A tungsten supplemented diet attenuates bacterial translocation in chronic portal hypertensive and cholestatic rats: role of xanthine dehydrogenase and xanthine oxidase

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Abstract

Background—Bacterial translocation (BT) plays a major role in the pathophysiological process of spontaneous infections in portal hypertension (PH) and cholestatic jaundice. The major mechanisms promoting BT in experimental animal models are the disruption of the intestinal ecological equilibrium and disruption of the intestinal mucosal barrier. The enzymes xanthine dehydrogenase (XD) and xanthine oxidase (XO) are often implicated as a significant source of oxidants which have a major impact on the impairment of intestinal barrier function.

Aim—To investigate the incidence of BT in rats with PH and obstructive jaundice, and to evaluate the impact of XD and XO.

Methods—Animals were subjected to sham laparotomy (SL), PH by calibrated stenosis of the portal vein, and common bile duct ligation (CBDL). They were fed either a standard pellet diet or a tungsten supplemented molybdenum-free diet. Four weeks after the operative procedure, intestinal colonisation and BT to portal vein, vena cava, mesenteric lymph nodes, liver, and spleen were determined. Intestinal XD and XO activity were measured enzymatically and histochemically.

Results—Significant ($p < 0.01$) intestinal bacterial overgrowth was present in all PH and CBDL groups compared with the SL group. In normally fed animals after SL, BT occurred in 12%. In PH and after CBDL, the rate of BT increased significantly ($p < 0.05$) to 28% and 54% respectively. In the jejunum of normally fed animals subjected to PH or CBDL, a significant increase in XO was observed ($p < 0.01$). Animals fed a tungsten supplemented diet showed a significant attenuation of BT to 14% in PH and 22% after CBDL ($p < 0.05$). Tungsten treatment completely suppressed jejunal XD and XO activities.

Conclusions—Significant intestinal bacterial overgrowth, BT, and XD to XO conversion occurred in PH and after CBDL. XD and XO inactivation by a tungsten supplemented molybdenum-free diet significantly reduced the incidence of BT without affecting intestinal bacterial overgrowth. These data strongly support the hypothesis that increased XD to XO

conversion may contribute to intestinal barrier failure in PH and after CBDL.

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Keywords: bacterial translocation; portal hypertension; chronic cholestasis; xanthine oxidase; xanthine dehydrogenase

Spontaneous bacterial peritonitis, cholangitis, bacteraemia, and sepsis are complications of portal hypertension (PH) and obstructive jaundice which account for high morbidity and death rates.^{1,2} In most of these infectious episodes, the exact pathophysiological mechanism remains unclear. In recent years, the gut has been considered to be the major source of infection.² It was proposed that PH and jaundice may disrupt intestinal mucosal integrity and facilitate the egress of intraluminal intestinal bacteria to distant organs, a process termed bacterial translocation (BT). This process includes the steps of attachment of microbes to the gut mucosa, penetration through the epithelium into the lamina propria, and transport to distal sites.^{3,4}

PH, either isolated or as a sequel to liver disease, leads to severe alterations in intestinal perfusion. The increased splanchnic vascular resistance (backward flow theory) as well as an increased portal venous flow (forward flow theory) may cause intermittent intestinal mucosal hypoperfusion and hypoxia.^{5,6} Hypoxia initiates oxidative damage of the intestinal integrity by increased conversion of the enzyme xanthine dehydrogenase (XD) to xanthine oxidase (XO). XD and XO are metalloflavoenzymes (four redox centres in the active site, molybdenum, two iron-sulphur clusters, and a flavin adenine dinucleotide centre) which are widely distributed among tissues of most species and are present in high concentrations in the intestinal epithelial cells.⁷⁻¹¹ Under normal conditions, XD serves as a rate limiting step in nucleic acid degradation by catalysing the oxidation of hypoxanthine to xanthine and uric acid. In pathological conditions, XD can be converted into XO through reversible thiol oxidation or irreversible proteolytic modification. This XO has been shown to generate reactive oxygen species, superoxide and hydrogen peroxide, which are involved in the

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Abbreviations used in this paper: BT, bacterial translocation; SL, sham laparotomy; PH, portal hypertension; CBDL, common bile duct ligation; XO, xanthine oxidase; XD, xanthine dehydrogenase.

peroxidation damage of cells.^{7 9 12 13} Mainly lipid peroxidation and activation of neutrophils are promoted by these oxygen derived free radicals which further ameliorate epithelial injury.¹⁴ In the intestinal tract, this epithelial injury may cause intestinal barrier failure, thus promoting BT.

In a previous study performed in portal hypertensive and common bile duct ligated rats, we were able to show that inhibition of XO by allopurinol can decrease the incidence of BT, attenuate intestinal mucosal lipid peroxidation (measured by intestinal mucosal malone dialdehyde levels), and reduce intestinal neutrophil derived myeloperoxidase activity.¹⁵

The aim of this study was to elucidate further the role of XD and XO in BT in chronic portal hypertensive and common bile duct ligated rats. In addition to the determination of intestinal bacterial colonisation and BT, we estimated XD and XO enzymatically and histochemically in the jejunum. Furthermore we examined the effect of a tungsten supplemented molybdenum-free diet. The result of feeding a diet containing tungsten is incorporation of wolfram instead of molybdenum into newly synthesised XD and XO, resulting in progressive loss of oxidase activity. Complete inactivation of XD and XO can be achieved when tungsten is fed for more than 14 days.^{13 16} This diet has been used to ameliorate the deleterious effects of oxygen radicals generated by ischaemia-reperfusion in the intestinal tract.^{16 17}

Methods

Four week old male Sprague-Dawley rats (130–145 g body weight) were used for these experiments. The animals were purchased from Himberg Breeding Laboratories, Vienna, Austria. All experiments were conducted with the permission of the animal research commission of the Ministry of Science of Austria following local guidelines for the care and use of laboratory animals. The rats were housed in an environmentally controlled vivarium with a 12 hour light/dark cycle; they received a standard pellet diet and water ad libitum. Sixty animals were randomly assigned to one of three equal groups of 20 animals subjected to sham laparotomy (SL), PH, or common bile duct ligation (CBDL). For all surgical procedures, anaesthesia was induced by intraperitoneal injection of 100 mg/kg ketamine.

Twenty rats in the SL group were anaesthetised, and under sterile conditions a 2 cm coeliotomy was performed. The portal vein and

common bile duct were dissected free, and the abdominal wall was then closed in two layers with absorbable interrupted sutures.

Chronic PH was produced in 20 rats by calibrated stenosis of the portal vein as previously described.¹⁸ Briefly, the portal vein was dissected free, and a ligature of 3-0 silk was placed around the vein. A 20 gauge blunt needle was placed alongside the vein, and the ligature was tied snugly to the needle and vein. The needle was removed to yield a calibrated stenosis of the portal vein.

Twenty rats were subjected to obstructive jaundice induced by CBDL. The common bile duct was dissected free and double ligated with a 3-0 silk ligature. All ligatures were placed in the same position in all rats.

After the laparotomy all animals were placed in stainless steel molybdenum-free cages. Ten animals from each group were fed a standard pellet diet and water ad libitum and ten received a tungstate enriched normal protein diet (ICN Biochemicals Inc, Biochemical Division, Cleveland, Ohio, USA) and distilled water.

EXPERIMENTAL DESIGN

After four weeks, animals were weighed and reanaesthetised with ketamine, and a laparotomy from the xiphoid to the pubis was performed. Using sterile technique, the skin flaps were retracted and the abdominal muscles treated with 70% ethanol before the abdominal cavity was opened. Portal pressure was measured by cannulating the superior mesenteric vein with a 21 gauge needle connected to a manometer filled with saline, and the height of the right atrium was taken as zero reference level.

Blood samples were collected from the inferior vena cava for the measurement of total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and lactate dehydrogenase with a Cobas Mira multianalyser (Hoffmann LaRoche, Basel, Switzerland). Aliquots of 0.1 ml of portal blood and inferior caval vein blood were plated on to blood agar. The central mesenteric lymph node complex was excised with sterile instruments. The liver and spleen were removed using separate instruments.

To measure intestinal colonisation, a 3 cm long piece of the jejunum was excised, opened on its antimesenteric border, and washed in sterile 0.9% saline. The mesenteric lymph node, parts of the liver, spleen, and ileum were weighed and placed in grinding tubes contain-

Table 1 Animal weight, portal pressure, total serum bilirubin and serum liver enzymes after sham laparotomy (SL), portal hypertension (PH), and common bile duct ligation (CBDL) in untreated and tungsten supplemented (T) animals

	SL	PH	CBDL	SL+T	PH+T	CBDL+T
Weight (g)						
At the start	130 (10)	140 (10)	145 (10)	140 (10)	135 (10)	140 (10)
After 4 weeks	350 (120)	330 (10)	330 (20)	340 (15)	315 (20)	295 (20)
Bilirubin (mg/%)	0.23 (0.1)	0.22 (0.2)	15.1 (2.7)*	0.3 (0.1)	0.37 (0.2)	6.6 (3.8)*†
AST (U/l)	47 (16)	45 (11)	234 (52)*	37 (4)	34 (10)	171 (28)*†
ALT (U/l)	33 (15)	24 (5)	118 (35)*	26 (4)	25 (4)	47 (15)*†
AP (U/l)	231 (41)	288 (45)	599 (71)*	261 (36)	269 (49)	515 (57)*
Portal pressure (mm Hg)	7.4 (1.1)	19.9 (2.1)*	18.9 (1.8)*	7.2 (0.5)	18.7 (2.2)*	17.9 (1.3)*

AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase.

* $p < 0.01$ v SL; † $p < 0.05$ v untreated (no tungsten supplement).

Table 2 Jejunal colonisation with Gram negative and positive bacteria

	Jejunal colonisation (log 10 per g tissue)					
	SL	PH	CBDL	SL+T	PH+T	CBDL+T
Gram positive	3.1 (1.3)	4.4 (1.4)**	4.7 (0.9)**	3.4 (1.1)	4.2 (0.5)**	4.9 (0.7)**
Gram negative	2.4 (0.6)	2.6 (0.4)	3.6 (0.7)**	2.6 (1.3)	2.1 (1.1)	3.4 (1.6)**

SL, sham laparotomy; PH, portal hypertension; CBDL, common bile duct ligation; +T, tungsten supplemented diet.

** $p < 0.01$ v SL.

Table 3 Incidence of bacterial translocation

	Incidence of bacterial translocation (number of positive cultures)					
	SL	PH	CBDL	SL+T	PH+T	CBDL+T
Vena cava		1/10	2/10		1/10	1/9
Vena porta		3/10	5/10		1/10	2/9
MLN	4/10	5/10	8/10	2/10	3/10	4/9
Liver		2/10	9/10		1/10	2/9
Spleen	2/10	3/10	3/10	2/10	1/10	2/9
Incidence (%)	12	28*	54*	8	14†	28†
Positive animals	4	5	9	3	2	4

SL, sham laparotomy; PH, portal hypertension; CBDL, common bile duct ligation; +T, tungsten supplemented diet; MLN, mesenteric lymph node.

* $p < 0.05$ v SL; † $p < 0.05$ v untreated (no tungsten supplement).

ing the 1:9 volume of sterile brain heart infusion (BioMerieux, Marcy l'Etoile, France), and homogenised with sterile ground glass stoppers. Serial dilutions of the homogenates were carried out, and 0.1 ml of each dilution was plated on blood agar to culture for aerobic and facultative Gram positive cocci, on Endo agar to culture for aerobic and facultative Gram negative bacilli, and on MRS agar to culture for lactobacilli (Oxoid Co, London, UK). All plates were incubated aerobically at 37°C for 48 hours. The plates were evaluated for bacterial growth by standard bacteriological techniques. Any growth of bacteria of the same biotype as cultured in the ileum was considered positive. Quantitative culture results were expressed as the number of colony forming units per g tissue (CFU/g), calculated from the dilutions of organ homogenates.

Enzymatic activities of XO and XD were determined spectrophotometrically in the jejunum (Spectronic 300 Array; Milton-Roy Company, Brussels, Belgium) measuring the production of uric acid from xanthine. Briefly, the homogenised tissue was placed in 50 mM potassium phosphate (pH 7.4) containing 0.25 M sucrose, 1 mM EDTA, 1 mM dithiothreitol, and 0.2 mM phenylmethanesulphonyl fluoride. The homogenates were centrifuged at 12 000 rpm for 20 minutes at 4°C. The supernatants were used for measurement of XO+XD and XO. Uric acid formation per minute at 295 nm in the presence of either NAD or xanthine was determined. XD and XO activities were expressed as mU/g dry weight of tissue samples. Dry weight of the jejunum was estimated by incubation of specimens of the jejunum for 48 hours at 50°C.

The histochemical distribution of xanthine oxidoreductase in the jejunum was analysed by a modified histochemical method as described by Kooij.¹⁹ Briefly, specimens of the jejunum were immediately frozen in liquid nitrogen and stored at -80°C. Cryostat sections of 8 µm thickness were cut at a cabinet temperature of -25°C and incubated for 30 minutes at 37°C using an incubation medium containing 18%

polyvinyl alcohol, 1.1 mM tetranitroblue tetrazolium, 0.45 mM 1-methoxyphenazine methosulphate and 0.5 mM hypoxanthine. After incubation, the polyvinyl alcohol-containing medium was removed from the sections by rinsing for 10 seconds in 0.1 M phosphate buffer. Sections were successively fixed in 4% formaldehyde in distilled water for five minutes at room temperature, washed in distilled water for one minute, and mounted in glycerol jelly. Sections were immediately evaluated by light microscopy and photographs of the sections were made because the final reaction product rapidly fades on exposure to light and heat.

STATISTICAL ANALYSIS

The results are expressed as mean (SD). Differences between the means of two groups were statistically tested using the Mann-Whitney U test, and analysis of variance was used to compare more than two groups. $p < 0.05$ or less was considered significant in all analyses.

Figure 1 Xanthine oxidase (XO) and xanthine dehydrogenase (XD) activities in the jejunum of untreated rats subjected to sham laparotomy, portal hypertension, and common bile duct ligation. In all tungsten supplemented groups, no XO or XD activity was detected, indicating their complete inactivation. ** $p < 0.01$ v sham laparotomy.

Figure 2 Histochemical distribution of xanthine oxidase in the jejunum of untreated animals. (A) sham laparotomy; (B) portal hypertension.

Results

After four weeks, 59 animals had survived the experiment. One rat with CBDL being fed the tungsten diet died on the 4th postoperative day. Four animals, two with PH, one after SL, and one with CBDL developed an incisional hernia. All sham operated animals had a patent portal vein and common bile duct. In rats with a calibrated portal vein stenosis, a stenotic but patent portal vein with dilated mesenteric veins was present. All animals with CBDL became visibly jaundiced with a cystic remnant of the common bile duct proximal to the ligatures. Table 1 summarises weight gain, portal pressure, and serum liver enzymes.

COLONISATION (TABLE 2)

Jejunum colonisation in all PH animals showed a significant Gram positive bacterial overgrowth when compared with the SL group ($p < 0.05$), and in all animals with CBDL a significant Gram positive and Gram negative bacterial overgrowth was seen. This bacterial overgrowth was related to greater jejunal colonisation with *Escherichia coli*, *Streptococcus* species, *Lactobacilli*, *Pasteurella*, and *Proteus* strains. There was no difference in jejunal colonisation between untreated PH and CBDL rats and PH and CBDL rats on the tungsten diet.

BACTERIAL TRANSLOCATION (TABLE 3)

In untreated and tungsten supplemented SL groups, BT occurred only to mesenteric lymph nodes and the spleen, with an incidence of 12% and 8% respectively. In untreated PH animals, the frequency of BT increased to 28%, and it was mainly *E coli* and *Streptococci* that were translocated ($p < 0.05$ v SL). Tungsten supplementation attenuated BT in PH animals to 14%; only two animals from this group showed positive cultures compared to five animals in the normally fed PH group.

CBDL in untreated animals resulted in a BT rate of 54%, and, in nine of 10 animals, translocated *E coli*, *Streptococci*, *Proteus*, and *Pasteurella* strains could be cultured ($p < 0.05$ v SL). In the tungsten supplemented CBDL group, BT was found in 22% (four of nine animals) and this was significantly lower ($p < 0.05$) than in the untreated CBDL group. *E coli* and *Streptococci* were cultured most often. The mean number of translocating bacteria was 10^2 CFU/g tissue in the SL groups and this increased to 10^3 and 10^5 CFU/g tissue in the PH and CBDL groups respectively, with the highest number of bacteria cultured in samples of the portal vein and liver in untreated animals with CBDL.

XO AND XD IN THE JEJUNUM

Assays of XO and XD in the jejunum showed significantly ($p < 0.01$) higher XO activity in untreated PH and CBDL animals than in the SL group (fig 1). As expected, in all tungsten supplemented animals, this treatment resulted in complete inactivation of XO and XD, and all measured XO and XD levels were zero. On histochemical examination, large amounts of the final reaction product of XO and XD were found in the epithelial cells of the villi and crypts of the jejunum, and low but distinct amounts in Goblet's cells (fig 2). The layers beneath the epithelial lining did not show any enzyme activity. In histological sections from tungsten supplemented animals, no XO and XD activity could be found, confirming the results obtained enzymatically.

Discussion

In patients with PH or obstructive jaundice, an incidence of infectious complications of between 8% and 25% has been reported in the literature.^{1 2 20} Spontaneous bacterial peritonitis,

cholangitis, bacteraemia, and sepsis account for death rates of up to 78%.^{1,2} Some 95% of these infections are caused by aerobic organisms normally present in the intestinal tract.²¹ However, in up to 30%, no infective focus in patients with severe sepsis could be identified.^{1,2,21} Recent studies have shown that PH and obstructive jaundice disrupt intestinal ecology and barrier function by producing structural changes in the bowel mucosa. The formation of portal systemic shunts, bacterial overgrowth resulting from the absence of intestinal bile flow, decreased bacterial clearance by the reticuloendothelial system, and impaired immunological defence are sequels to PH and/or cholestasis.^{3,22-26} These changes predispose to alterations in the intestinal ecology and the egress of bacteria from the intestinal tract to distant organs, a process termed BT. However, the exact mechanism leading to BT has not yet been clarified.

PH, either isolated or as a sequel to liver disease, is characterised by a pathological increase in portal pressure and the formation of a network of portosystemic collaterals, diverting the portal blood stream to the systemic circulation.^{6,27} PH further increases microvascular intestinal blood flow, but this is associated with a 41–51% decrease in intestinal arterial pressure, leading to intermittent intestinal mucosal hypoperfusion and abnormal tissue oxygenation.²⁸ In particular, the microcirculatory architecture of the villus characterised by a single arteriole that passes unbranched from the submucosal plexus to the tip of the villus may act to increase the susceptibility of the epithelium to hypoxic injury.²⁹ In such tissues, when intermittent hypoxia and reoxygenation is imposed, the enzyme XD can be converted into XO, and XO from the inactive to the active form.^{9,16,30}

XD and XO are ubiquitous cytoplasmic molybdenum-containing enzymes which belong biochemically to the group of hydroxylases.^{11,19} They exist in separate but interconvertible forms, and the liver and intestine have the highest XD/XO activity.⁹ Under physiological conditions, XD/XO is involved in the degradation of alkaline phosphatase to urate by converting hypoxanthine via xanthine into uric acid. XD and XO also oxidise endogenous purines and pyrimidines.³¹ Normally, the XD form accounts for 80–96% of the enzyme, and only 4–20% is in the XO form.^{19,32} Under various conditions, such as ischaemia-reperfusion or infection, increased conversion of XD into XO occurs. Like XD, XO also oxidises accumulated hypoxanthine, but produces as a byproduct reactive oxygen metabolites such as oxygen radical, superoxide anions, and hydrogen peroxide.⁹ These reactive oxygen species have cytotoxic effects resulting from peroxidation of lipid components of epithelial cells and mitochondrial membranes which have the highest content of polyunsaturated fatty acids.³³⁻³⁶ Further, these reactive oxygen species act as chemoattractants for polymorphonuclear neutrophils by inducing extravasation of neutrophils from the bloodstream into the tissue. These neutrophils probably worsen tissue injury because of their ability to release

toxic substances and activation of cytokines.^{37,38}

Many previous studies have documented that XD/XO inhibition or inactivation under various pathological conditions successfully attenuates the generation of reactive oxygen species, reducing the extent of tissue injury.^{17,33,39} We have shown in previous studies that competitive inhibition of XO with allopurinol in PH and after CBDL attenuated the incidence of BT, intestinal mucosal lipid peroxidation, and intestinal neutrophil derived myeloperoxidase activity.^{15,40}

In the present animal model of PH and CBDL, we particularly investigated the impact of intestinal XD and XO activities on intestinal bacterial colonisation and BT. In addition, we evaluated the effect of a tungsten supplemented molybdenum-free diet which has been reported to inactivate XD and XO.^{13,17}

To investigate possible changes in intestinal colonisation, we chose a segment of the jejunum and flushed it with sterile saline to remove non-adherent bacteria. Normally, this part of the intestine is sparsely populated with microbes, and it is believed that the magnitude of translocation is highly associated with bacterial density in the gut.⁴¹ Furthermore it has been suggested that attachment of bacteria is greater in the upper intestinal tract, and BT occurs to a slightly greater extent in the small intestine than in the colon.⁴ This could be, in part, because of a large surface area. PH and CBDL resulted in a significantly higher number of adherent bacteria in the jejunum but there was no difference in the type and number of bacteria cultured in the jejunum between normally fed and tungsten supplemented animals in this study. Whereas after CBDL the absence of intestinal bile, lack of secretory IgA, and impaired intestinal immunity are the possible causes of intestinal bacterial overgrowth, in PH the mechanisms promoting bacterial overgrowth are thought to be related to intestinal motor dysfunction and subsequent intestinal stasis.⁴²⁻⁴⁶ BT occurred after SL in 12% of normally fed animals and 8% of the tungsten supplemented group. Positive cultures were only found in mesenteric lymph nodes and spleen. These incidences correspond to previous findings of BT in normal animals, and it is believed that this phenomenon of BT to lymph nodes and spleen is a normal process whereby gut associated lymphoid tissue samples foreign antigens.⁴⁷ In PH in normally fed animals, a significant increase in BT was found in this study, indicating systemic invasion of enteric bacteria. In addition to bacteria in mesenteric lymph nodes and spleen, positive bacterial cultures could be obtained from the portal vein, vena cava, and liver.

Tungsten supplementation in PH resulted in lower incidences of BT without affecting the number of bacteria colonising the intestine. The same results for BT were found after CBDL, when nine of 10 untreated animals had a total of 54% of positive bacteriological cultures performed from the vena cava, portal vein, mesenteric lymph nodes, liver, and

spleen. In the tungsten supplemented CBDL group, only four of nine animals showed a total of 22% of positive bacteriological cultures. Similarly to the PH groups, treatment did not influence the extent of bacterial overgrowth which was significantly higher than in the SL groups.

These findings suggest that intestinal bacterial overgrowth per se cannot be the single cause of BT in PH and CBDL. However, significantly higher numbers of viable bacteria were recovered from mesenteric lymph nodes, spleen, and lung of animals in which bacteria were injected into the upper intestinal tract than in those that received injection into the colon.⁴ This suggests that the lower part of the gut has a more efficient mechanism for killing translocating bacteria than the relatively clean upper intestinal tract, and that an increase in microbes in the jejunum may highly predispose to BT.

Enzymatic and histochemical determination of XD and XO in the jejunum showed a significant increase in XO in normally fed animals with PH and CBDL, but no significant changes in XD when compared with the SL group. This indicates an increased conversion and/or activation of XO, possibly caused by altered perfusion of the intestine in PH and after CBDL. Whereas after SL, the percentage of jejunal XO to total XO/XD was 28%, it increased to 41% in PH and 44% after CBDL respectively. These results are in accord with reports in the literature, in which increased conversion of XD into XO under hypoxaemic conditions has been described.⁴⁸⁻⁵⁰ The tungsten supplemented molybdenum-free diet fed for 4 weeks completely inactivated XD and XO in normal, PH, and CBDL animals, and this was associated with significantly lower incidences of BT.

All these results were obtained in animal experiments, and it should be remembered that human XD/XO activities are relatively low and vary individually compared with values in animals.^{9,30,51-53} However, preliminary results from a clinical study in children, in which we investigated intestinal XO and XD activities in gut specimens resected for various congenital or acquired diseases, showed quite high levels of XD and XO that were comparable with activities found in animals. A possible explanation for the differences between the reported low levels of XD/XO in humans and our clinical study could be the fact that the reported levels of XD/XO were determined post mortem whereas we were able to harvest specimens during surgery.^{52,53}

Our findings support the thesis that bacterial species originating from the gut may be a significant source of infection in PH and obstructive jaundice. Inactivation of XD and XO by a tungsten supplemented diet resulted in a significant attenuation of BT in animals with PH and CBDL without influencing intestinal bacterial colonisation. Therefore high levels of XO in the intestine may contribute to intestinal barrier failure and promote BT by production of reactive oxygen species, activation of cytokines, and attraction of polymor-

phonuclear neutrophils. Furthermore, XO inactivation seems to also have a beneficial effect on liver function in chronic cholestasis. In the tungsten supplemented CBDL group, significantly lower levels of serum bilirubin, aspartate aminotransferase and alanine aminotransferase were present than in the untreated CBDL group. This suggests that XD and XO, heterogeneously distributed within the liver, may contribute to hepatocellular injury in chronic cholestasis.^{14,54}

However, the clinical relevance of this study remains speculative, firstly, because rats are very different with respect to the distribution of XO activity in the intestine, and secondly, because the side effects of long term XD/XO inactivation have not yet been investigated. Further studies on BT in humans with PH or chronic cholestasis are warranted, especially with regard to the role of XO and its inhibition.

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