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Patulin reduces glutathione level and enzyme activities in rat liver slices

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In the present study, an attempt was made to identify glutathione (GSH) adducts of patulin in precision-cut rat liver slices, which were used as a model system to study the metabolism and biological effects of this mycotoxin. Patulin disappeared in the slices but none of the GSH adducts, previously demonstrated in the chemical reaction of patulin with GSH, could be detected by HPLC. After incubation with various concentrations of patulin, a concentration-dependent decline of the GSH level was observed in the slices. For example, only 25% of the GSH of controls was found with 200 μM patulin. The activities of glutathione-*S*-transferase (GST) and of drug metabolizing phase I and phase II enzymes, assayed by the hydroxylation and conjugation of testosterone, were also reduced. On the other hand, incubation with patulin markedly increased lipid peroxidation in the slices. The effects of patulin on enzyme activities and lipid peroxidation may be a consequence of the GSH decline, which cannot be accounted for by a direct reaction of patulin with GSH due to the high concentration of GSH in hepatocytes. The decrease of GSH level and GST activity may be related to the putative mutagenic and carcinogenic potential of patulin.

Keywords: Glutathione / Glutathione-*S*-transferase / Liver slices / Patulin

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1 Introduction

Patulin is a mycotoxin produced by various species of the genera *Penicillium, Aspergillus*, and *Byssochlamys*. It is sometimes found in considerable concentrations in ripe apples and other fruits [1–3], and therefore constitutes an important contaminant of fruit products, such as apple juice and cider [4]. Patulin is believed to exert its toxic [5, 6], chromosome-damaging [7–9], mutagenic [10], and possibly carcinogenic [11, 12] activity mainly by covalent binding to cellular nucleophiles, in particular to the thiol groups of proteins and glutathione (GSH) [8, 12, 13]. Although it has long been known that patulin is able to react with GSH, the chemical structures of the GSH adducts of patulin have only recently been elucidated in our laboratory [14]. Figure 1 depicts the major reaction pathways and adduct structures. It was disclosed that up to three GSH molecules can react with one molecule of patulin. Whereas the adducts with two or three thiols have low or no further reactivity, the initial reaction of patulin with one thiol leads to an adduct which is even more reactive towards thiol and amino groups than patulin itself [15]. Therefore, reaction with GSH may cause both an increase and a decrease of the electrophilic reactivity of patulin.

In the present study, we have attempted to demonstrate the formation of GSH adducts of patulin under *in vivo*-like metabolic conditions. Precision-cut rat liver slices were chosen as *in vitro* system, because liver cells have high activities of various drug-metabolizing enzymes and a high content of GSH. Since hepatocytes remain in their natural environment in precision-cut liver slices, this *in vitro* technique closely mimicks the *in vivo* situation in the liver [16]. All three phases of metabolism, *i. e.*, functionalization, conjugation, and transport of conjugates, are continuously operative, and metabolites can be analyzed both in the incubation medium and in the slices. Moreover, the integrity of the tissue allows the assessment of biological effects elicited by the compound. Here, we report on the effects of patulin on the GSH content and lipid peroxidation as well as on the activities of glutathione-*S*-transferase (GST) and phase I and phase II enzymes.

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Abbreviations: GSH, glutathione; **LDH**, lactate dehydrogenase

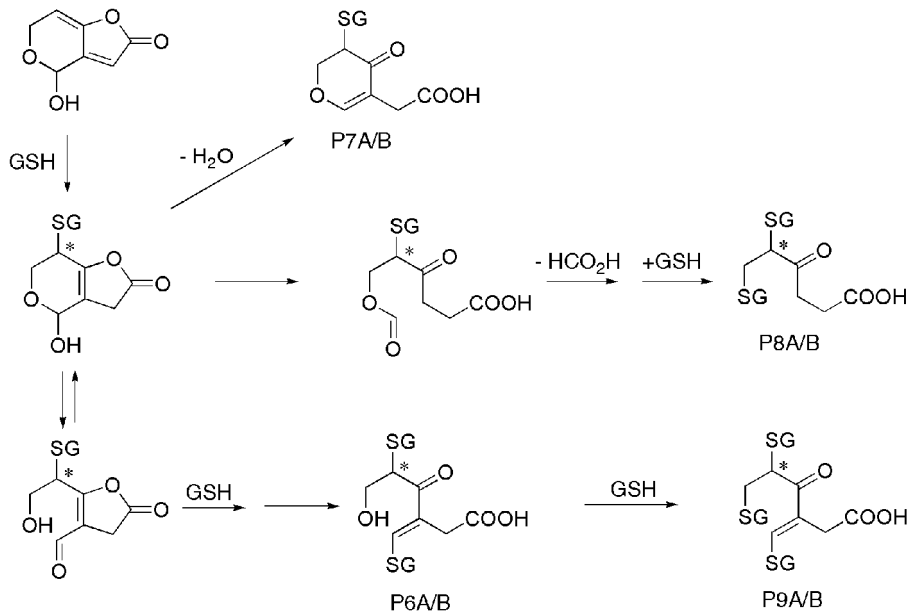

Figure 1. Major pathways of the reaction of patulin with GSH. The nomenclature of the adducts is according to Fliege and Metzler [14], the asterisks denote chiral carbon atoms.

2 Materials and methods

2.1 Chemicals and animals

Patulin was isolated from cultures of *Penicillium expansum* strain D19 (obtained from the Federal Research Centre for Nutrition and Food, Karlsruhe, Germany) as described previously [17] with a purity of >99% as determined by HPLC with diode-array detection and by gas chromatography-mass spectrometry after trimethylsilylation. All other chemicals were obtained from Sigma-Aldrich (Taufkirchen, Germany) and were of the highest purity available. Male Sprague-Dawley rats purchased from Harlan Winkelmann GmbH (Borchen, Germany) were maintained on a 12-h light/12-h dark cycle and had access to water and food *ad libitum*.

2.2 Chemical reaction of patulin with GSH

GSH (10 mM) was incubated with various concentrations of patulin (0.02, 0.2, and 2 mM) in a total volume of 1 mL 0.1 M potassium phosphate buffer, pH 7.4, at 37°C for 6 h and the reaction mixture analyzed by HPLC similar to the procedure described previously [14]. A HP1100 series HPLC equipped with a binary HPLC pump, a photodiode-array detector, and a HP Chem Station for data collection and handling was used (Waldbronn, Germany). Separation was carried out on a reversed-phase Prodigy 5ODS(2) column (250 mm × 4.6 mm ID, particle size, 5 µm). Eluents were (A) distilled water adjusted to pH 3 with formic acid and (B) 15% acetonitrile in distilled water with 0.5 mL/L formic acid, with a flow rate of 1 mL/min. The gradient changed linearly from 5% B to 10% B during the first 20 min and then to 100% B over the next 40 min. The diode-array detector was set to 275 and 300 nm. Under these conditions, the retention time of patulin was 24.5 min and the GSH adducts of patulin eluted between 30 and 45 min.

2.3 Preparation and incubation of cell fractions

Microsomes and cytosol were prepared from the livers of untreated male Sprague-Dawley rats according to the method of Lake [18]. Protein concentration was determined by the method of Bradford [19] using bovine serum albumin as standard. Incubations of rat liver cell fractions were carried out in a total volume of 1 mL 0.1 M potassium phosphate buffer, pH 7.4, at 37°C for 40 min in the presence and absence of the appropriate cofactors. Concentrations were 0.1 mM for patulin (dissolved in DMSO, final DMSO concentration, 1%), 1 mg/mL for microsomal or cytosolic protein, 4 mM for UDPGA, and 10 mM for NADH. For oxidative metabolism, a NADPH-generating system (0.5 U isocitrate dehydrogenase, 9.4 mM isocitrate, 1.2 mM $NADP^+$, and 4.3 mM $MgCl_2$) was used. In some experiments with microsomes, 10 mM GSH was added after the initial incubation and HPLC analysis conducted after an additional 40 min at 37°C. The same HPLC conditions as described in Section 2.2 were used, with patulin eluting after 24.5 min and UDPGA after 6.2 min. The activity of the microsomes for oxidative metabolism and for glucuronidation was demonstrated with the model compounds testosterone and umbelliferone, respectively.

2.4 Preparation and incubation of liver slices

Precision-cut tissue slices were prepared from the livers of three untreated male Sprague-Dawley rats as described previously [20]. Rats were killed by cervical dislocation and their livers immediately placed in cold Krebs-Henseleit buffer (4°C, pH 7.4). Tissue cylinders were prepared by slowly turning and advancing a sharpened metal tube of 8 mm diameter into the liver lobes which were spread out on a wax support. Tissue slices of about 200 μm were prepared from the individual cylinders, using a mechanical tissue slicer (Vitron, Tucson, AZ, USA). The slicer was operated with ice-cold oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit buffer. Up to 50 slices are obtained from one liver. The time between removal of the liver and start of the incubations must not exceed 60 min in order to maintain high viability of the slices. Incubations of slices were carried out according to the method of Fisher *et al.* [21]. Briefly, slices floating in Krebs-Henseleit buffer were placed onto stainless-steel mesh half cylinders (one slice per cylinder) and loaded horizontally into glass scintillation vials containing 1.7 mL Waymouth's medium, supplemented with 10% fetal calf serum and gentamycin (84 μg/mL). Vials were closed with plastic-lined caps with a 2-mm hole in the center and were placed in a dynamic roller culture incubator at 37°C. The vials rotated in the incubator, constantly exposing both surfaces of the slice to gas and liquid throughout the culture period. After a preincubation period of 1–2 h, the roller inserts with the slices were loaded into vials containing fresh medium with the test compounds dissolved in DMSO (final DMSO concentration, 1%) and further incubated for 6 h. This incubation time was chosen because pilot studies had shown that the effects of patulin (see below) were more pronounced than after 10 or 24 h. Thirty slices of each rat liver were used for the various experiments: six slices each were incubated with 0, 50, 100, and 200 μM patulin to study the formation of GSH adducts (see Section 2.5) and the effects on GSH content (Section 2.6), lactate dehydrogenase (LHD) activity (Section 2.7), GST activity (Section 2.8) and lipid peroxidation (Section 2.10). Pilot studies had shown that patulin concentrations of 10 and 20 μM were without effects, whereas 500 μM patulin was too toxic. For the testosterone assay (2.9), three slices were incubated with 200 μM testosterone alone and three with 200 μM testosterone plus 200 μM patulin. Incubations were also conducted without liver slices. After incubation, the slices and the medium were separately stored at −80°C until analyzed.

2.5 GSH adducts

Three of the six slices used for each concentration of patulin were combined and homogenized in 300 μL of 0.1 M phosphate buffer, pH 6.5, at 4°C. Proteins were precipitated with 60 μL of cold 15% aqueous trichloroacetic acid, centrifuged, and the supernatant was freeze-dried and redissolved in 20 μL phosphate buffer. The complete sample was then injected and analyzed by HPLC for the presence of GSH adducts, using the conditions described in Section 2.2.

2.6 GSH content

The GSH content and the activities of LDH and GST were determined in the homogenate of each of the three remaining slices. Each slice was homogenized individually in 0.2 mL of ice-cold 0.1 M phosphate buffer, pH 6.5. 100 μL of the homogenate was used for measuring LDH and GST activity (see Sections 2.7 and 2.8). The other 100 μL were mixed with 20 μL cold 15% aqueous trichloroacetic acid and then centrifuged to sediment the precipitated proteins prior to the determination of reduced GSH, which was measured using an adaptation of the method of Ellman [22]: 50 μL of the supernatant were mixed with 200 μL Ellman's reagent (39.6 mg 5,5′-dithiobis-(2-nitrobenzoic acid) in 10 mL ethanol, followed by 1 : 10 dilution with 0.5 M Tris/0.01 M EDTA buffer, pH 8.9) in a microtiter plate. Absorbances at 405 nm were read within 5 min and concentrations were calculated using GSH as standard. Although other small thiol-containing molecules, *e.g.*, cysteine, are also measured by Ellman's reagent in the supernatant of the precipitated proteins, their amount is minute in comparison with GSH and can therefore be neglected. The GSH content of cell-free incubations was measured by the same method as used for the supernatants of slice homogenates.

2.7 LDH activity

A 50 μL aliquot of the slice homogenate or of the incubation medium was added to 0.1 M phosphate buffer, pH 7.0, containing 1 mM pyruvate and 0.2 mM NADH in a total volume of 0.5 mL. NADH was measured by its absorbance at 340 nm and the linear decrease of NADH concentration used to calculate the LDH activity. Cytotoxicity was expressed as percent of LDH released into the incubation medium.

2.8 GST activity

GST activity was determined according to the method of Habig *et al.* [23]. A 50 μL aliquot of the slice homogenate was added to 0.1 M phosphate buffer, pH 6.5, containing 1 mM GSH and 1 mM 1-chloro-2,4-dinitrobenzene in a total volume of 0.5 mL. Dilutions leading to a linear increase of the reaction product measured at 340 nm were used to calculate the enzyme activity.

2.9 Testosterone assay

The influence of patulin on the hydroxylation and conjugation of testosterone in the liver slices was assessed as described earlier for other compounds [20]. Briefly, three slices from each of three livers were incubated for 6 h with 200 μM testosterone and another three slices with 200 μM testosterone plus 200 μM patulin. The three slices were homogenized together in 1 mL 0.1 M potassium phosphate buffer, pH 7.4. For the analysis of unconjugated metabolites, 0.5 mL aliquots of the homogenate or of the pooled incubation medium were extracted twice with 0.5 mL each of cold ethylacetate, the residue of the extract dissolved in 40 μL methanol and 10 μL analyzed by HPLC (see below). For the analysis of total (*i. e.*, unconjugated plus conjugated) metabolites, another 0.5 mL aliquot of the homogenate was mixed with 200 μL 0.15 M acetate buffer, pH 5.0, and incubated with 10 μL of a β-glucuronidase/sulfatase preparation from *Helix pomatia* at 37°C for 16 h prior to extraction with ethylacetate. The amount of conjugates was calculated as the difference between total and unconjugated metabolites. HPLC analysis was conducted on the same column as described in Section 2.2 with the following eluents: (A) distilled water adjusted to pH 3.0 with formic acid, and (B) acetonitrile. The flow rate was 1.0 mL/min and the gradient changed in a linear manner from 20% B to 75% B within 35 min and subsequently to 100% B in 6 min. Absorbance of the eluent was monitored at 240 and 280 nm. Under these conditions, patulin eluted after 5.5 min and testosterone after 21.9 min, whereas the hydroxylation products of testosterone eluted between 6.5 and 18.5 min [20]. Quantification was based on the peak areas, assuming the same absorption coefficient for testosterone and the metabolites.

2.10 Lipid peroxidation

Lipid peroxidation was measured as the release of 2-thiobarbituric acid-reactive substances into the culture medium using a modified method of Fraga *et al.* [24]. Assays were carried out in a total volume of 0.86 mL containing an aliquot of 0.1 mL of the incubation medium, 0.1 mL 3% aqueous sodium dodecyl sulfate, 0.4 mL 0.1 N aqueous HCl, 0.06 mL 10% aqueous phosphotungstic acid, and 0.2 mL 0.7% aqueous 2-thiobarbituric acid. The mixture was heated for 45 min at 100°C, cooled on ice, and extracted with 1 mL *n*-butanol. The organic extract was measured fluorometrically (excitation at 515 nm and emission at 550 nm) using 1,1,3,3-tetramethoxypropane as standard.

2.11 Statistics

Differences between treatments were analyzed statistically by using Student's *t*-statistic for unpaired data. Data are given as mean ± standard deviation.

3 Results

3.1 Incubation of patulin with GSH and subcellular fractions

When 2 mM patulin was incubated with 10 mM GSH as decribed in Section 2.2 and then analyzed by HPLC, 14 reaction products were observed. The major products were cochromatographed with the authentic reference compounds from the study of Fliege and Metzler [14] and identified as products P6A, 6B, 7A, 7B, 8A, and 8B (Fig. 1). A and B designate diastereomeric forms of the same reaction product. When 0.02 mM patulin was incubated with 10 mM GSH under the same conditions, the major products detected were P6A/B and P9A/B. Unchanged patulin was not found in either incubation. When the concentration of GSH was determined in the same incubations and another one with 0.2 mM patulin, no decrease of GSH could be detected with patulin concentrations of 0.02 and 0.2 mM, whereas 2 mM patulin reduced the concentration of GSH by 3.3 mM. The results of these experiments confirm the high reactivity of patulin towards GSH leading to adducts with up to three GSH per patulin as reported earlier [14].

In order to clarify whether patulin is prone to hydroxylation or to conjugation with glucuronic acid, patulin was incubated with microsomes from rat liver in the presence of the corresponding cofactors and the incubation mixtures were analyzed by HPLC (see Section 2.3). Neither oxidative metabolites nor glucuronides but near-quantitative amounts of unchanged patulin were detected. When GSH was added to microsomal incubations of patulin after 40 min, the same pattern of GSH adducts was formed as in buffer alone and described above. In contrast to the results obtained with microsomes, neither patulin nor any new products were detectable by HPLC in incubations of patulin with cytosol. The complete disappearance of patulin was observed both in the presence and absence of NADH.

3.2 Incubation of patulin with liver slices

Concentrations of 0, 50, 100, and 200 μM patulin were incubated with rat liver slices, and the formation of GSH adducts as well as the effects of patulin on GSH level, various enzyme activities, and lipid peroxidation were studied. Three liver slices each from three different rats were used for each endpoint.

3.2.1 Failure to find GSH adducts of patulin

When the entire samples obtained from three pooled liver slices after incubation with patulin, homogenization and protein precipitation were analyzed by HPLC without extraction, neither GSH adducts of patulin nor unchanged patulin could be detected. When the corresponding incuba-

tion media were extracted with ethylacetate, 2 – 6% of the patulin incubated with the slices was found unchanged in the extract, and no GSH adducts were observed in the extracted medium. In control incubations of patulin without slices, 40 – 50% of the applied patulin was recovered unchanged in the extract. Experiments with patulin and the reference adducts P7B and P9B showed that the extraction of patulin into ethylacetate was virtually quantitative, whereas adducts remained completely in the aqueous media. The limit of detection of the adducts was 1 nmol/mL medium.

3.2.2 Effects of patulin on the release of LDH, on GSH level, and on the activity of GST

The activities of LDH and GST as well as the GSH content were determined in the homogenate of the liver slices, whereas LDH was also measured in the incubation medium. Intact hepatocytes do not release LDH to any significant extent. Therefore, the LDH activity in the culture medium reflects the leakage from hepatocytes and can be used as a marker for cytotoxicity. No increased release of LDH was noted with 50 μM patulin, whereas 200 μM patulin caused a leakage of LDH of about 16% as compared with controls (Fig. 2).

In order to correct for the variation of the different slices and also for the cytotoxic effects of patulin, the measured values of the GSH concentrations and GST activities were related to the LDH activity of each slice and expressed as percent of control incubations without patulin. These standardized values are depicted in Figs. 3 and 4. Increasing concentrations of patulin caused a marked and concentration-dependent decrease of the GSH level in the slices (Fig. 3). The decline of GSH was about 20% with 50 μM

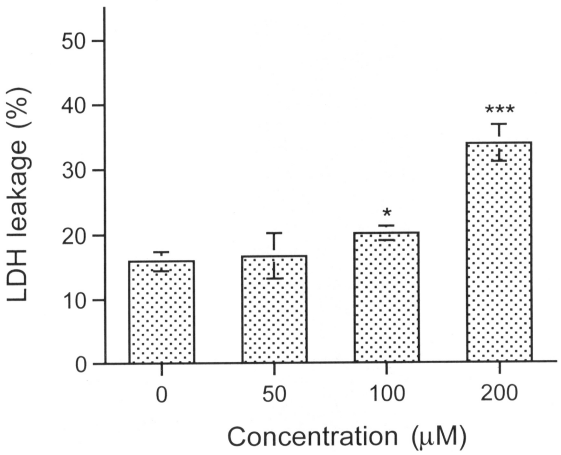

Figure 2. Cytotoxicity of patulin in rat liver slices after 6 h incubation and determined as release of LDH into the incubation medium. Data represent the mean ± standard deviation of three rats and three independently incubated slices from each rat. Level of significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

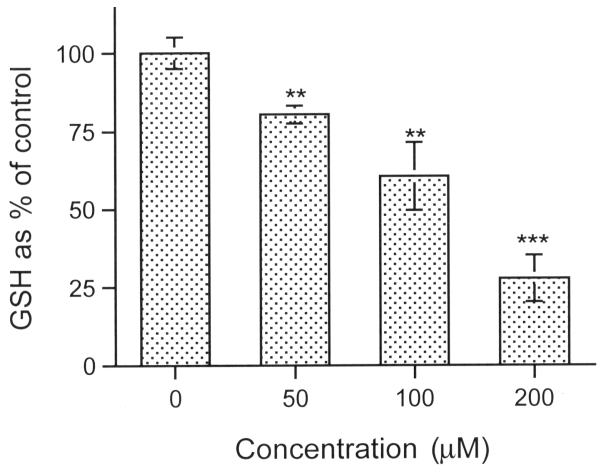

Figure 3. GSH content of rat liver slices after 6 h incubation with patulin. The GSH content was standardized for the LDH content of the respective slice. Data represent the mean ± standard deviation of three rats and three independently incubated slices from each rat. Level of significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

patulin and 75% with 200 μM patulin. A concentration-dependent decrease was also noted for the activity of GST with increasing patulin concentration (Fig. 4). The decrease was not statistically significant when the average of the three experiments was calculated (Fig. 4), but was significant within each experiment (data not shown).

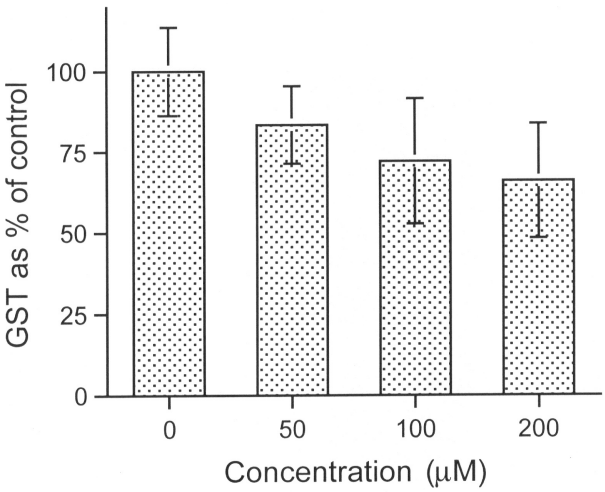

Figure 4. Activity of GST in rat liver slices after 6 h incubation with patulin. The GST activity was standardized for the LDH content of the respective slice. Data represent the mean ± standard deviation of three rats and three independently incubated slices from each rat.

3.2.3 Effect of patulin on the hydroxylation and conjugation of testosterone

Testosterone is hydroxylated by various forms of cytochrome P450 in a regio- and stereospecific manner [25].

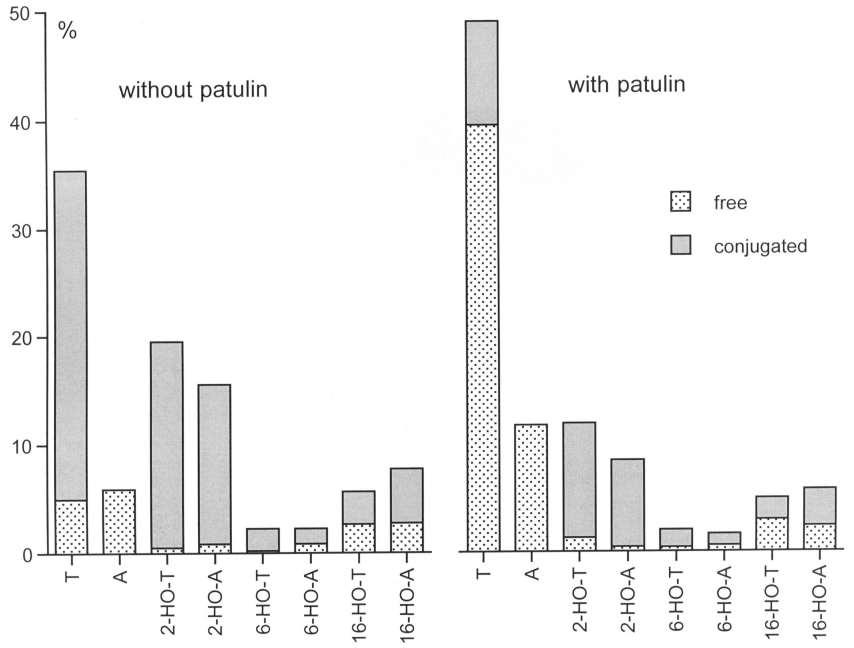

Figure 5. Pattern of unconjugated (stippled bars) and conjugated (shaded bars) testosterone metabolites in rat liver slices incubated for 6 h in the absence (left chart) and presence (right chart) of patulin. Data are expressed as % of the sum of all detected compounds and represent the mean of three experiments with different rat livers. T, testosterone; A, androstenedione; HO, hydroxy.

We have demonstrated in previous studies that testosterone is oxidized to androstenedione and both steroids are preferentially hydroxylated at positions 2α, 16α, and 6β in liver slices from male Sprague-Dawley rats [20]. Furthermore, testosterone and its metabolites are conjugated by phase II enzymes in the slices [20].

In the present study, the effect of patulin on the metabolism of testosterone was determined in liver slices. The pattern of oxidative testosterone metabolites was determined by HPLC in the slices as well as in the media. The extent of conjugation was calculated by measuring the metabolites prior to and after enzymatic hydrolysis of conjugates. In incubations without and with patulin, about 96% of the testosterone-related material were found in the medium and 4% in the slices. In the medium, oxidative testosterone metabolites were decreased from 31 to 19% and conjugation products from 27 to 16% in the presence of patulin. This suggests that patulin inhibits both the oxidation and the conjugation of testosterone. This effect was even more obvious when the slices were analyzed in detail (Fig. 5). Comparison of the pattern of oxidative testosterone metabolites formed in the absence and presence of patulin showed that all hydroxylation products were decreased by patulin while testosterone and androstenedione were increased. Interestingly, conjugation of testosterone and, to a lesser extent, of hydroxylated testosterone metabolites was more affected by patulin than conjugation of hydroxylated androstenedione metabolites (Fig. 5).

3.2.4 Effect of patulin on lipid peroxidation

The extent of lipid peroxidation in liver slices was determined by measuring the 2-thiobarbituric acid-reactive substances released into the incubation medium. Patulin caused a concentration-dependent increase in lipid peroxidation, which was already clearly observable with 50 μM patulin (Fig. 6A). With 200 μM patulin, the extent of lipid peroxidation was the same as with 1 mM *tert*-butylhydroperoxide used as positive control (Fig. 6A). In slices which did no longer contain viable cells due to storage at −80°C for prolonged time, lipid peroxidation could only be induced by *tert*-butylhydroperoxide but not patulin (Fig. 6B). This indicates that *tert*-butylhydroperoxide acts directly on the lipid membranes whereas patulin has an indirect effect requiring viable cells.

4 Discussion

The main objective of this study was to clarify the fate of the mycotoxin patulin in precision-cut rat liver slices. Of particular interest was the question whether GSH adducts of patulin, previously identified when patulin reacts with GSH under cell-free conditions, can also be demonstrated in rat liver slices. This *in vitro* technique closely mimicks the metabolic *in vivo* situation in the liver [16], because hepatocytes remain in their natural environment and maintain high activities of drug-metabolizing enzymes and a high intracellular concentration of GSH over prolonged periods of time. Therefore, all three phases of metabolism, *i.e.*, functionalization, conjugation, and transport of conjugates, are continuously operative, and metabolites can be excreted into the incubation medium.

Neither GSH adducts nor any oxidative or reductive metabolites nor glucuronides of patulin were detected in the

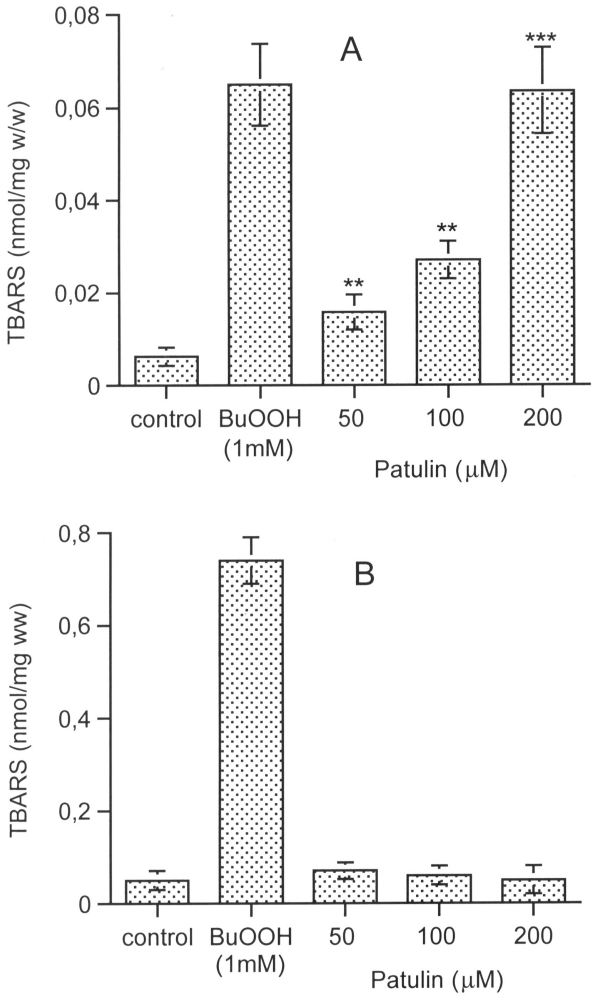

Figure 6. Lipid peroxidation induced by patulin (A) in viable and (B) in dead rat liver slices and measured as 2-thiobarbituric acid-reactive substances (TBARS) released into the incubation medium. Viable slices were incubated for 6 h in medium and dead slices for 2 h in 0.1 M phosphate buffer pH 7.4. Data represent the mean ± standard deviation of three experiments (see text for details). BuOOH, *tert*-butylhydroperoxide, used as positive control. Level of significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

medium and in the slices. No unchanged patulin was found in the slices and less than 10% in the medium. Thus, patulin is degraded in liver slices but the degradation products remain unknown. It is conceivable that GSH adducts were formed initially but were further metabolized or otherwise degraded, *e.g.*, by reaction with cellular thiol or amino groups [15, 17]. From our experimental conditions, it is estimated that patulin itself or its GSH adducts would have been detectable if patulin had reached concentrations of at least 10 μM in the slices. A similar disappearance of patulin was reported by Rychlik [26], who incubated 100 μg patulin with 9 mL of blood and recovered only 6% of the administered patulin after 2 min. The failure to find metabolites of patulin in liver slices was consistent with the observation that patulin was not metabolized by hepatic microsomes.

Patulin had a marked and concentration-dependent effect on the GSH content and on various enzyme activities in liver slices. For example, the GSH level in patulin-treated slices decreased to about 25% of that in untreated slices. Normalizing the GSH level to the activity of LDH in the slices ensured that cytotoxicity was accounted for. Likewise, the activity of GST and the hydroxylation and conjugation of the model compound testosterone were diminished in liver slices after treatment with patulin in a concentration-dependent manner. Since the high level of intracellular GSH is commonly considered as an important protection against the oxidation of protein thiol groups, the partial inhibition of GST and of the testosterone-metabolizing enzymes, *i.e.*, cytochrome P450 and glucuronosyltransferase, may be a consequence of the depletion of GSH by patulin. The lower GSH level could also explain the observed increase of lipid peroxidation in patulin-treated slices, which occurs only in viable slices and is therefore not due to a direct interaction of patulin with cell membranes.

The mechanism of the depletion of intracellular GSH caused by patulin in rat liver slices is presently unkown. Since the GSH concentration in the liver is about 10 mM, a direct reaction of patulin with GSH cannot account for the GSH decrease, which was much larger than would be expected from the stoichiometry of the reaction. Therefore, other mechanisms must be involved. Several studies on the cytotoxicity of patulin have disclosed alterations of the function of cell membranes. In addition to the inhibition of Na^+/K^+-ATPase [27], patulin caused an increased intracellular electronegativity as the result of a selective increase in plasma membrane permeability to K^+ and a net efflux of positive charge [28]. This K^+ efflux could be due to oxidation of critical membrane sulfhydryls. Reglinski *et al.* [29] used Ellman's reagent to induce an oxidative stimulus on the exofacial membrane sulfhydryl groups, which was followed by a depletion of intracellular GSH and other thiols. A mechanism was proposed linking oxidation of exofacial sulfhydryls associated with transport proteins to endofacial sulfhydryls associated with enzymes, and it was further hypothesized that this leads to depletion of cytosolic thiols [29]. The ability of patulin to disturb plasma membrane function is consistent with later work by Arafat and Musa [30], who showed that patulin induced inhibition of protein synthesis by two mechanisms, *i.e.*, inhibition of amino acid uptake into the cells and their incorporation into proteins. It would be interesting in future studies to examine the effect of antioxidants on the patulin-mediated depletion of GSH.

In conclusion, our study has demonstrated that noncytotoxic concentrations of patulin give rise to a pronounced depletion of intracellular GSH in rat liver slices and inhibit

various drug-metabolizing enzymes. These effects may not only represent early events in the cytotoxic action of patulin but may also be of relevance for the putative carcinogenic and genotoxic activity of this mycotoxin, because depletion of GSH and compromized detoxification of other carcinogens must be expected to increase DNA damage due to oxidative stress and adduct formation.

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