ethylating intermediates or by the formation of the nasal carcinogens acetaldehyde [16] or ethylene seems possible.

Acknowledgements—This work was supported by progetto Finalizzato "Oncologia" of C.N.R.

Istituto di Mutagenesi e Differenziamento
C.N.R.
Via Svezia 10
56100 - Pisa
Italy; and
* *Dipartimento di Patologia Generale; and*
† *Istituto di Clinica Otorinolaringoiatrica*
Università degli Studi
56100 - Pisa
Italy

V. LONGO
G. M. PACIFICI*
G. PANATTONI†
F. URSINO†
P. G. GERVASI‡

REFERENCES

1. Buiatti M, Geddes M, Carnevale F and Merler E, Nasal cavity and paranasal sinus tumors in woodworkers and shoemakers in Italy compared to other countries. In: *Nasal Tumors in Animals and Men* (Eds. Reznik G and Stinson SF), Vol. I, pp. 111–149. CRC Press, Inc., Boca Raton, Florida, 1983.
2. Lijinky W, Species differences in nitrosamine carcinogenesis. *J Cancer Res Clin Oncol* **108**: 46–55, 1984.
3. Dahl AR, Activation of carcinogens and other xenobiotics by nasal cytochrome P-450. In: *Microsomes and Drug Oxidation* (Eds. Boobis AR, Cold Well J, de Matteis F and Eleombe CR), pp. 299–309. Taylor and Francis, London, 1985.
4. Brittebo EB and Tjalve H, Metabolism of *N*-nitrosamines by the nasal mucosa. In: *Nasal Tumors in Animals and Men: Anatomy, Physiology and Epidemiology* (Eds. G. Reznik and S. F. Stinson), Vol. III, pp. 233–250. CRC Press Inc., Boca Raton, Florida, 1983.
5. International Agency for Research on Cancer, Some *N*-nitroso compounds. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 17, pp. 83–124. IARC, Lyon, 1978.
6. Longo V, Citti L and Gervasi PG, Metabolism of diethylnitrosamine by nasal mucosa and hepatic microsomes from hamster and rat: species specificity of nasal mucosa. *Carcinogenesis* **7**: 1323–1328, 1986.
7. Ding X and Coon MJ, Cytochrome P-450-dependent formation of ethylene from *N*-nitrosoethylamine. *Drug Metab Disp* **16**: 265–269, 1988.
8. Gervasi PG, Longo V, Ursino F and Panattoni G, Drug metabolizing enzymes in respiratory mucosa of humans. Comparison with rats. In: *Proc. 6th Int. Conf. Biochemistry and Biophysics of Cytochrome P-450*. Taylor and Francis, London, in press.
9. Longo V, Citti L and Gervasi PG, Biotransformation enzymes in nasal mucosa and liver of Sprague–Dawley rats. *Toxicol Lett* **44**: 289–297, 1988.
10. Farrelly JG, A new assay for the microsomal metabolism of nitrosamines. *Cancer Res* **40**: 3241–3244, 1980.
11. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
12. Lai DY and Arcos JC, Dialkylnitrosamine bioactivation and carcinogenesis. *Life Sci* **27**: 2149–2165, 1980.
13. Yoo JH, Guengerich FP and Yung CS, Metabolism of *N*-nitrosodialkylamines by human liver microsomes. *Cancer Res* **88**: 1499–1504, 1988.
14. Ding X, Koop DR, Crump BL and Coon MJ, Immunochemical identification of cytochrome P-450 isozyme 3a (P-450 ALC) in rabbit nasal and kidney microsomes and evidence for differential induction by alcohol. *Molec Pharmacol* **30**: 370–378, 1986.
15. Kato R, Mixed function oxidases in microsomes from human liver. In: *Hepatic Cytochrome P-450 Monooxygenase System* (Eds. Schenkman JB and Kupfer D), pp. 141–145. Pergamon Press, Oxford, 1982.
16. Feron VJ, Kruysse A and Woutersen RA, Respiratory tract tumors in hamsters exposed to acetaldehyde vapor alone or simultaneously to benz(a)pirene or diethylnitrosamine. *Eur J Cancer Clin Oncol* **18**: 13–31, 1982.

‡ Correspondence should be addressed to Dr P. G. Gervasi.

Biochemical Pharmacology, Vol. 38, No. 11, pp. 1869–1872, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

Gonadotropin-dependent metabolism of 7,12-dimethylbenz(*a*)anthracene in the ovary of rhesus monkey

(*Received* 12 *October* 1988; *accepted* 10 *January* 1989)

7,12-Dimethylbenz(*a*)anthracene (DMBA)* is a potent inducer of skin and breast tumors in the rat. This compound affects several steroidogenic organs in another way. DMBA causes necrosis in the two inner zones of the rat adrenal cortex [1] and in the germinal epithelial cells in the seminiferous tubuli of the testis [2] in this same animal. The adrenal necrosis is dependent on the presence of a fully functional pituitary gland, probably related to ACTH-induced maturity of the middle layer of the adrenal cortex and does not occur in immature animals [3].

Cell death caused by DMBA treatment may be prevented by coadministration of certain inhibitors and inducers of cytochrome P-450 [4]. Antioxidants also prevent this cytotoxicity partially, as well as preventing the extensive cytotoxic effect of 7-hydroxymethyl-12-methylbenz(*a*)anthracene in rat adrenal cell cultures [5], suggesting the involvement of a peroxidative mechanism, generating reactive oxygen, in the development of cellular necrosis. In addition, covalent binding of DMBA-metabolites to adrenal microsomal protein was increased 7-fold in the presence of peroxidase, indicating that, using endogenous hydrogen peroxide, peroxidases may activate

* Abbreviations used: ACTH, adrenocorticotropic hormone; DMBA, 7,12-dimethylbenz(*a*)anthracene; Hepes, *N*-2-hydroxy-ethylpiperazin-*N*-2-ethane sulfonic acid; PAH, polycyclic aromatic hydrocarbons; PMSG, pregnant mare's serum gonadotropin.

polycyclic aromatic hydrocarbons (PAH) by one electron oxidation [6]. In rat testis microsomes, addition of exogenous peroxidase increased covalent binding of DMBA metabolites to protein 3-fold, a process which was inactivated by cytochrome P-450 inhibitors [7]. In the testis spermatogenic cells active in DNA-synthesis are the target for cellular destruction by DMBA [2, 3]. These cells lack DMBA monooxygenase activity, suggesting that reactive metabolites are produced by cytochrome P-450-containing Leydig cells, whereafter these metabolites redistribute into the tubuli cells [8].

In addition to its effects on the adrenal cortex, DMBA treatment caused similar cell destruction in the rat ovarian corpus luteum, where the necrosis was restricted to the most recently formed corpora lutea, derived from the previous ovulation [9]. Necrosis appeared only in those animals which were in the proestrus or estrus stages of the estrus cycle [9], when the level of gonadotropins is elevated [10, 11]. In ovaries of mice the major target for the cytotoxic action of DMBA was shown to be the primordial oocytes [12, 13], whereas the same cells in rat ovaries were less sensitive [12]. Together, the effects on the germ cells in the male and the oocytes or ovarian lutein cells in the female caused by exposure to DMBA and to other PAH are likely to result in decreased fertility in both sexes in a species- and strain-dependent manner [14].

Although PAH (e.g. DMBA, benzo(*a*)pyrene and 3-methylcholanthrene) require metabolic activation in order to produce oocyte toxicity, there is no relationship between the rate of PAH metabolism by ovarian microsomes and oocyte destruction following administration by intraperitoneal injection [14]. However, upon intraovarian injection of benzo(*a*)pyrene, which does not cause occyte toxicity unless it is metabolized, metabolism in the ovary itself was shown to produce the ovotoxic metabolites [15]. Primordial occyte toxicity has been shown to be blocked by simultaneous administration of the cytochrome P-450 inhibitor α-naphthoflavone [16]. Hence, cytochrome P-450-dependent monooxygenase activities located in the ovary [17, 18] which are under endogenous hormonal regulation in the rat [19, 20], are proposed to play an important role in the cytotoxic and genotoxic effects of PAH within the ovarian tissue.

In human ovaries the level of DMBA monooxygenase activity was shown to be maximal in follicular granulosa cells from women prestimulated *in vivo* with gonadotropin and, in the case of unstimulated ovaries, in the granulosa-lutein cells, which differentiate from follicular granulosa following ovulation [21]. The present study concerns the corresponding localization of cytochrome P-450-dependent DMBA monooxygenase(s) in a non-human primate. The monkey model provides a good complement to studies with ovarian tissue from man and rat. *In vivo* treatment with various inducers and effectors are possible to perform with this model and, in contrast to the situation in rat, different structures of ovarian tissue can easily be obtained in appropriate quantities.

The metabolism of DMBA in primary cultures of rhesus monkey ovarian cells was investigated with special reference to the consequences of unilateral intraovarian injection of gonadotropin (PMSG) and, thus, the hormonal requirements for biotransformation of this particular carcinogen.

Materials and methods

Treatment of monkeys in vivo *with PMSG.* Two sexually mature rhesus monkeys (National Center for Toxicological Research, AR) received intraovarian injections of 200 i.u. PMSG (Sigma Chemical Co., St Louis, MO) in 0.3 ml saline unilaterally and an equal volume of saline in the other ovary under anesthesia 48 hr prior to ovariectomy. Both individuals were in the proliferative phase (days 6 and 3) of their menstrual cycles at the time of PMSG treatment.

Cell isolation. Following collection, the ovarian tissue was sliced with a pair of scissors and treated with a suspension of collagenase (2.5 mg/ml, Worthington CLS II) and DNAse (50 μg/ml, Sigma) in phosphate-buffered saline containing 0.2% glucose and 0.5% bovine serum albumin, as described before for human samples [21]. Culture Medium 199 (with Earle's Unmodified Salts, L-glutamine, and without $NaHCO_3$, containing 10 mM Hepes (*N*-2-hydroxy-ethylpiperazin-*N'*-2-ethane sulphonic acid), 4 mM $NaHCO_3$ 6 mM NaCl, 5% foetal calf serum and gentamicin (50 mg/l) was used. All solutions with which the cells had contact were checked for pH (7.4) and osmolarity (290 mOsm/l).

Cell survival was monitored by Trypan blue exclusion in a haemocytometer.

After filtration on a 100 μm nylon filter, the cells were placed on top of a discontinuous Percoll gradient ranging from 20–70% isoosmotic Percoll in phosphate-buffered saline. Centrifugation at 400 g_{av} for 20 min resulted in the appearance of two or three bands at the different interphases.

Measurements of DMBA monooxygenase activity. Cells from separate bands were put onto culture plates at a density of 50,000 cells/well with 0.5 ml Hepes-buffered culture Medium 199 (Gibco) containing 5% fetal calf serum. Cultures were left to establish for 16 hr prior to addition of [G-^{3}H]-DMBA (46 Curies/mmol) (Amersham, U.K.) (5 μM final concentration) in fresh medium, as described earlier [21]. Incubations were terminated after 7 hr by addition of 2 vol. of 0.15 M KOH–85% DMSO to 1 vol. of culture medium. Unmetabolized substrate was subsequently extracted with 8 vol. *n*-hexane and DMBA monooxygenase activity was determined according to van Cantfort *et al.* [22].

Ovarian cells from untreated Sprague–Dawley rats, isolated in parallel, were used as a control.

Results

Cell separation on Percoll gradients. The distribution of monkey ovarian cells dispersed with collagenase on the Percoll gradient is shown in Table 1. Most of the cells from all samples were recovered at the interphase between 30 and 50% Percoll (ρ = 1.040–1.064 g/ml). 2–2.5 times as many cells (6–14 × 10^6 viable cells) were recovered from the hormone-treated ovaries.

Table 1. Distribution of cells from the untreated and PMSG-treated ovaries of rhesus monkeys on the discontinuous Percoll gradient

Ovarian tissue	Distribution (% of the total cells recovered) at the interphase between 20/30 % Percoll (iso-osm)	30/50 % Percoll (iso-osm)	50/60 % Percoll (iso-osm)
Monkey I			
untreated	5	95	0
PMSG-treated	<1	80	21
Monkey II			
untreated	26	74	<1
PMSG-treated	<1	98	2
Rat	22	78	<1

Dispersed cell preparations from four monkey ovaries were separated on Percoll gradients. The values represent the distribution of cells at the different interphases, expressed as percent of the total viable cells recovered. The distribution of rat ovarian cells in the same system is shown for comparison.

Effect of in vivo *treatment with PMSG on DMBA monooxygenase activity in cell cultures.* Cells isolated from the untreated monkey ovaries did not metabolize DMBA to any significant degree in the present investigation. However, in both monkeys cells from the gonadotropin-treated ovaries demonstrated a capacity to metabolize DMBA (Table 2). The bulk of this activity was exhibited by those cells recovered at the interphase between 30 and 50% Percoll. In the corresponding band of rat ovarian cells, DMBA metabolism occurred at an even higher rate (Table 2).

Discussion

The present results demonstrate that the increase in DMBA monooxygenase activity in primary cultures of human ovarian cells prepared after gonadotropin stimulation *in vivo* [21] is also observed in another primate, i.e. the rhesus monkey. However, the monkey cell fraction exhibiting DMBA metabolism migrated to a higher density on the Percoll gradient than did the corresponding human fraction. The metabolically active monkey ovarian cells were recovered at the same density as human theca cells [21]. Further morphological investigations are required to characterize the different ovarian cell types and to conclude whether the same cell type from different species will migrate differently on the gradient.

In the untreated ovaries examined in this study, no DMBA metabolism could be detected. In addition, ovarian cells obtained from the untreated rat ovary and recovered at the same interphase on the Percoll gradient accounted for 97% of the total DMBA monooxygenase activity measured.

The localization of and hormonal effects on PAH-metabolizing enzymes is likely to be an important parameter in the generation of cytotoxic and genotoxic effects, such as sterility or decreased fertility, teratogenicity and cancer [14, 23–26]. An early study performed by Ford and Huggins [2] on the effect of DMBA administration on female reproduction in rats demonstrated a 14% decrease in the number of pups delivered in the treated group. This decrease in offspring may be due to corpus luteum deficiency. Luteal phase defects have been suggested as one cause of human infertility [27]. DMBA administration 16 days prior to breeding, as performed in the study with rats, is too short a period to reflect oocyte destruction, since in the rat a preovulatory follicle chosen to ovulate is recruited from the pool of small preantral follicles at least 19 days earlier [28]. However, the preovulatory or growing follicles may also be affected. In addition, steroid metabolism may be altered. Moreover, hyperstimulation with gonadotropin brings about enlargement of rat ovaries, which become cystic [3]. In women an increased incidence of ovarian tumors is positively correlated with, for example, pituitary hyperstimulation in menopause [23] and clinical gonadotropin therapy [29, 30]. This tumor formation is not due to activation of exogenously administered hydrocarbons, but the rate of formation and incidence is probably increased in the presence of such compounds [cf. 31].

The present investigation confirms our earlier studies on the potentiating effect of gonadotropins on ovarian cytochrome P-450-dependent PAH metabolism [18–20], demonstrating a similar increase in activity in man and rhesus monkey, after *in vivo* stimulation.

Table 2. DMBA monooxygenase activity in primary cultures of cells from the untreated and PMSG-treated ovaries of rhesus monkeys

Ovarian tissue	DMBA-monooxygenase activity (pmol/h × 10^6 cells)* at the interphase between 20/30 % Percoll (iso-osm)	30/50 % Percoll (iso-osm)	50/60 % Percoll (iso-osm)
Monkey I			
untreated	<5	<5	ND
PMSG-treated	ND	52.6 ± 4.98	6.53 ± 1.02
Monkey II			
untreated	<5	<5	ND
PMSG-treated	ND	153 ± 15.7	ND
Rat	30.5 ± 2.91	258 ± 32.7	ND

Activities were determined in primary cultures of ovarian cells recovered at the interphases indicated. For details, cf. Methods. The metabolism in rat ovarian cultures is shown for comparison.

* Mean ± SD from quadruplicate determinations.
ND = not determined.

Acknowledgements—The authors thank Mr John Bailey for excellent performance of the operations on the rhesus monkeys. The research visit by Margot Bengtsson was supported by the Swedish Council for Planning and Coordination of Research. This study was part of the project entitled "The development of ovarian toxicity screening methods: Studies on the classification of ovarian follicles as an indication of ovarian toxicity" headed by D. R. Mattison.

MARGOT BENGTSSON*,†,‡
DONALD R. MATTISON†

† *Division of Reproductive and Developmental Toxicology*
National Center for Toxicological Research
Jefferson
Arkansas 72079, U.S.A.
and
Division of Reproductive Pharmacology and Toxicology
Department of Obstetrics and Gynecology
University of Arkansas for Medical Sciences
Little Rock
Arkansas 72205, U.S.A.
and
‡ *Present address:*
Department of Biochemistry
Arrhenius Laboratory
University of Stockholm
S-106 91 Stockholm
Sweden.

* To whom correspondence should be addressed.

REFERENCES

1. Huggins CB and Morii S, Selective adrenal necrosis and apoplexy induced by 7,12-dimethylbenz(*a*)-anthracene. *J Exp Med* **114**: 741–760, 1961.
2. Ford E and Huggins CB, Selective destruction in testis induced by 7,12-dimethylbenz(*a*)anthracene. *J Exp Med* **118**: 27–40, 1963.
3. Huggins CB, *Experimental leukemia and mammary cancer. Induction, prevention and cure.* University of Chicago Press, Chicago, 1979.
4. Huggins CB and Fukunishi R, Induced protection of adrenal cortex against 7,12-dimethylbenz(*a*)-anthracene. Influence of ethionine. Induction of menadione reductase. Incorporation of thymidine-^{3}H. *J Exp Med* **119**: 923–942, 1964.

5. Hallberg E and Rydström J, Toxicity of 7,12-dimethylbenz(*a*)anthracene and 7-hydroxymethyl-12-methylbenz(*a*)anthracene and its prevention in cultured rat adrenal cells. Evidence for a peroxidative mechanism of action. *Toxicology* **47**: 259–275, 1987.
6. Montelius J and Rydström J, Peroxidase-dependent covalent binding of 7,12-dimethylbenz(*a*)anthracene metabolites to rat adrenal microsomes. In: *Extrahepatic Drug Metabolism and Chemical Carcinogenesis* (Eds. Rydström J, Montelius J and Bengtsson M), pp. 123–129. Elsevier, Amsterdam, 1983.
7. Georgellis A and Rydström J, Evidence for a free-radical-dependent metabolism of 7,12-dimethylbenz(*a*)anthracene in rat testis. *Toxicol Appl Pharmacol* **87**: 141–154, 1987.
8. Georgellis A and Rydström J, submitted to *Chem Biol Interact* 1989.
9. Wong T-W, Warner NE and Yang NC, Acute necrosis of adrenal cortex and corpora lutea induced by 7,12-dimethylbenzanthracene and its implication in carcinogenesis. *Cancer Res* **22**: 1053–1057, 1962.
10. Butcher RL, Collins WE and Fugo NW, Plasma concentrations of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-day estrous cycle of the rat. *Endocrinology* **94**: 1704–1708, 1974.
11. Smith MS, Freeman ME and Neill JD, The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: Prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. *Endocrinology* **96**: 219–226, 1975.
12. Mattison DR, Difference in sensitivity of rat and mouse primordial oocytes to destruction by polycyclic aromatic hydrocarbons. *Chem Biol Interact* **28**: 410–433, 1979.
13. Mattison DR, Shiromizu K and Nightingale MR, Oocyte destruction by polycyclic aromatic hydrocarbons. In: *Reproductive Toxicology* (Ed. Mattison DR), pp. 191–202. Alan R. Liss, New York, 1983.
14. Mattison DR and Nightingale MR, The biochemical and genetic characteristics of murine ovarian aryl hydrocarbon (benzo(*a*)pyrene) hydroxylase activity and its relationship to primordial oocyte destruction by polycyclic aromatic hydrocarbons. *Toxicol Appl Pharmacol* **56**: 399–408, 1980.
15. Takizawa K, Yagi H, Jerina DM and Mattison DR, Experimental ovarian toxicity following intraovarian injection of benzo(*a*)pyrene or its metabolites in mice and rats. In: *Reproductive Toxicology* (Ed. Dixon RL), pp. 69–94. Raven Press, New York, 1985.
16. Mattison DR and Thorgeirsson SS, Ovarian aryl hydrocarbon hydroxylase activity and primordial oocyte toxicity of polycyclic aromatic hydrocarbons in mice. *Cancer Res* **39**: 3471–3475, 1979.
17. Mattison DR and Thorgeirsson SS, Gonadal aryl hydrocarbon hydroxylase in rats and mice. *Cancer Res* **38**: 1368–1373, 1978.
18. Bengtsson M, Montelius J, Mankowitz L and Rydström J, Metabolism of polycyclic aromatic hydrocarbons in the rat ovary. Comparison with metabolism in adrenal and liver tissues. *Biochem Pharmacol* **32**: 129–136, 1983.
19. Bengtsson M and Rydström J, Regulation of carcinogen metabolism in the rat ovary by the estrous cycle and gonadotropin. *Science* **219**: 1437–1438, 1983.
20. Bengtsson M, Dong Y, Mattison DR and Rydström J, Mechanisms of regulation of rat ovarian 7,12-dimethylbenz(*a*)anthracene hydroxylase. *Chem Biol Interact* **63**: 15–27, 1987.
21. Bengtsson M, Hamberger L and Rydström J, Metabolism of 7,12-dimethylbenz(*a*)anthracene by different types of cells in the human ovary. *Xenobiotica* **18**: 1255–1270, 1988.
22. Van Cantfort J, DeGraeve J and Gielen JE, Radioactive assay for aryl hydrocarbon hydroxylase. Improved method and biological importance. *Biochem Biophys Res Commun* **79**: 505–512, 1977.
23. Basler A and Rohrborn G, Chromosome aberrations in oocytes of NMRI mice and bone marrow cells of chinese hamsters induced with 3,4-benzopyrene. *Mutat Res* **38**: 327–332, 1976.
24. Mattison DR and Thorgeirsson SS, Smoking and industrial pollution, and their effects on menopause and ovarian cancer. *Lancet* **1**: 187–188, 1978.
25. Mattison DR, The mechanisms of action of reproductive toxins. In: *Reproductive Toxicology* (Ed. Mattison DR), pp. 65–79. Alan R. Liss, New York, 1983.
26. Gram TE, Okine LK and Gram RA, The metabolism of xenobiotics by certain extrahepatic organs and its relation to toxicity. *A Rev Pharmacol* **26**: 259–291, 1986.
27. March MM, Update: Luteal phase defects. *Fertil News* **21**, 3–6, 1987.
28. Richards JS, Maturation of ovarian follicles: Actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. *Physiol Rev* **60**: 51–89, 1980.
29. Bamford PM and Steele SJ, Uterine and ovarian carcinoma in a patient receiving gonadotrophin therapy. Case report. *Br J Obstetr Gynaecol* **89**: 962–964, 1982.
30. Carter ME and Joyce DN, Ovarian carcinoma in a patient hyperstimulated by gonadotropin therapy for *in vitro* fertilization: A case report. *J In Vitro Fertil Embryo Transf* **4**: 126–128, 1986.
31. Rao AR, Effects of carcinogen and/or mutagen on normal and gonadotropin-primed ovaries of mice. *Int J Cancer* **28**: 105–110, 1981.

Biochemical Pharmacology, Vol. 38, No. 11, pp. 1872–1875, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

6-Hydroxydopamine toxicity to dopamine neurons in culture: potentiation by the addition of superoxide dismutase and *N*-acetylcysteine

(*Received* 17 *August* 1988; *accepted* 12 *December* 1988)

6-Hydroxydopamine (6OHDA) is a neurotoxin specific for catecholamine neurons of both the central and the peripheral nervous systems. There is strong evidence that 6OHDA neurotoxicity is correlated with its property to auto-oxidize rapidly at neutral pH and produce H_2O_2 and hydroxyl and superoxide radicals [1, 2]. The binding of quinones, formed during the auto-oxidation of 6OHDA, to proteins could also be contributing to the cytotoxicity of 6OHDA [3].

Cysteine and its derivative, *N*-acetylcysteine (NAC), are therapeutic agents that are widely used because of their antioxidant properties and ability to restore hepatic