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Sensitivity of taurine uptake to oxygen-derived reactive substances in MDR and non-MDR cells

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Summary. In human KB and LoVo cell lines, high affinity taurine uptake was strongly reduced in both a time and dose-dependent manner by cumene hydroperoxide (CH) and to a lesser extent by hydrogen peroxide (H_2O_2). Uptake-inhibition was greater in multidrug resistant (MDR) cells than in their non-MDR counterparts. Basal taurine efflux was unaffected by the oxidants. Lipid peroxidation levels closely correlated with the uptake inhibition levels, and were greater in MDR cells than in their non-MDR counterparts. The two oxidants reduced the Vmax and, to a lesser extent, the affinity of the transporter for taurine. They also reduced low affinity taurine uptake and, to a lesser extent, taurine diffusion. The composition of the medium used for cell treatment, especially its pyruvate content, greatly affected the H_2O_2 effect. H_2O_2- or CH-induced reduction of the high affinity taurine uptake was unaffected by protein kinase C (PKC) inhibitors and by the calmodulin antagonist W-13, ruling out the involvement of PKC and perhaps of calmodulin kinases in their effect.

Keywords: Amino acids – Taurine uptake – MDR cells – Hydrogen peroxide – Cumene hydroperoxide – Malondialdehyde – Lipid peroxidation – Pyruvate – PKC – Calmodulin kinases

Abbreviations: CH, cumene hydroperoxide; CIM, CO_2 independent medium, Gibco; FCS, foetal calf serum; H_2O_2, hydrogen peroxide; 4-HNE, 4-hydroxynonenal; MDA, malondialdehyde; MDR, multidrug resistance; PKC, protein kinase C; ROS, reactive oxygen-derived substances

Introduction

Mammalian cells and tissues are protected from the deleterious consequences of oxidative stress by complex systems of enzymes and antioxidants (Packer, 1994; Chaudiere and Ferrari-Liou, 1999). Reactive oxygen species (ROS) affect these various protective systems differently. Depending on the

tissue and the oxidant tested, an increase or an inhibition of the activity of superoxide dismutase, catalase or glutathione peroxidase (Lai et al., 1996; Pinteaux et al., 1996; Röhrdanz and Kahl, 1998) with an increase in ROS levels, has been observed, whereas vitamin E levels were slightly decreased (Li et al., 1994; Vaage et al., 1997).

Concentration of taurine (2-aminoethane sulfonic acid), an ubiquitous sulfonic β-aminoacid), is notably higher (up to the millimolar range or more) in tissues subjected to oxidative stress (such as brain, retina, heart, lung, intestine, white blood cells, . . .) than in cells weakly sensitive to such stress such as fibroblasts (Jacobsen and Smith, 1968; Fukuda et al., 1982; Chesney, 1987; Huxtable, 1992). Recent results have shown that taurine behaves like an antioxidant (Pasantes-Morales et al., 1985; Aruoma et al., 1988; Koyama et al., 1992; Gordon et al., 1992; Banks et al., 1992; Kaplan et al., 1993; Ogasawara et al., 1993; Cozzi et al., 1995; Venkatesan et al., 1997; Shi et al., 1997; You and Chang, 1998a and b; Dawson et al., 1998; Boldyrev et al., 1999). In particular, taurine protects cardiac cells against anthracyclins toxicity (Hamaguchi et al., 1988 and 1989) which is due to the formation of free radicals by this drug (Winterbourn, 1981; Hodnick and Sartorelli, 1994; Yee and Pritsos, 1997). The cell taurine concentration depends on its uptake and efflux. Taurine uptake involves transporter(s) and diffusion. The activity of some transporters or ions channels is decreased by ROS (reviewed in Kourie, 1998). To the best of our knowledge, no study has addressed the question of the effect of oxidative stress on taurine transport. We have therefore studied the effect of two different oxidants, hydrogen peroxide (H_2O_2) and cumene hydroperoxide (CH), on taurine uptake by cultured human tumour cells. Clinical cancer treatments show the existence of two types of tumours: those which share acquired or intrinsic resistance to the cytotoxicity of various chemically and structurally unrelated chemotherapeutic agents such as anthracyclines, *vinca*-alcaloïds, taxans, and others (multidrug resistance or MDR phenotype), and those which are sensitive to these chemotherapeutic drugs (non-MDR). Effect of H_2O_2 and CH on taurine uptake was therefore studied on MDR cells and their respective non-MDR counterparts.

Materials and methods

Chemicals and culture media

[^{3}H] Taurine (NET 541, 29 Ci/mmol) was from Dupont NEN and unlabeled taurine from Janssen Chimica. Vinblastine, doxorubicin, cumene hydroperoxide (CH), hydrogen peroxide (H_2O_2), sodium pyruvate and hexadecylphosphocholine were purchased from Sigma. Chelerythrine and N-(4-aminobutyl)-5-chloro-1-naphtalenesulfonamide hydrochloride (W-13) were obtained from Calbiochem, whereas Bioxytech® LPO-586™ was from Oxis International, Inc (Portland, USA). All other chemicals were of analytical grade. DMEM/Ham F12 (Dulbecco modified Eagle medium/Ham F12 nutrient mixture (1/1), F-4815) was purchased from Seromed, RPMI 1640 (R 7755) from Sigma, CO_2 independent medium (CIM, 18045-054) from Gibco BRL and foetal calf serum (FCS) from Dutscher (Bischwiller, France). Osmolarities of the different media, measured with a Roebling osmometer, were in the physiological range (290–315 mOsm).

Cell lines

Two types of human cells were used: KB and LoVo cell lines. The KB-3-1 cell line was originally established from a human oral carcinoma. The multidrug resistant (MDR) KB-V-1 cell line was derived from the KB-3-1 cells by exposure to increased doses of vinblastine up to 1 μg/ml (Akiyama et al., 1985). KB-V-1 cells were further stepwise selected in our laboratory by resistance to 0.2-μg/ml doxorubicin in the presence of 1 μg/ml vinblastine. KB-3-1 and KB-V-1 cell lines were kindly provided by Dr. M. M. Gottesman (NCI – NIH Bethesda MD – USA). The LoVo cells, derived from a supraclavicular metastasis of a colon carcinoma, were obtained from Pharmacia & Upjohn Cie (Milano, Italy). Two drug-sensitive variants were selected in our laboratory from the LoVo parental cell line, which differed in morphology. These were named "LoVo S fusoid" and "LoVo S small cell" variants. Preliminary results suggest that these two non-MDR LoVo variants differ in their degree of differentiation (Lelong-Rebel et al., in preparation). The MDR LoVo D/X cell, derived from the original LoVo cells by treatment with increasing doses of doxorubicin up to 0.1 μg/ml (Grandi et al., 1986), were further selected in our laboratory with up to 0.4 μg/ml doxorubicin, and were named LoVo Dox. The three LoVo cell lines contain the P-glycoprotein (P-gp or PGY1), the multidrug resistance associated protein (MRP) and the lung resistance protein (LRP). The three proteins are localised differently in the MDR LoVo Dox and the two non-MDR LoVo cell lines (Lelong-Rebel et al., in preparation).

Cell culture

KB and LoVo cell lines were grown as monolayer cultures in 75 cm^2 vented flasks (Costar n° 3,376) at 37°C in a 5% CO_2 atmosphere in DMEM/HamF12 supplemented with a mixture of 50 U/ml penicillin and 50 μg/ml streptomycin, 5% (v/v) heat inactivated selected FCS, 365 mg/l L-glutamine and 55 mg/l sodium pyruvate (1 mM final concentration). KB-V-1 culture medium also contained 1 μg/ml vinblastine and 0.2 μg/ml doxorubicin, and LoVo Dox culture medium 0.4 μg/ml doxorubicin. Cells were passaged at 3/4 confluence. Mycoplasmas were routinely checked using Hoechst 33258.

For experiments, cells were seeded in 35 mm Petri dishes (Costar n° 3,035) and grown in 2 ml of their respective culture media up to confluence. Media were changed every other day. The last change was made 24 to 48 h before taurine uptake measurements.

Cell viability was assessed by Trypan blue dye exclusion, or by the MTT test as described by Lelong and Rebel (1998a). Osmolarities of all the media used for culture and experiments were checked with a Roebling osmometer.

Oxidative stress

Nearly confluent cultures were washed twice with DMEM/Ham F12 and then maintained for 24 hours in 2 ml of DMEM/Ham F12 (without vinblastine and/or doxorubicin) with or without 5% (v/v) FCS (*treatment medium*). Oxidative stress was induced by addition to the treatment medium of H_2O_2 or cumene hydroperoxide (CH). 500-mM stock solutions of H_2O_2 and CH were diluted with water and ethanol respectively just before use, then added to the culture media to obtain various concentrations between 0 and 1 mM. H_2O_2 stock solutions were prepared extemporarly from the commercial solution carrefully kept at +4°C in absence of light. Then, cells were maintained for various times in a 5% CO_2 incubator. When the effect of the oxidants was studied using PBS as *treatment medium* (137 mM NaCl, 2.70 mM KCl, 0.50 mM $MgCl_2$ ($6H_2O$), 0.68 mM $CaCl_2$ (2 H_2O), 6.46 mM Na_2HPO_4 (2 H_2O) and 1.47 mM KH_2PO_4), nearly confluent cultures were washed twice with PBS (at 37°C). Then, 2 ml of warm PBS containing the oxidants was added and cells were maintained at 37°C for various times in an oven. Whatever the treatment medium used, oxidative stress was stopped by washing the cells twice with the *incubation medium*

used for taurine uptake measurement. Control cells were treated with an equal concentration of the solvents (H_2O or ethanol). In all experiments, the final concentration of solvents never exceeded 0.2%.

Taurine uptake measurements

High-affinity taurine uptake was measured as described by Lelong and Rebel (1998b). Briefly, oxidatively stressed cells were preincubated for 5 min in a rotary water bath at 37°C in 3 ml of *incubation medium*, which was CIM except when otherwise stated. Then, 100 μl of CIM containing a mixture of 0.4 μCi [^{3}H] taurine and 1.33 μM of unlabeled taurine were added to the incubation medium and cells were incubated for 10 min. Then, medium was removed, cells were washed three times with ice cold NaCl 0.9% and frozen on their Petri dishes. After addition of 1 ml of NaOH 0.1 N, dishes were left for 1 h at 37°C, cultures were homogenized and aliquots taken for protein determination according to Lowry et al. (1951) and for radioactivity measurements by scintillation counting with Aquasol (Packard, Nef 934-G). Previous analysis have shown that low affinity uptake and diffusion were the main uptake systems when KB or LoVo cells were incubated in CIM containing 200 μM or 2 mM (instead of 1.33 μM) unlabeled taurine respectively (Wersinger et al., 2000, and submitted). Similar incubation conditions were used when these uptake systems were checked. Then, cells were proceeded as indicated for the high-affinity uptake.

Taurine efflux measurements

Confluent cultures grown on Petri dishes were loaded in their culture media with 0.4 μCi [^{3}H] taurine for 4 h. Cells were then treated as follows: a first set of dishes was washed three times with ice cold NaCl 0.9% then frozen (control); other sets were washed three times with 3 ml of CIM, incubated for 10 min in 3 ml of CIM, then washed three times with ice cold NaCl 0.9% and frozen. Cells were homogenised in 1 ml of NaOH 0.1 N. Aliquots were removed for radioactivity and protein determination. The efflux data were calculated as the differences (in %) between the radioactivity per mg of protein in control cells, versus the incubated cells.

Determination of lipid peroxidation generated by H_2O_2 and cumene hydroperoxide (CH)

Fluorescent chromolipid levels were determined, according to Goto et al. (1992) with slight modifications. Briefly, high density cells grown on 60 mm Petri dishes were treated with H_2O_2 or CH as described before. After oxidative stress, cells were washed twice with ice cold NaCl 0.9%. Then, Petri dishes were placed on liquid nitrogen for 1 min according to Demediuk et al. (1985) to avoid artefactual production of lipid peroxides. Dishes were then kept at −80°C until peroxide determination. After addition of 2 ml of sterile double distilled water to each Petri dish, cells were sequentially frozen at −20°C for 1 hour, thawed for 1 hour at 37°C, then frozen again for 1 hour at −20°C. Thereafter, cells were thawed at room temperature. Following homogenisation by pipetting, an aliquot was taken for protein determination according to Lowry et al. (1951) and 1 ml of cell lysate was extracted with 4 ml of chloroform/methanol (2/1). About 5 ml of water was then added and the mixture centrifuged at 200 g for 10 min at 4°C. The fluorescence emissions of both the aqueous and chloroform/methanol layers were determined using 360-nm excitation and 430-nm emission according to Dillard and Tappel (1984). Blank fluorescence emissions obtained by extraction of 1 ml water with 4 ml chloroform/methanol were substracted. In parallel, lipid peroxidation end-products malondialdehyde (MDA)

and 4-hydroxynonenal (4-HNE) levels were determined in the cell lysates using the Bioxytech® LPO-586™ kit, according to the manufacturer's protocol. Measurements were performed in quadruplicate.

Statistical analysis

Each experimental measurement was performed at least in quadruplicate. Results are expressed as means ± SEM of taurine uptake (pmol/min/mg of protein). The kinetic parameters of taurine uptake were calculated by linear regressions of the Eadie-Hoffstee plots and confirmed by a non-linear regression program on Kaleidagraph (version 3.0.8 D, Abelbeck Software). Statistical significance of the experimental results was obtained by Variance Analysis with a Fisher's test. $P < 0.05$ was considered to denote statistical significance.

Results

Whatever the experimental conditions used to induce oxidative stress (effect of oxidant concentration or of time of exposure, . . .), cell viability, assessed by Trypan blue and MTT, remained similar in control and treated cultures (results not shown). Protein/DNA ratios were identical between the two KB cell lines (0.348 ± 0.02) and between the three LoVo cell lines (0.180 ± 0.002). This ratio was unaffected by H_2O_2 or CH. Taurine uptake was therefore expressed in pmol/min/mg of protein.

In the following sections, strictly similar results were often obtained using LoVo S fusoid cells and LoVo S small cells. Results under the name LoVo S refer therefore to these two cell lines.

Effect of H_2O_2 on high-affinity taurine uptake

High-affinity taurine uptake was linear for 60 min whether cells were treated with H_2O_2 or CH (results not shown). Taurine uptake was therefore measured using 10 min incubation with [^{3}H] taurine.

Figure 1 shows the effect of increasing concentrations of H_2O_2 on KB and LoVo cells treated in PBS. Taurine uptake was reduced in chemosensitive cells when the concentration of H_2O_2 was higher than 10 μM. MDR cells were more sensitive, as taurine uptake was already inhibited in the presence of 1 μM H_2O_2. Though the slope of the inhibition curve was similar, KB cells were slightly less sensitive to H_2O_2 than LoVo cells. The reduction of KB and LoVo taurine uptake in the presence of 25 μM H_2O_2 increased with time, and was significant for an exposure time as short as 1 hour (Fig. 2).

Effect of H_2O_2 on LoVo high-affinity taurine uptake in different treatment media

H_2O_2 (100 μM) reduced taurine uptake similarly in PBS, CIM and RPMI 1640, three media lacking sodium pyruvate (Table 1). In contrast, 100 μM H_2O_2 failed to affect taurine uptake in DMEM/Ham F12, a culture medium that

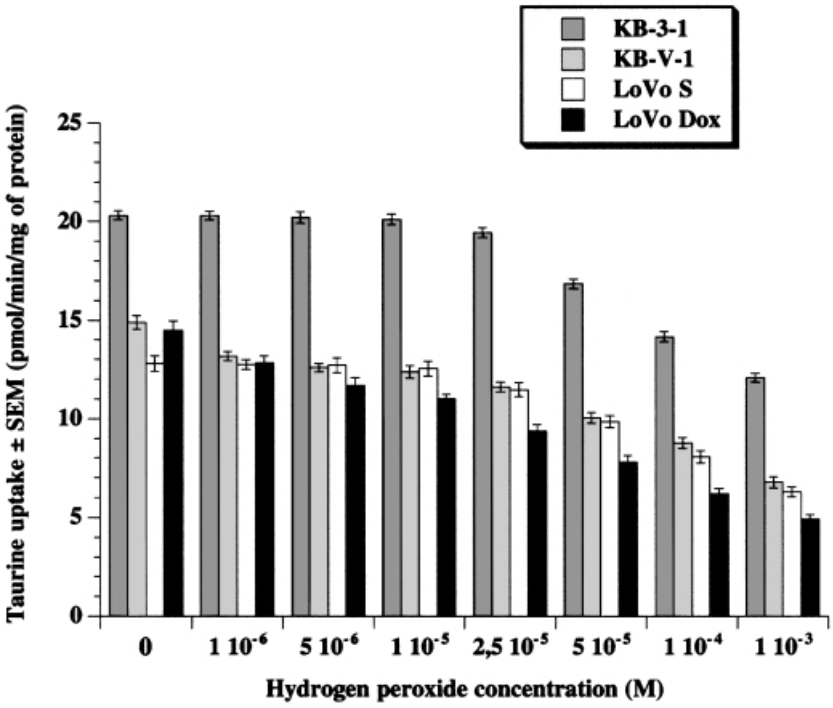

Fig. 1. Effect of increasing concentrations of H_2O_2 on high affinity taurine uptake. Confluent cells were treated for 1 hour with various concentrations of H_2O_2 in PBS as described in Materials and methods. ■ = KB-3-1; ▒ = KB-V-1; □ = LoVo S; ■ = LoVo Dox. Significance in decrease of uptake compared to the respective control: KB-3-1 and LoVo S: 50 μM and 100 μM H_2O_2 ($p < 0.05$) and 1 mM H_2O_2 ($p < 0.01$); KB-V-1 and LoVo Dox: 1 μM to 25 μM H_2O_2 ($p < 0.05$) and 50 μM to 1 mM H_2O_2 ($p < 0.01$). Differences in reduction of uptake between the MDR cells and their chemosensitive counterparts were always significant ($p < 0.05$)

contains 1 mM of sodium pyruvate under our culture conditions. Adding sodium pyruvate to PBS, CIM or RPMI 1640 notably reduced the H_2O_2 effect on taurine uptake. The H_2O_2 effect was totally neutralised when the *treatment media* contained 2 mM sodium pyruvate. No significant differences in the uptake sensitivity towards H_2O_2 were observed between cells treated in CIM, RPMI 1640 or PBS (Table 1). Treatment of KB-3-1 cells with H_2O_2 in PBS or DMEM/Ham F12 gave identical results to those obtained with LoVo sensitive cells. Similarly, addition of pyruvate to the PBS *treatment medium* inhibited the H_2O_2 effect on KB-3-1 taurine uptake (not shown).

Table 2 shows that in the absence of an oxidative stress (control cells), the presence of pyruvate in the *treatment medium* or in the *incubation medium* did not change the taurine uptake of LoVo Dox cells. Pyruvate inhibited the action of H_2O_2 when present in the *treatment medium*, but not if it were present in the *incubation medium*. Taurine uptake was not affected when cells were treated with H_2O_2 in DMEM/Ham F12. The presence of serum in the *treatment medium* changed neither the taurine uptake nor the H_2O_2 effect. Similar results were observed for LoVo S cells (data not shown).

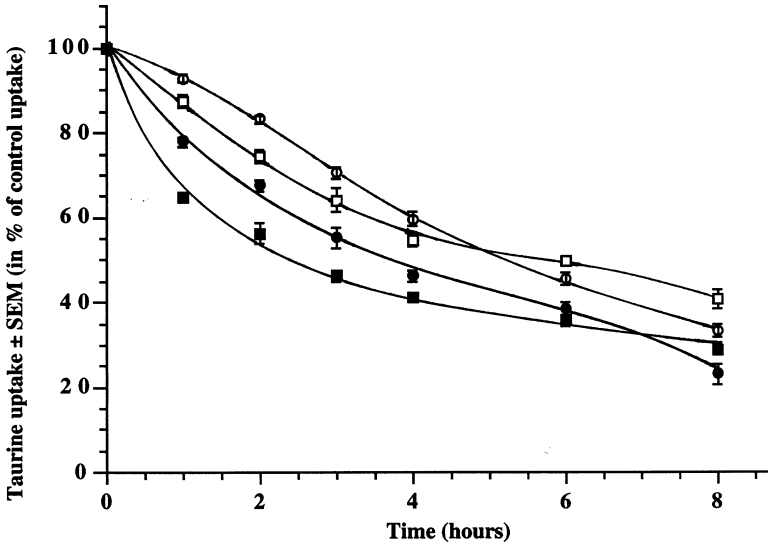

Fig. 2. Time-dependent reduction of high affinity taurine uptake by a single concentration of H_2O_2. Confluent cells were treated for various times without (control; uptake of 100%) or with 25μM H_2O_2 added to PBS as described in Materials and methods. When not shown, error bars are smaller than symbols. ❍ = KB-3-1; ● = KB-V-1; □ = LoVo S; ■ = LoVo Dox. Significance in decrease of uptake compared to the respective control: KB-3-1 and LoVo S: 2 h to 4 h ($p < 0.05$) and 6 h to 8 h ($p < 0.01$); KB-V-1 and LoVo Dox: 1 h and 2 h ($p < 0.05$) and 3 h to 8 h ($p < 0.01$). Differences in reduction of uptake between the MDR cells and their chemosensitive counterparts were always significant ($p < 0.05$)

Effect of cumene hydroperoxide (CH) on high-affinity taurine uptake

CH induced a concentration dependent reduction of KB and LoVo taurine uptake (Fig. 3). As observed with H_2O_2-treated cells, MDR cell lines were slightly more sensitive to CH-induced oxidative stress. Reduction of taurine uptake in KB and LoVo cells by 10μM CH increased with time, and this was significant for an exposure time as short as 15 min for MDR cells and 30 min for sensitive cells (Fig. 4). Figure 4A also shows that the uptake inhibition was greater when KB cells were treated in DMEM/Ham F12 than in PBS. In contrast, reduction of the uptake in LoVo cells was not affected by the type of *treatment medium* used, and was similar in PBS and DMEM/Ham F12 (Fig. 4B). Strictly similar results were obtained when cells were treated in the presence or the absence of 5% FCS (data not shown).

Effect of the oxidants on the kinetic parameters of high-affinity taurine uptake

One hour exposure of KB and LoVo cells to PBS with H_2O_2 (100μM), or 30 min with CH (100μM), slightly increased the Km of the high-affinity taurine uptake (Table 3). A more noticeable decrease of the Vmax was found, which correlated directly with the inhibition of uptake. Whatever

Table 1. Effect of H_2O_2 on LoVo taurine uptake in various treatment media

H_2O_2	Treatment medium	Pyruvate	Taurine uptake ± SEM (pmol/min/mg of protein)		
			LoVo S small cells	LoVo S fusoid	LoVo Dox
−	PBS	–	14.56 ± 0.44	12.56 ± 0.34	19.45 ± 0.51
+	PBS	–	9.31 ± 0.50 (63.93%)*	8.57 ± 0.47 (68.24%)*	7.43 ± 0.31 (38.21%)**
+	PBS	1 mM	13.10 ± 0.51 (89.94%)	10.69 ± 0.42 (85.10%)	17.40 ± 0.60 (89.45%)
+	PBS	2 mM	14.54 ± 0.48 (99.89%)	12.56 ± 0.41 (99.98%)	19.47 ± 0.55 (100.12%)
−	DMEM/Ham F12	–	14.35 ± 0.54	12.35 ± 0.33	18.54 ± 0.45
+	DMEM/Ham F12	–	14.13 ± 0.48 (98.49%)	12.17 ± 0.44 (98.55%)	18.19 ± 0.33 (98.11%)
−	RPMI	–	14.30 ± 0.47	12.30 ± 0.38	18.31 ± 0.47
+	RPMI	–	10.62 ± 0.52 (74.25%)*	9.27 ± 0.39 (75.34%)*	9.31 ± 0.42 (50.87%)**
+	RPMI	1 mM	13.67 ± 0.49 (95.61%)	11.61 ± 0.47 (94.35%)	17.13 ± 0.80 (93.56%)
+	RPMI	2 mM	14.30 ± 0.31 (99.97%)	12.32 ± 0.50 (100.20%)	18.38 ± 0.41 (100.40%)
−	CIM	–	14.21 ± 0.35	12.21 ± 0.37	18.28 ± 0.34
+	CIM	–	10.07 ± 0.24 (70.84%)*	8.76 ± 0.41 (71.71%)*	8.34 ± 0.31 (45.62%)**
+	CIM	1 mM	13.61 ± 0.49 (95.80%)	11.53 ± 0.41 (94.42%)	17.08 ± 0.74 (93.45%)
+	CIM	2 mM	14.21 ± 0.55 (99.99%)	12.39 ± 0.44 (101.47%)	18.27 ± 0.42 (99.97%)

24 hours before confluence, cells were washed twice with the treatment medium and cells were cultured for 24 hours in the same treatment medium (2 ml/dish) without FCS. Then, 100 μM H_2O_2 or vehicle (0.2% H_2O) were added to the treatment medium for 1 hour. For PBS treatments, cells were cultured for 24 h in DMEM/Ham F12 without FCS, then washed twice with PBS ± pyruvate and treated in PBS ± pyruvate ± 100 μM H_2O_2 for 1 hour. Treated cells were washed twice with CIM at 37°C to stop the action of H_2O_2, and taurine uptake was measured as described in Materials and methods. Control cells were treated with an equal concentration of the solvent (0.2% H_2O). (−): absence of H_2O_2; (+): presence of H_2O_2. Values in brackets show taurine uptake in % of the respective control uptakes. Significance in reduction of uptake compared to control cells: **($p < 0.01$) and *($p < 0.05$).

the origin of the oxidative stress (H_2O_2 or CH), its effect on Km and Vmax was similar.

Effect of oxidants on basal (non-stimulated) taurine efflux

The basal taurine efflux was not significantly changed by H_2O_2 or CH, whatever the *treatment medium* used (PBS or DMEM/Ham F12). No difference in the sensitivity of the efflux from non-MDR or MDR cells to the two oxidants was observed (Table 4). The high efflux observed in MDR cells was not related to a cell injury as these cells did not take up Trypan blue, the uptake of this dye being related to an increase of the plasma membrane permeability.

Table 2. Effect of pyruvate supplementation during H_2O_2 cell treatment or taurine uptake measurement

Treatment	Treatment medium	Incubation medium			
		PBS	PBS + pyrvuvate	CIM	CIM + pyruvate
Control	PBS	25.12 ± 0.55	25.10 ± 0.50	20.19 ± 0.57	20.17 ± 0.51
	PBS + pyruvate	25.11 ± 0.48	25.13 ± 0.54	20.18 ± 0.45	20.18 ± 0.49
	CIM	25.13 ± 0.49	25.12 ± 0.49	20.17 ± 0.48	20.20 ± 0.52
	CIM + pyruvate	25.12 ± 0.53	25.14 ± 0.44	20.15 ± 0.49	20.19 ± 0.63
	DMEM/Ham F12 ± 5% FCS	25.21 ± 0.72	25.23 ± 0.62	20.21 ± 0.62	20.18 ± 0.65
H_2O_2	PBS	9.68 ± 0.45 (38.54%)**	9.78 ± 0.44 (38.96%)**	7.90 ± 0.44 (39.13%)**	7.99 ± 0.41 (39.61%)**
	PBS + pyruvate	22.48 ± 0.68 (89.52%)	22.57 ± 0.63 (89.81%)	18.21 ± 0.51 (90.24%)	18.28 ± 0.70 (90.58%)
	CIM	11.27 ± 0.48 (44.85%)**	11.30 ± 0.53 (44.98%)**	9.13 ± 0.42 (45.27%)**	9.16 ± 0.43 (45.35%)**
	CIM + pyruvate	23.89 ± 0.56 (95.10%)	23.89 ± 0.55 (95.03%)	19.02 ± 0.51 (94.39%)	19.24 ± 0.51 (95.30%)
	DMEM/Ham F12 ± 5% FCS	23.68 ± 0.60 (93.93%)	23.71 ± 0.59 (93.98%)	19.04 ± 0.55 (94.21%)	19.10 ± 0.59 (94.70%)

24 hours before confluence, LoVo Dox cells were washed twice with the treatment medium, then maintained for 24 h in the same treatment medium (2 ml/dish). LoVo Dox cells were treated for 1 hour with 100 μM H_2O_2 or vehicle (0.2% H_2O) added to the treatment media. Then, loVo Dox cells were washed twice with the incubation medium to stop the action of H_2O_2, and taurine uptake was measured as described in Materials and methods, but by using the incubation media indicated in the table. Control cells were treated with an equal concentration of the solvent (0.2% H_2O). Results are expressed as taurine uptake ± SEM (pmol/min/mg of protein). Values in brackets show taurine uptake in % of the respective control uptakes. Significance in reduction of uptake compared to control cells: **($p < 0.01$).

Effect of the oxidants on the low affinity and non-saturable uptake systems of LoVo cells

Taurine uptake is mediated in the three LoVo cell lines by a high-affinity, a low-affinity and a non-saturable (diffusion) uptake system (Wersinger et al., 2000). Low-affinity uptake was significantly reduced by H_2O_2 and strongly reduced by CH. Diffusion was less affected by the oxidants, being only weakly reduced. Sensitivity of these last two uptake systems to the oxidants was slightly greater for the LoVo Dox cells than for the LoVo S cells (Fig. 5).

Quantification of lipid peroxidation induced by H_2O_2 and cumene hydroperoxide (CH)

When KB and LoVo cells were incubated in PBS with H_2O_2 or CH, a strong increase of malondialdehyde + 4-hydroxynonenal (MDA + 4-HNE) (Figs. 6 and 7) as well as fluorescent lipid peroxides (Figs. 8 and 9) was observed. This increase was time and concentration dependent. Addition of H_2O_2 to cells treated in DMEM/Ham F12 ± 5% FCS did not induce a significant lipid peroxidation increase whatever the H_2O_2 concentration used (data not

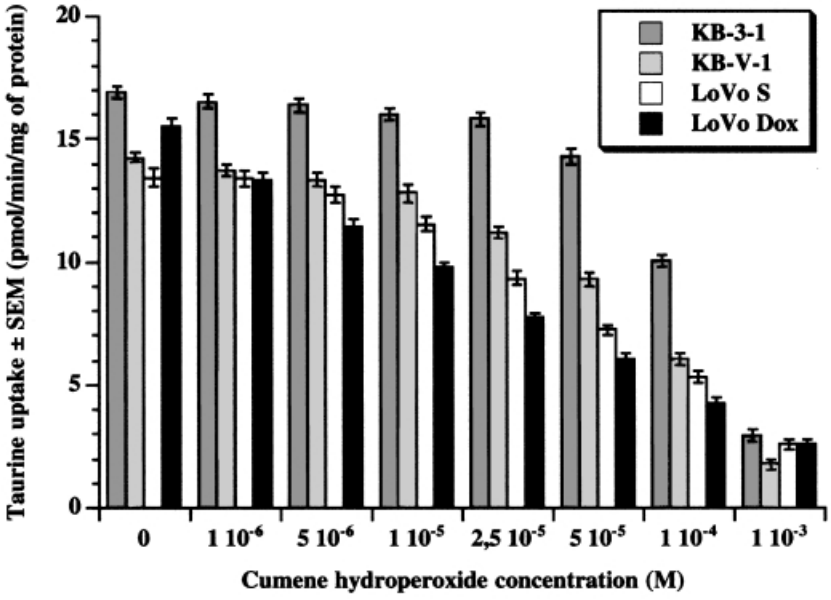

Fig. 3. Effect of increasing concentrations of cumene hydroperoxide (CH) on high affinity taurine uptake. Confluent cells were treated for 30 min with various concentrations of CH in PBS as described in Materials and methods. ■ = KB-3-1; □ = KB-V-1; □ = LoVo S; ■ = LoVo Dox. Significance in reduction of uptake compared to the respective control: KB-3-1: 50 μM and 100 μM CH ($p < 0.05$) and 1 mM CH ($p < 0.01$); LoVo S and KB-V-1: 10 μM to 50 μM CH ($p < 0.05$) and 100 μM to 1 mM CH ($p < 0.01$); LoVo Dox: 1 μM to 10 μM CH ($p < 0.05$) and 25 μM to 1 mM CH ($p < 0.01$). Differences in reduction of uptake between the MDR cells and their chemosensitive counterparts were always significant except for 1 mM ($p < 0.05$)

Table 3. Effect of oxidative stress on kinetic parameters of high affinity taurine uptake

Kinetic parameters of taurine uptake	Cell treatment	KB-3-1	KB-V-1	LoVo S	LoVo Dox
Km ± SEM (μM)	Control	21.9 ± 1.6	22.8 ± 1.5	16.1 ± 1.5	24.6 ± 1.2 (a)
	H_2O_2	28.5 ± 1.5 (b)	31.6 ± 1.3 (b)	21.9 ± 1.4 (b)	35.9 ± 1.4 (b)
	CH	30.6 ± 1.8 (c)	35.5 ± 1.5 (c)	24.1 ± 1.6 (c)	41.9 ± 1.9 (c)
Vmax ± SEM	Control	220.3 ± 6.2	108.4 ± 5.8 (a)	123.4 ± 5.2	63.2 ± 5.1 (a)
(pmol/min/mg	H_2O_2	152.1 ± 5.4 (b)	65.2 ± 4.8 (b)	78.4 ± 5.1 (b)	28.8 ± 4.4 (b)
of protein)	CH	130.2 ± 5.1 (c)	49.7 ± 4.4 (c)	50.4 ± 5.0 (c)	18.2 ± 3.4 (c)

Confluent cells were washed twice with PBS then treated in PBS with either vehicle (control), 100 μM H_2O_2 or 100 μM CH. After two washes with CIM, initial uptake rates were determined after 10 min preincubation and 10 min incubation in CIM with different concentrations of unlabeled taurine from 0 up to 50 μM. The concentration of radiolabeled taurine was maintained constant at 5 nM. Kinetic parameters of taurine uptake were calculated by linear regressions of the Eadie-Hoffstee plots and confirmed by a non-linear regression program on Kaleidagraph (version 3.0.8 D, Abelbeck Software). Results are means ± SEM of the kinetic parameters determined in three independent experiments. Vmax: pmol/min/mg of protein; Km: μM. (*a*), significant differences between MDR and non-MDR cells ($p < 0.05$); (*b*), significant differences between control and H_2O_2 treated cells; (*c*), significant differences between control and CH treated cells ($p < 0.05$).

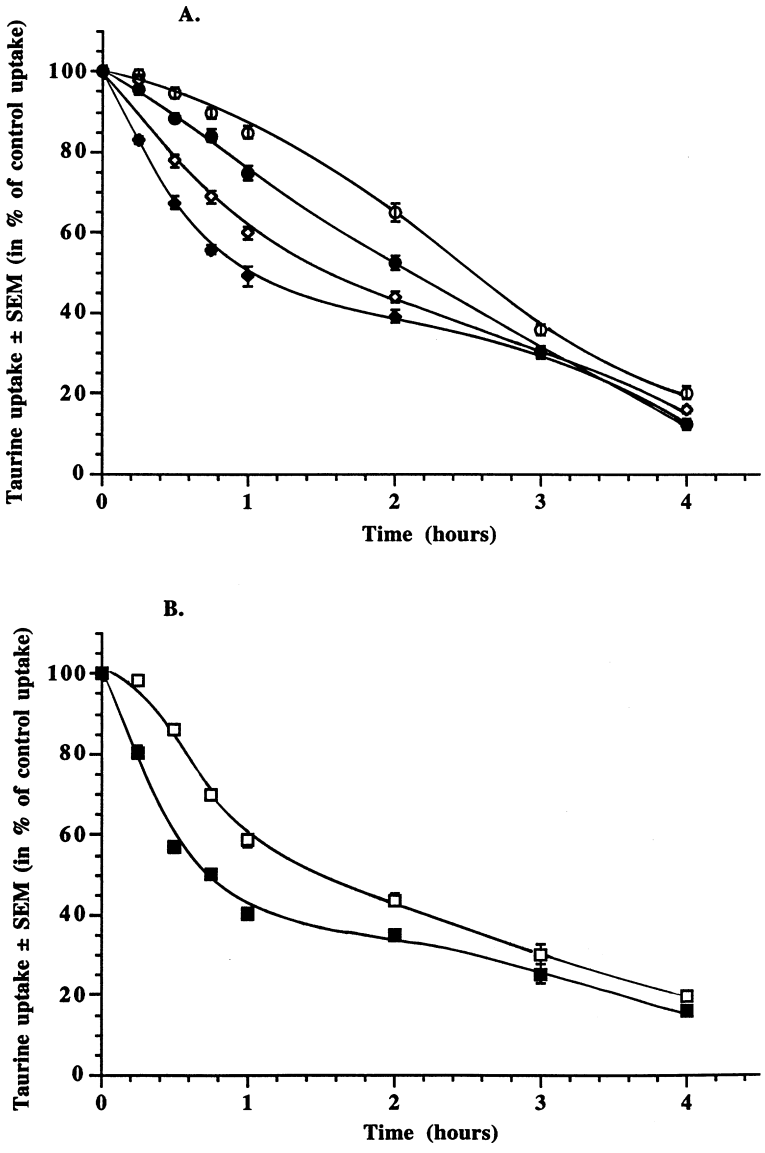

Fig. 4. Time-dependent reduction of high affinity taurine uptake of KB (**A**) and LoVo (**B**) cell lines by a single concentration of cumene hydroperoxide (CH). Confluent cells were treated for various times without (control; uptake of 100%) or with 10 μM CH either in PBS or in DMEM/Ham F12 as described in Materials and methods. When not shown, error bars are smaller than symbols. ❍ = KB-3-1 in PBS; ✧ = KB-3-1 in DMEM/Ham F12; ● = KB-V-1 in PBS; ◆ = KB-V-1 in DMEM/Ham F12; □ LoVo S in PBS or DMEM/Ham F12; ■ = LoVo Dox in PBS or DMEM/Ham F12. Significance in reduction of uptake compared to the respective control: KB-3-1 and KB-V-1 in PBS: 45 min to 2 h ($p < 0.05$) and 3 h to 4 h ($p < 0.01$); KB-3-1 in culture medium and LoVo S: 30 min to 2 h ($p < 0.05$) and 3 h to 4 h ($p < 0.01$); KB-V-1 in culture medium and LoVo Dox: 15 min to 45 min ($p < 0.05$) and 1 h to 4 h ($p < 0.01$). KB-3-1 and KB-V-1 uptake reduction was always significantly different in PBS and culture medium except for 4 h ($p < 0.05$). Differences in reduction of uptake between the MDR cells and their chemosensitive counterparts were always significant except for 3 h and 4 h ($p < 0.05$)

Table 4. Effect of oxidants on basal taurine efflux

Cell lines	Cell treatment	[^{3}H] taurine (cpm/mg of protein) ± SEM in cells		Taurine efflux (% of control)
		No incubation in CIM (control)	After 10 min incubation in CIM	
LoVo S small cells	Control	44.58 ± 0.95	44.52 ± 0.82	0.12%
	H_2O_2	42.54 ± 0.91	42.39 ± 0.75	0.34%
	CH	42.15 ± 0.82	41.95 ± 0.81	0.48%
LoVo S fusoid cells	Control	29.28 ± 0.68	29.20 ± 0.68	0.28%
	H_2O_2	27.93 ± 0.95	27.79 ± 0.85	0.49%
	CH	28.81 ± 0.98	28.63 ± 0.64	0.61%
LoVo Dox cells	Control	62.52 ± 0.75	50.55 ± 1.21 (*)	19.15% (*)
	H_2O_2	60.39 ± 0.88	47.97 ± 1.23 (*)	20.56% (*)
	CH	61.96 ± 0.95	49.02 ± 0.81 (*)	20.88% (*)

Basal taurine efflux was measured as described in "Materials and methods". Taurine efflux was calculated as difference between radioactivity found in control cells minus that of cells incubated 10 min, represented as percent of the activity in control cells (100%). (*), significant differences in efflux between incubated cells and control cells ($p < 0.05$).

shown). In contrast, addition of CH to cells treated in DMEM/Ham F12 ± 5% FCS strongly increased MDA + 4-HNE levels and fluorescent lipid peroxidation products as a function of time and CH concentration. For LoVo S and LoVo Dox, this increase was strictly similar to that observed with cells treated in PBS. For KB-3-1 and KB-V-1 cells, this increase was slightly higher when cells were treated in DMEM/Ham F12 than in PBS (data not shown). Oxidative stress increased more the amount of reactive aldehydes than the amount of lipid peroxides (Table 5). MDR cells were more sensitive to H_2O_2 than their chemosensitive counterparts. Indeed, a peroxide increase was observed in the presence of 1μM H_2O_2 in MDR cells, whereas 50μM was required to obtain a similar result in non-MDR cells (Fig. 7). The sensitivity of cells to CH (Fig. 9) was slightly different, LoVo Dox being more affected (an increase in peroxide was observed in the presence of 1μM CH) than the LoVo S and KB-V-1 cells (an increase in peroxide required 25μM CH) and KB-3-1 cells (an increase in peroxide required 100μM CH). If at the end of the incubation period the difference between MDR and non-MDR cells remained noticeable in H_2O_2-treated cells, it became smaller in CH-treated cells (Table 5).

Effect of protein kinase C inhibitors and of the calmodulin inhibitor W-13 on ROS-induced decrease of high-affinity taurine uptake

The PKC inhibitors chelerythrine and hexadecylphosphocholine did not change the taurine uptake in control cells. They did not affect the uptake inhibition observed in the presence of H_2O_2 (Fig. 10) or CH (data not shown). The calmodulin inhibitor W-13 reduced taurine uptake. Effect of W-13 and

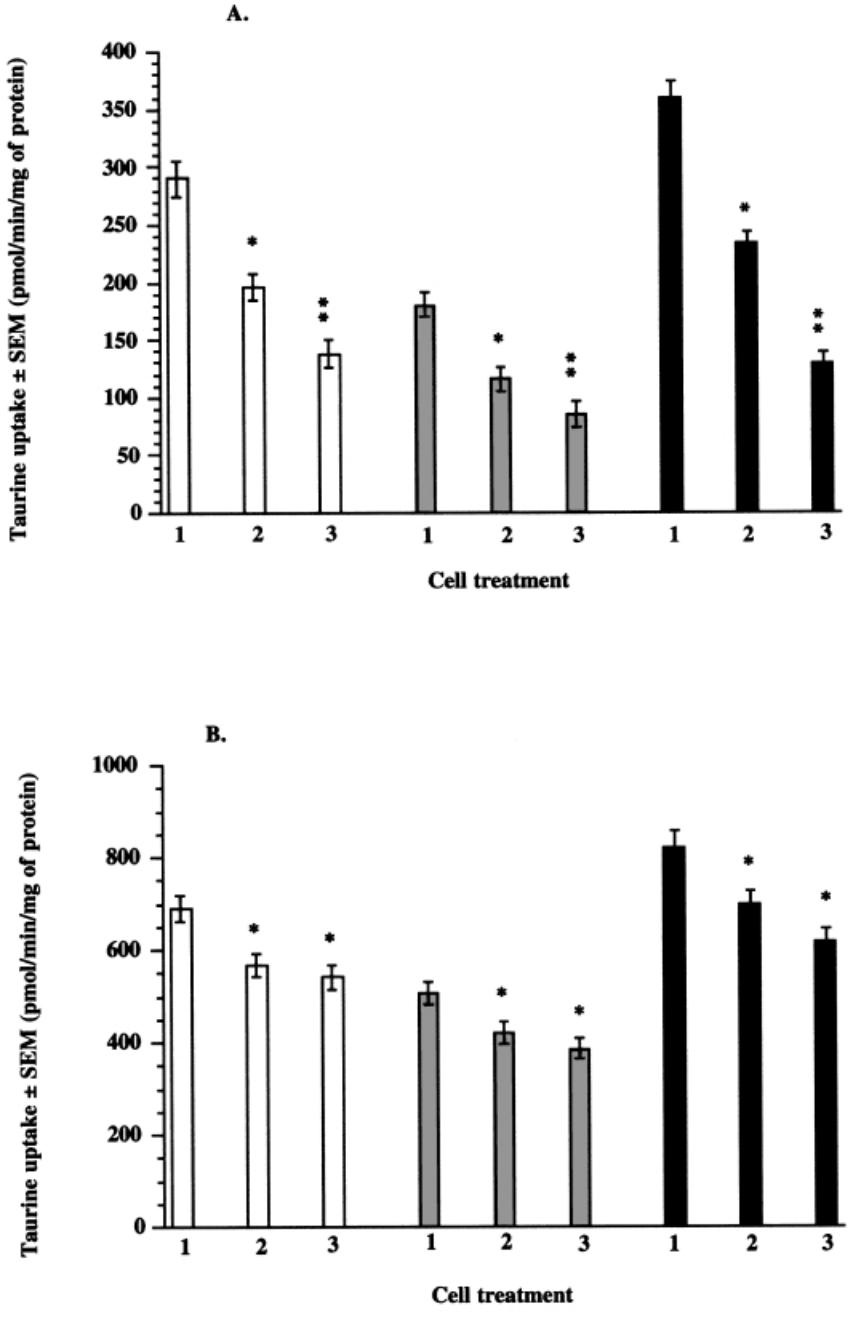

Fig. 5. Effect of oxidants on the low affinity (**A**) and the non-saturable (diffusion, **B**) uptake systems. Confluent LoVo cells were treated in PBS with 100 μM H_2O_2 for 1 hour or with 100 μM CH for 30 min, as described in materials and methods. Taurine uptake was measured as described in Materials and methods, using 5 min preincubation and 10 min incubation in CIM supplemented with 200 μM (low affinity, **A**) or 2 mM (diffusion, **B**) unlabeled taurine. 1. Control. 2. H_2O_2 treated cells. 3. CH treated cells. □ = LoVo S small cells; ▩ = LoVo S fusoid cells; ■ = LoVo Dox. **($p < 0.01$) and *($p < 0.05$) = significantly different compared to the respective control

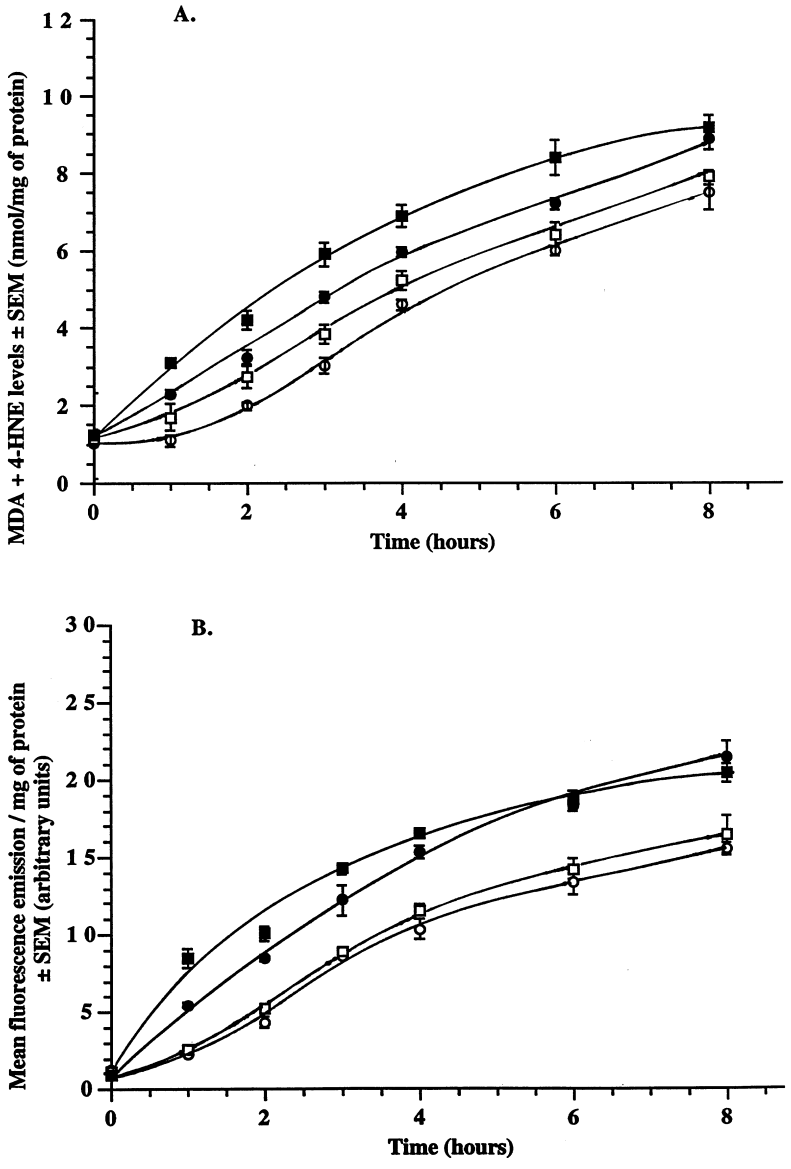

Fig. 6. Peroxidative effect of hydrogen peroxide: effect of exposure time. ○ = KB-3-1; ● = KB-V-1; □ = LoVo S; ■ = LoVo Dox. Cells were exposed to 25 μM H_2O_2 in PBS for various times. Malondialdehyde + 4 hydroxynonenal levels (graph **A**) were determined using the Bioxytech LPO 586 kit as a described in Materials and methods. They are expressed in nmol/mg of protein. Fluorescent chromolipids produced by lipid peroxidation (graph **B**) were determined as described in the Materials and methods and are expressed as mean fluorescence emission at 530 nm of the aqueous phase per mg of protein. Statistical significance compared to non-treated cells: $p < 0.05$ at 1 h and $p < 0.01$ after 2 h for non-MDR cells; $p < 0.01$ after 1 h for MDR cells

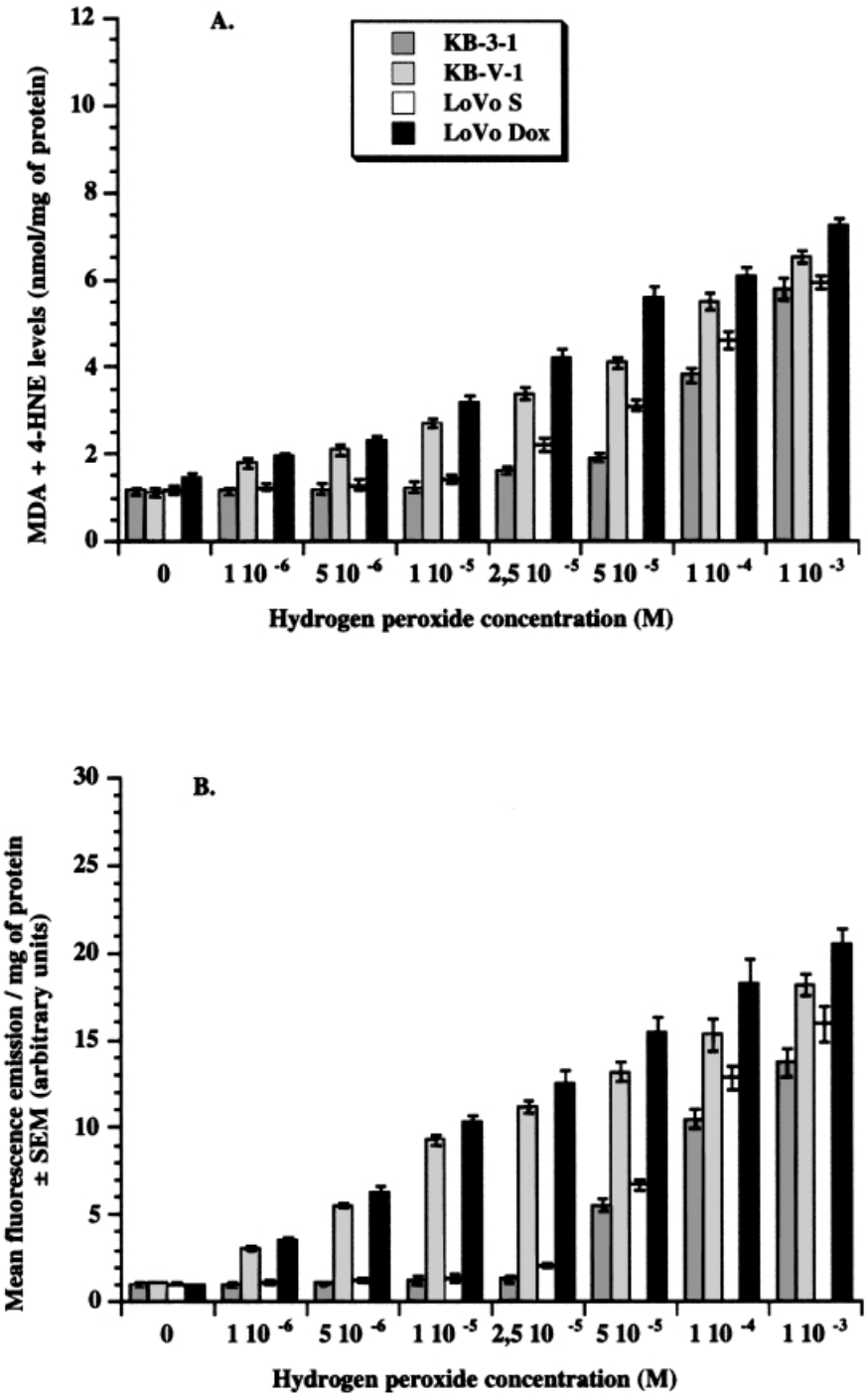

Fig. 7. Peroxidative effect of hydrogen peroxide: effect of H_2O_2 concentration. ■ = KB-3-1; ▨ = KB-V-1; □ = LoVo S; ■ = LoVo Dox. Cells were exposed to various concentrations of H_2O_2 for 1 h in PBS. Peroxidative products (malondialdehyde + 4 hydroxynonenal levels in graph **A**; fluorescent chromolipids produced by lipid peroxidation in graph **B**) were determined as indicated in Fig. 6. Statistical significance compared to non-treated cells: $p < 0.05$ at 50 μM H_2O_2 and $p < 0.01$ at 100 μM to 1 mM H_2O_2 for non-MDR cells. For MDR cells, $p < 0.05$ at 10 μM H_2O_2 and $p < 0.01$ for 50 μM to 1 mM H_2O_2 when MDA + 4-HNE was determined. For MDR cells when fluorescent lipid peroxides were determined, $p < 0.05$ at 5 μM H_2O_2 and $p < 0.01$ at 10 μM H_2O_2 for LoVo Dox; $p < 0.05$ at 10 μM H_2O_2 and $p < 0.01$ for 25 μM to 1 mM H_2O_2 for KB-V-1 cells

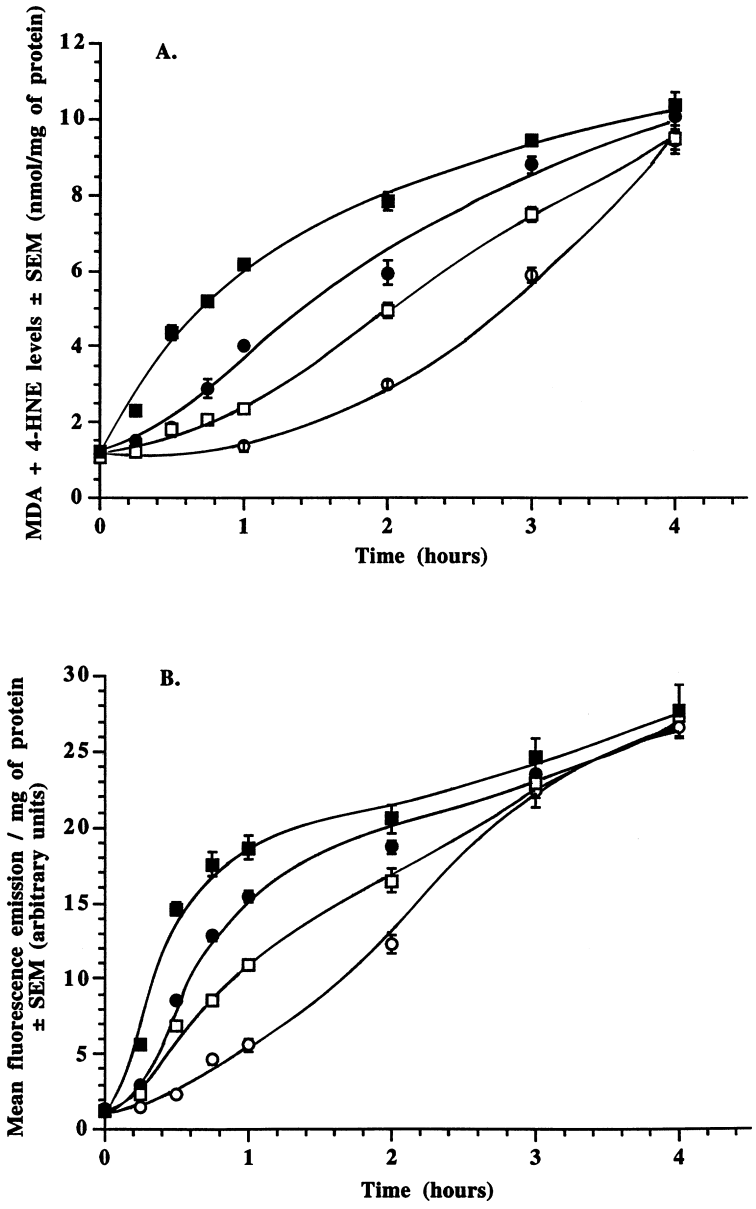

Fig. 8. Peroxidative effect of cumene hydroperoxide: effect of exposure time. ○ = KB-3-1; ● = KB-V-1; □ = LoVo S; ■ = LoVo Dox. Cells were exposed to 10μM CH in PBS for various times. Peroxidative products (malondialdehyde + 4 hydroxynonenal levels in graph **A**; fluorescent chromolipids produced by lipid peroxidation in graph **B**) were determined as indicated in Fig. 6. Statistical significance compared to non-treated cells: $p < 0.05$ at 1 h and $p < 0.01$ after 2 h for non-MDR cells; $p < 0.01$ after 1 h for MDR cells

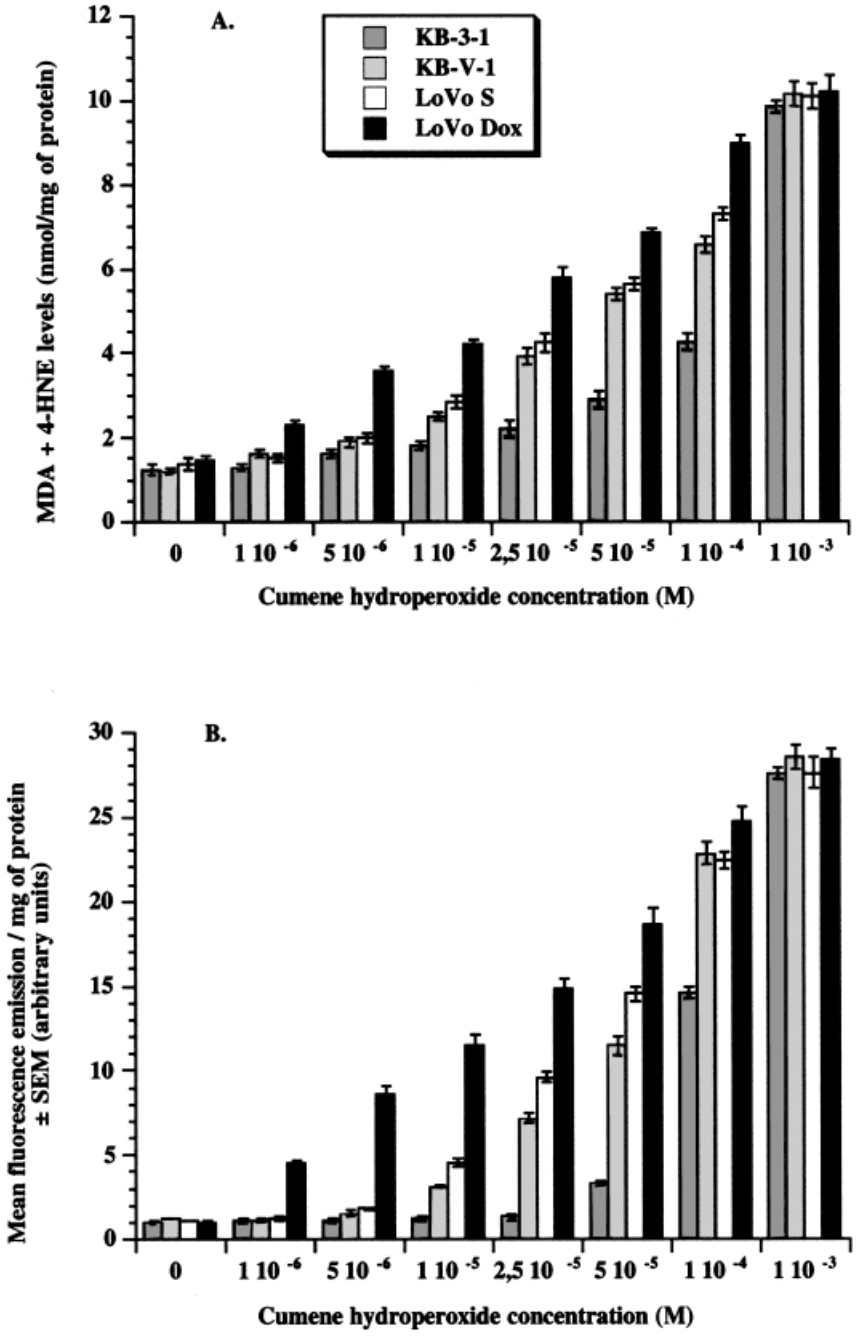

Fig. 9. Peroxidative effect of cumene hydroperoxide: effect of CH concentration. ■ = KB-3-1; ▨ = KB-V-1; □ = LoVo S; ■ = LoVo Dox. Cells were exposed to various concentrations of CH for 30 min in PBS. Peroxidative products (malondialdehyde + 4 hydroxynonenal levels in graph **A**; fluorescent chromolipids produced by lipid peroxidation in graph **B**) were determined as indicated in Fig. 6. Statistical significance compared to non-treated cells: $p < 0.05$ at 50 μM CH and $p < 0.01$ at 100 μM to 1 mM CH for non-MDR cells. For MDR cells, $p < 0.05$ at 10 μM CH and $p < 0.01$ for 25 μM to 1 mM CH. For MDR cells when fluorescent lipid peroxides were determined, $p < 0.05$ at 5 μM CH and $p < 0.01$ at 10 μM CH for LoVo Dox; $p < 0.05$ at 10 μM CH and $p < 0.01$ at 25 μM to 1 mM CH for KB-V-1 cells

Table 5. Comparative effect of H_2O_2 and cumene hydroperoxide on increase of lipoperoxides or reactive aldehydes and taurine uptake inhibition

Oxidant	Cell lines	Lipoperoxides (% increase)	Reactive aldehydes (% increase)	Taurine uptake (% inhibition)
H_2O_2	KB-3-1	1,405	673	71
	KB-V-1	1,930	780	77
	LoVo S	1,480	718	60
	LoVo Dox	1,850	833	67
CH	KB-3-1	2,070	743	81
	KB-V-1	2,155	850	84
	LoVo S	2,115	743	80
	LoVo Dox	2,155	875	88

Percent increase of lipoperoxides and reactive aldehydes (MDA + 4-HNE) was calculated from Figs. 6 to 9 as the value obtained after 8 h (*H_2O_2*) or 4 h (*CH*) in percent of 0 h values with cells incubated in PBS.

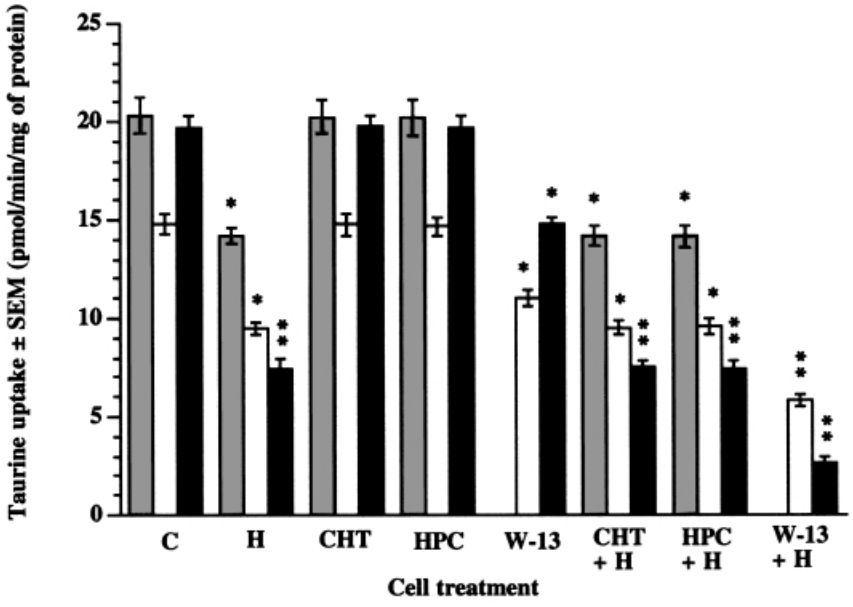

Fig. 10. Effect of PKC inhibitors or W-13 on H_2O_2-induced reduction of taurine uptake. Confluent LoVo cells were washed twice with PBS. Cells were pretreated in PBS for 30 min with 1 μM chelerythrine (*CHT*) or 1 μM hexadecylphosphocholine (*HPC*), or for 15 min with W-13 before addition of 100 μM H_2O_2 (*H*) or vehicle (control, *C*) to PBS (H_2O) for 1 h. After 2 washes with CIM, taurine uptake was measured as described in Materials and methods. ▒ = KB-3-1 cells; □ = LoVo S; ■ = LoVo Dox. **($p < 0.01$) and *($p < 0.05$) = significantly different compared to the respective controls

H_2O_2 (Fig. 10), or of W-13 and CH (data not shown) were additive. Identical results were obtained when CIM containing or not 5% FCS was used as *treatment medium* instead of PBS (data not shown). Similar results were obtained with KB-V-1 cells. MDR and non-MDR cells gave similar patterns.

Discussion

Many physiological events are modulated by ROS either directly by oxidation of cysteine or methionine in proteins, or guanine in nucleic acids, or indirectly by adduct formation between proteins or nucleic acids and aldehydes (malondialdehyde, 4-hydroxy-nonenal) originating from lipid peroxidation (Packer, 1994; Yu and Anderson, 1997; Mylonas and Kouretas, 1999). Oxidative stress occurs in physiological conditions after an overproduction of ROS or a decrease of antioxidant substances or enzymes. We have tested the effects of H_2O_2, one of the major ROS produced by mammalian cells, and of cumene hydroperoxide (CH), a very powerful oxidant that can mimic the situation occurring during a strong oxidative stress.

H_2O_2 and CH strongly reduced the taurine uptake of KB and LoVo cell lines. CH was a more powerful inhibitor of the uptake than H_2O_2. This could be related to the amount and kind of active compounds formed by H_2O_2 or CH. However, we cannot exclude that the observed difference could also be due to the different sites of action of the two oxidants. H_2O_2 can easily enter cells, acting on many different intracellular organelles (Vroegop et al., 1995) and therefore having a diffuse action. In contrast, oxidation by CH occurs essentially at the plasma membrane (Vroegop et al., 1995), where the taurine transporter is localised.

The effect of H_2O_2 largely depended on the composition of the *treatment medium*, especially on its pyruvate content. A correct knowledge of this last concentration (which differs in the same type of medium from one manufacturer to another) must be known to avoid discrepancies in results. In fact, H_2O_2 affected taurine uptake only in culture media (RPMI 1640, CIM) or physiological buffers (PBS) deprived of pyruvate. This is not surprising since pyruvate has been shown to chemically scavenge hydrogen peroxide producing acetate, CO_2 and H_2O (Holleman, 1904; Bunton, 1949). Protection of cultured cells by pyruvate against H_2O_2 toxicity has been extensively reported (Andrea et al., 1985; O'Donnell-Tormey et al., 1987; Salahudeen et al., 1991; Nath et al., 1994; Kashiwagi et al., 1997; Desagher et al., 1997; Herz et al., 1997; Giandomenico et al., 1997; Jaimes et al., 1997; Ruiz et al., 1998; Ramakrishnan et al., 1998). However, the fact that the reduction of taurine uptake produced by H_2O_2 in LoVo cells, when CIM or RPMI 1640 (which do not contain pyruvate) were used as *treatment media*, is smaller than in PBS but greater than in PBS containing sodium pyruvate, shows that pyruvate is not the only compound in culture media which reduces hydrogen peroxide-driven oxidation. In contrast to H_2O_2, the effect of CH was not affected by the presence or absence of pyruvate. This suggests that H_2O_2 is not the main active compound in oxidative stress driven by CH. The CH effect on taurine uptake was similar in DMEM/Ham F12 or PBS for LoVo cells or higher in DMEM/Ham F12 than in PBS for KB cells, but we do not have an explanation for this at the present time.

H_2O_2 and CH induced a similar cellular peroxide level, whether the *treatment medium* contained 5% FCS or not. The effect of the two oxidants on taurine uptake was also similar in *treatment medium* containing FCS or not.

This could be related to the fact that FCS contains only a small amount of antioxidants such as vitamin E or selenium (Leist et al., 1996).

Though free radicals and peroxides alterate membrane proteins, only few data concerning membrane transporters can be found. Peroxides inhibit taurine uptake. Similar inhibition by ROS or peroxides of nervous cell glutamate uptake (Piani et al., 1993; Voltera et al., 1994; Springer et al., 1997; Muller et al., 1998), dopamine transport (Berman et al., 1996) and of calcium channel in coronary artery (Grover et al., 1992) was previously reported. An irreversible closure of outwardly rectifying chloride channel was also observed (Jeulin et al., 2000). The decrease of the high-affinity taurine uptake we observed during the oxidative stresses induced by H_2O_2 or CH was coupled to a notable decrease of Vmax and to a lesser increase of Km. The reduced number of active high-affinity taurine transporters (Vmax) could result from a direct inactivation of the transporter activity linked to an oxidation of cysteyl (Dumaswala et al., 1996) and/or tyrosyl (Kulanthaivel et al., 1989) residues, that are essential for taurine transporter activity. Taurine uptake inhibition correlates better with the lipid peroxides change than with the reactive aldehydes variations (Table 5). This could result from an alteration of the correct insertion of the transporter into the oxidized plasma membrane bilayer. Though this cannot be completely excluded, it is probable that the effect of the two oxidants was too fast to affect the synthesis of the transporter. Our results also show a moderate decrease in the affinity of the transporter for its substrate. Previous reports have shown that oxidants can affect the activity of various ion transporters or ion channels such as sodium or chloride channels, or ATPases such as the Na/K ATPase (reviewed in Kourie, 1998). It is well documented that high-affinity taurine transport is a sodium and chloride driven transport (Huxtable, 1992). Taurine transport could therefore be also reduced by oxidants as a consequence of their effect on transmembrane sodium and chloride gradients.

Basal taurine efflux from KB and LoVo cells was not modified by H_2O_2 and CH, showing that the reduction in uptake observed in the presence of oxidants is only related to a reduction in the activity of the uptake transporter. Our results contrast with the previous data of Rego et al. (1996), who observed an increase of the basal taurine efflux induced by oxidants in retinal cells. Nobody has presently an idea about the taurine basal efflux mechanism that differs notably from a cell type to another (discussed in Wersinger et al., 2000) and could therefore be affected differently by oxidants. Our results cannot concern stimulated efflux (such as the swelling activated anion channel that is involved in taurine efflux) which in contrast to the basal efflux, is probably mediated by a carrier. Moreover, as all the experiments reported were done in isoosmotic conditions (osmolarity 300 ± 5 mOsm), this type of efflux cannot be measured.

Taurine uptake is mediated in KB and LoVo cells by a high-affinity transport system, a low-affinity transport system, and diffusion (Wersinger et al., 2000; Wersinger et al., submitted). The last two uptake systems were also reduced by the oxidative stress induced by CH and to a lesser extent by H_2O_2. This shows that ROS affected not only the transporter-mediated taurine

uptake but also the general properties of the plasma membrane which govern the diffusion.

Other authors have shown that oxidants such as H_2O_2 stimulate certain protein kinases such as protein kinase C (PKC) (O'Brian et al., 1988; Gopalarishna and Anderson, 1989; Taher et al., 1993; Brawn et al., 1995; Chakraborti et al., 1995; Konishi et al., 1997; Takeishi et al., 1999). PKC stimulation by phorbol esters inhibits taurine uptake in various cell types (Kulanthaivel et al., 1991; Jones et al., 1991; Brandsch et al., 1993; Tchoumkeu-Nzouessa and Rebel, 1995 and 1996; Mollerup and Lambert, 1996; Loo et al., 1996; Nakamura et al., 1996; Kim et al., 1996; Han et al., 1996 and 1999; Lambert, 1998; Wersinger et al., submitted). PKC-induced inhibition of taurine uptake is related to phosphorylation of the transporter (Han et al., 1996 and 1999). Our results showing H_2O_2 or CH-induced decrease of taurine uptake could therefore be explained by an effect of H_2O_2 (or CH) "activating" PKC. However, the inability of the PKC inhibitors chelerythrine and hexadecylphosphocholine (tested at a concentration which reverses PKC activation by phorbol 12-myristate 13-acetate in KB and LoVo cells, Wersinger et al., submitted) to reverse the H_2O_2 or CH effect exclude the participation of PKC in the uptake inhibition induced by the two oxidants. However, taurine uptake inhibition induced by ROS could also result from increased phosphorylation of the taurine transporter by another mechanism. Indeed, H_2O_2 decreases protein phosphatase activity probably by oxidation of a catalytically active cysteine (Denu and Tanner, 1998; Robinson et al., 1999), a mechanism which is not affected by PKC inhibitors. It is presently difficult to explain the result obtained with W-13. W-13 is used as an inhibitor of calmodulin-dependent phosphodiesterase. We have presently no idea how this compound could affect (activation or inhibition) calmodulin kinases or calmodulin-dependent phosphoprotein phosphatase. Moreover, we do not know if these last two systems can be affected by H_2O_2.

Higher was the peroxidative index (level of lipid peroxides and of reactive aldehydes), more important was the taurine uptake inhibition (see Table 5). The greater uptake inhibition observed in CH-treated cells was probably related to the amount of lipid peroxides formed. Though analysis of the lipid composition of MDR cells and their chemosensitive counterparts show only minor changes (summarised in Alon et al., 1991 and Callaghan et al., 1992), the fatty acid composition of MDR and non-MDR cells being very similar (Callaghan et al., 1992), we cannot exclude that the type of lipid peroxide formed in presence of CH is different from the one obtained with H_2O_2. Our results show that production of lipid peroxides and production of reactive aldehydes induced by oxidants was greater in MDR cells than in their non-MDR counterparts, except at high concentrations of CH. It is possible that MDR and non-MDR cells differ in their antioxidant contents (α-tocopherol, glutathion, glutathion peroxidase, catalase, superoxide dismutase or others), rendering the MDR cells more sensitive to oxidative stress.

The inhibition of the three taurine uptake systems we observed during an oxidative stress is probably valuable for the majority of mammalian cells. This explains the tissue decrease in taurine concentrations found in pathological

conditions involving an overproduction of ROS (Desai et al., 1992; Jiang et al., 1993; Muscaritoli et al., 1999). However, this was not general (Moreno et al., 1993) and could be influenced by the nutritional status of the patients (Gray et al., 1994). Taurine is an "indirect antioxidant", protecting cells mainly through hypochloric acid scavenging, inhibition of glycation (Ogasawara et al., 1993) (a process that is at the origin of ROS formation (Hayase et al., 1996)) and decrease of calcium overload. Some chemotherapeutic agents such as doxorubicin are a source of free radicals (Winterbourn, 1981; Hodnick and Sartorelli, 1994; Yee and Pritsos, 1997). We have previously shown that doxorubicin did not affect the uptake of taurine by MDR and non-MDR LoVo cells (Wersinger et al., 2000) or KB cells (Wersinger et al., submitted). Moreover, supplementation of the culture medium with taurine does not change the growth inhibitory effect of this drug (Wersinger et al., in preparation). Our results show that the inhibition of taurine uptake in tumoral cells has few effects on this kind of cells. This is certainly related to the absence in these cells of myeloperoxidase, the enzyme responsible of HOCl production, and of a calcium channel activated by ROS (Herson et al., 1999). Finally, protein glycation can be excluded as the concentration of glucose is not at the level where this phenomenon is observed. The decrease in intracellular taurine concentration which could result from an oxidative stress will be surely more important in some normal cells such as cardiomyocytes, inflammatory cells (neutrophils, polymorphonuclear leucocytes, . . .), increasing the deleterious action of the free radicals. Indeed, taurine supplementation which will counterbalance the intracellular taurine decrease, counteracts the pathological events linked for example to ischemic cardiac failure (Hamaguchi et al., 1988 and 1989; Azuma et al., 1992; McCarty, 1999), ozone stress (Banks et al., 1992; Schuller-Levis, 1995), inflammatory bowel disease (Son et al., 1998), diabetes (You and Chang, 1998b), drug-induced hepatotoxicity (Timbrell et al., 1995) or bleomycin-induced fibrosis (Giri and Wang, 1992; Gordon et al., 1992).

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