmol/l (30 min) in the control group. Also, there were no significant differences in the potassium concentration between the controls and the polidocanol group, with values of 4.8 ± 0.6 mmol/l and 5.16 ± 0.49 mmol/l, respectively at 90 min

After the administration of polidocanol (Table 1), significant differences were found for total protein. In this respect, highly significant differences were present in the polidocanol-treated organs at 90 min (i.e., 60 min after polidocanol administration, when values for control were 3.03 ± 0.71 g/dl versus polidocanol 4.53 ± 0.38 g/dl, $p = 0.01$), and also, after 60 min, a significant difference was present between the two groups ($p = 0.03$) (Table 1).

Polidocanol administration also led to significant changes in hematological parameters. At 60 min after polidocanol administration, hemoglobin levels dropped to 7.9 ± 1.0 g/dl versus 9.9 ± 0.3 g/dl for control ($p = 0.01$) at 90 min. Also, the hematocrit decreased from $33.2 \pm 1.8\%$ pre-polidocanol (at 30 min, i.e., prior to polidocanol administration) to $19.8 \pm 1.6\%$ (60 min after administration), with significant differences at time points 60 min (control $27.8 \pm 1.1\%$ versus polidocanol

Table 3 Development of organ weight parameters of the hemoperfused liver preparation. *LGnat* weight (g) after harvesting,*LGkons* weight (g) after conservation and before perfusion; *LGperf* weight (g) after perfusion

Parameter	Control		Polidocanol	
	Mean	SD	Mean	SD
LGnat	2212	252	2495	49
LGkons	2269	251	2604	46
LGperf	2392	280	3441	181

$21.2 \pm 2.4\%$, $p = 0.01$) and 90 min (control $26.5 \pm 2.1\%$ versus polidocanol $19.8 \pm 1.6\%$, $p = 0.01$). In contrast to these changes, other hematological parameters did not differ throughout the perfusion.

Analysis of liver enzymes demonstrated a significant difference for alanine aminotransferase (ALT or GPT) values at 60 min with levels of 122.2 ± 30.3 U/I for the polidocanol group versus 78.8 ± 14.8 U/I for the control group ($p = 0.015$). Also, significant changes were present at 90 min with levels of 146.4 ± 28.7 U/I for the polidocanol group and 96.2 ± 19.5 U/I for the control group ($p = 0.02$) (Fig. 2).

The most striking differences were found for the hepatic bile flow, which was calculated per 1000 g liver weight. Here, the flow dropped in the polidocanol group to 0.2 ± 0.24 ml/min per 1000 g after administration of the compound compared with 4.1 ± 1.44 ml/min per 1000 g in the control group, and both the 60-min ($p = 0.01$) and the 90-min ($p = 0.03$) values differed significantly between the control and the polidocanol groups (Fig. 3).

To assess the cellular interactions of polidocanol, histological examination was performed and revealed qualitative differences between the polidocanol-treated livers and the control livers: In comparison with the control group, the polidocanol-treated livers exhibited a distorted morphology with hemorrhagic lesions (Fig. 4).

Discussion

The common mechanism of action of sclerosing substances is the reaction with endothelial surfaces leading to subsequent thrombosis, and later to intimal fibrosis and obliteration of the vessel lumen (Guex 1993).

As polidocanol is routinely used for sclerosing varices and other vascular pathologies in the gastrointestinal tract (Morales and Baum 2003; Vlavianos and Westaby 2001) and it has recently been proposed for use in the treatment different hepatic diseases (Kaneko et al. 2002; Ormeci et al. 2001), the present study addressed its effects when administered to the hepatic vascular system because the liver plays an important role in the metabolism of exogenous substances due to its anatomical position between the gastrointestinal tract and the systemic circulation.

Analysis of the different parameters demonstrated a specific impact of polidocanol on hepatic function. In contrast to stable hemodynamic perfusion parameters in both groups and constant electrolyte values, a first effect of polidocanol was found in the development of organ weight with a significant increase after administration when compared with the control group. This increase may be caused by the reaction of polidocanol with the

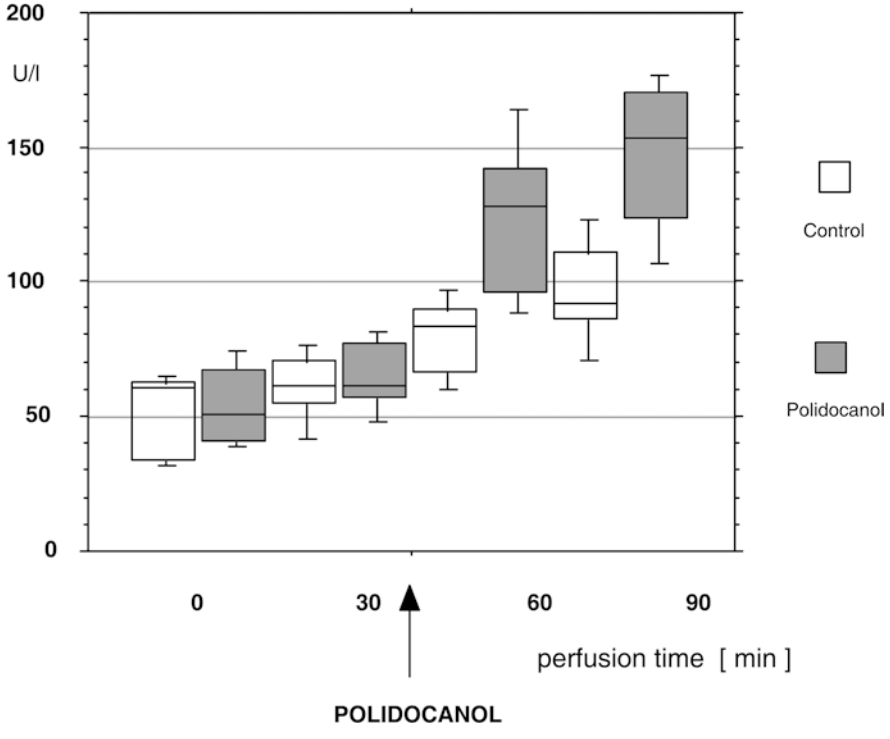

Fig. 2 Alanine aminotransferase (ALT/GPT) levels. Polidocanol administration (25 ml of a 0.5% preparation) to the liver perfusion system led to a significant increase in ALT values (medians and percentiles)

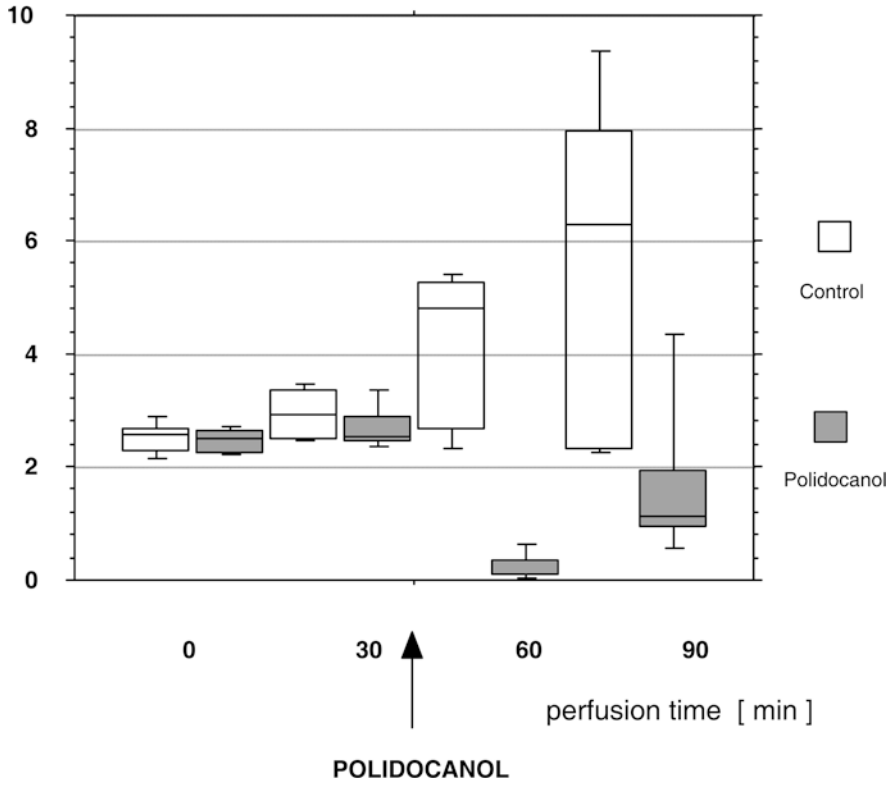

Fig. 3 Bile flow. A significant decrease in bile production and secretion is found after polidocanol administration (medians and percentiles)

endothelial structure with consequent stasis and increase in intra-organ blood contents. In this respect, the polidocanol administration also led to a drop in the hematocrit, which indicates a shift of blood cells to the liver parenchyma due to stasis that is caused by polidocanol-endothelium reactions.

Next to the observation of changes in organ function, the morphology of the polidocanol-treated organs was also assessed and compared with that of the control organs in order to assess the polidocanol-endothelium reaction. We found that the architecture of the polidocanol-treated organs was distorted as the interaction of the sclerosing agent with the endothelial cells led to a disruption of the endothelial barrier with subsequent hemorrhagic lesions in the liver parenchyma due to the known rapid over-hydration of endothelial cells and consequent vascular injury (Guex 1993).

Next to these changes, polidocanol also induced a significant effect on bile production and flow. Bile flow dropped dramatically in the polidocanol group 60 min after administration, indicating that the damage caused by treatment with polidocanol did not only lead to a destruction of the liver architecture with hemorraghic lesions, as assessed by histology, but also to a functional damage. This significant functional damage was not globally affecting all liver functions but was specifically found in parameters such as ALT/GPT or total protein, whereas parameters such as electrolytes (sodium, potassium), urea or creatinine did not significantly differ between the two groups throughout the perfusion period.

The present findings of polidocanol liver toxicity may be compared with previously published data of polidocanol toxicity. In this respect, it was shown that intravenous and intraperivenous injections of polidocanol in rats leads to a marked inflammation of muscle with signs of focal necrosis of the tissues surrounding the sclerosed femoral vein of rats (Morsiani et al. 1987). Also, the submucosal injection of increasing concentrations of polidocanol was reported to produce microscopic inflammation in the gastric mucosa of rabbits (Robertson et al. 1989).

Previous reports have also pointed to side-effects such as hemoglobinuria or intralesional hemolysis, which may lead to acute renal failure (Marrocco-Trischitta et al. 2001).

As polidocanol has been proposed for use as a sclerosing compound in treatment of diverse hepatic diseases such as intrahepatic cysts (Kaneko et al. 2002; Ormeci et al. 2001), the present data demonstrating a potential deleterious effect if polidocanol enters the hepatic vascular system have to be taken into account and polidocanol instillation may be performed under great caution.

The present approach used a model of isolated perfused liver. In contrast to other techniques such as cultured hepatocytes (Guillouzo 1998; Ulrich et al. 1995) or precision-cut liver slices (Parrish et al. 1995), the present model displays the closest approach to the in vivo situation, as it gives the opportunity to asses a large panel of hepatic functions (Groneberg et al. 2002e). In this respect, the major advantage of the present model is represented by the preservation of the three-dimensional organ structure with the cell-to-cell interaction of parenchymal and stromal cell types. A further major

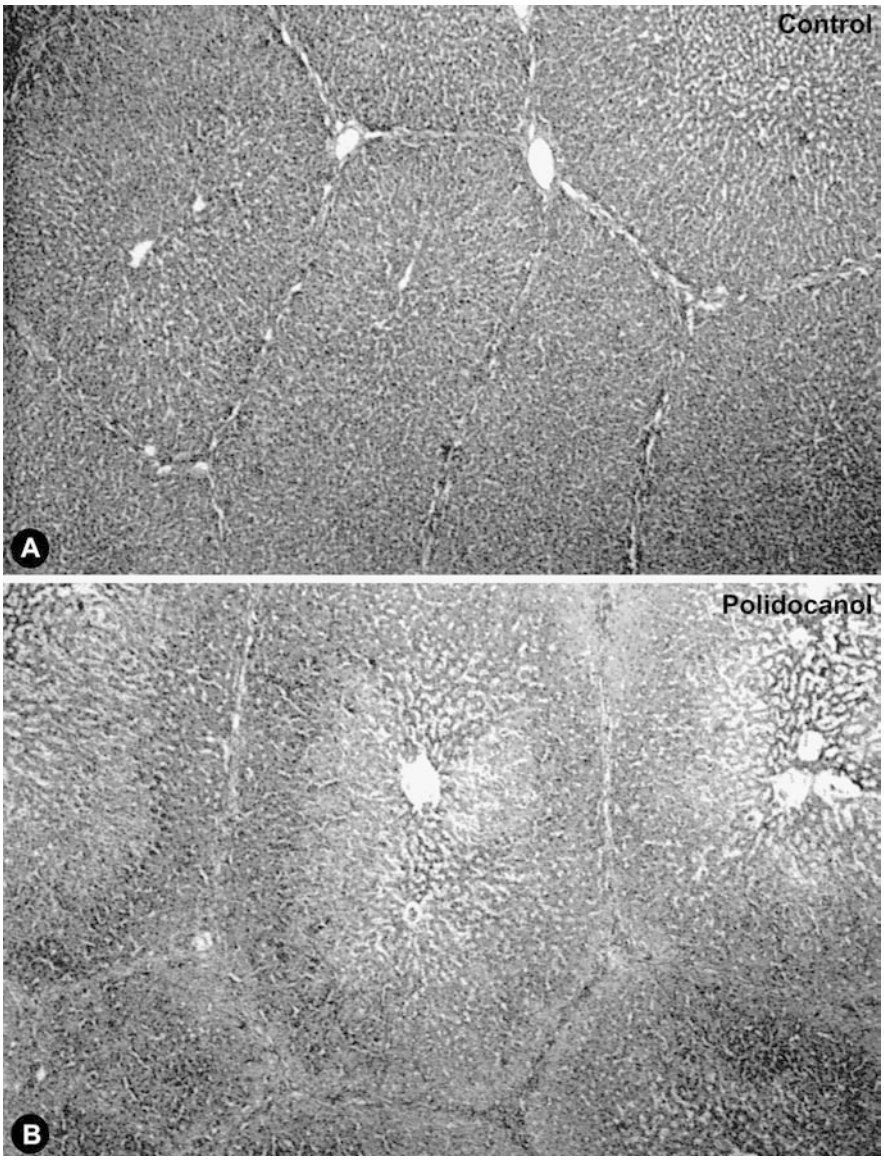

Fig. 4A,B Histology. Histological examination of the perfused liver prparation was performed to assess the cellular damage due to polidocanol using hematoxylin staining. Comparison with control livers (**A**) revealed that polidocanol-treated organs (**B**) exhibited a distorted morphology with enlargement of extracellular spaces and hemorrhagic lesions. *Original magnification* **A,B** x50

advantage is the possibility of real-time bile collection and analysis (Conway et al. 1985; Meren et al. 1986). A large number of studies using porcine liver perfusion systems have been described so far (Abouna et al. 1969; Drapanas et al. 1966; Elmslie et al. 1971; Ham et al. 1969; Jablonski et al. 1971). However, as modern ethical standards require reductions of laboratory animal numbers and the use of porcine organs in high numbers is very expensive, an alternative perfusion model was chosen in the present studies that uses organs from slaughterhouse pigs to reduce laboratory animal numbers. This model was developed from existing models of isolated perfused slaughterhouse porcine kidneys (Dittrich et al. 2004; Fehrenberg et al. 2004; Hochel et al. 2003), hearts (Modersohn et al. 2001) and skin (Grosse-Siestrup et al. 2002d), and has been previously established (Grosse-Siestrup et al. 2001). Also, a valid use for assessing organ toxicity was demonstrated recently (Grosse-Siestrup et al. 2002b). Together with these previously established models of slaughterhouse organ perfusion, the present ex vivo approach to investigate hepatic effects using slaughterhouse livers seems to offer an attractive alternative to animal experiments. Future studies involving modern techniques of molecular biology such as microdissection-assisted single cell reverse transcription-polymerase chain reaction (Peiser et al. 2002), cloning techniques (Hanze et al. 2002) and gene-depletion (Kerzel et al. 2003; Rubio-Aliaga et al. 2003) or gene array techniques (Groneberg et al. 2003b) in combination with classical techniques such as immunohistochemistry (Groneberg et al. 2003a; Heppt et al. 2002), in situ hybridization (Fischer et al. 2001, 2002; Jacob et al. 2002), northern blotting (Groneberg et al.2001, 2002a), western blotting (Lim et al. 2000) or electron microscopy (Groneberg et al. 2002b), will help to further characterize the liver perfusion model.

To guarantee the validity of the results, two controls were performed: (1) Prior to polidocanol administration,

the organs were perfused over a period of 60 min to generate an internal control, and (2) next to the internal pre-administration control, a second group of organs was perfused to which only a vehicle (NaCl) was administered. From the comparison of the different groups, the validity and integrity of the system was demonstrated.

In conclusion, the observation reported herein draws attention to the risk of hepatic effects of polidocanol. Using a previously established model to assess whole organ function and acute toxicity, it may be suggested that polidocanol exerts deleterious effects on liver functions via its known sclerosing effects on endothelial cells and consequent blood stasis and congestion. Further investigations are needed to confirm and extend this conclusion on the molecular level. Clinicians should be aware of this problem and therefore, the use of polidocanol for sclerosing intrahepatically should be restricted to specialized centers. Furthermore, the present ex vivo approach to investigate hepatic effects using slaughterhouse livers seems to offer an attractive alternative to animal experiments.

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