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EFFECT OF *N*-ACETYLCYSTEINE ON SOME ASPECTS OF CYCLOPHOSPHAMIDE-INDUCED TOXICITY AND IMMUNOSUPPRESSION

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Abstract—Cyclophosphamide (CPA)-induced bladder toxicity and lethality were inhibited by *N*-acetylcysteine (NAC) when given systemically at a 4:1 ratio prior to CPA. This dose of NAC did not affect the production or the formation of free alkylating agents from CPA *in vivo* or *in vitro*. The immunosuppressive effect of CPA against T cell response systems, such as graft vs host reaction, antibody to sheep red blood cells and (PHA) phytohemagglutin stimulation was unaffected by NAC. It is concluded that the metabolic products of CPA for cytotoxicity as expressed as cystitis and lethality are different from the alkylating agents, which appear to affect immunological phenomena.

Cyclophosphamide (CPA) is a pharmacologically inert substance which has to be activated by the liver microsomes to become an effective therapeutic agent [1, 2]. It is believed that the active pharmacological principle comes under the general classification of alkylating agents. Alkylating agents are highly reactive compounds which have found utility as antitumor and immuno-suppressive drugs [3, 4]. Although the specific mechanism of action of CPA as an antitumor or immunosuppressive agent is not entirely known, it appears to be unique in that it acts on more than one pathway of the immune response [5, 6]. This broad utility has led to extensive clinical use of CPA with the concomitant undesirable effects from this compound of sterility, infection, alopecia and hemorrhagic cystitis [4]. Hemorrhagic cystitis has been extensively studied in rats and other species which receive high doses of CPA. Recently it was reported that some of the undesirable effects in hemorrhagic cystitis can be prevented with the use of *N*-acetylcysteine (NAC) [7–9]. Although it is desirable to inhibit the toxic effects, to do so at the expense of the therapeutic activity would be an exercise in futility. Unfortunately, many compounds have been found to inhibit undesirable effects of CPA [10] but they also attenuated the desirable action of the drug. In this report we hope to demonstrate that two undesirable activities, namely hemorrhagic cystitis and lethality, can be prevented with NAC at doses which do not affect the blood levels of alkylating agents or the immunosuppressive and antiproliferative activities of CPA.

MATERIALS AND METHODS

Materials

Cyclophosphamide and *N*-acetylcysteine (as Mucomyst) were gifts of Mead Johnson & Co., Evansville, IN. The cyclophosphamide was diluted with sterile saline, and the *N*-acetylcysteine for experiments *in vivo* was diluted in 3% gum arabic. For experiments *in vitro*, *N*-acetylcysteine was diluted with sterile saline. A new bottle of Mucomyst was used with each experiment.

Seakem agarose was used for plaque tests. Phytohemagglutinin M (PHA) was obtained from Difco and diluted 1:10 with RPMI buffer immediately prior to use.

Methods

Hemorrhagic cystitis. The method previously described [7] was employed using female Wistar rats weighing between 150 and 175 g (obtained from Hilltop Lab Animals, Chatsworth, CA). Thirty min prior to an intravenous injection of 100 mg/kg of CPA in the rat, test drugs were administered orally in varying doses. Twenty-four or 48 hr after the administration of CPA, the animals were sacrificed, bladders removed, expressed and weighed wet, then dried overnight at 100° and re-weighed. Since all animals were within a narrow limited weight range, the data are expressed on the basis of bladder weight only. The experiments on mice were performed on Swiss Webster 20–25 g male mice obtained from Curd Caviary, Los Angeles, CA. The CPA was administered intraperitoneally (i.p.) and the animals were sacrificed 48 hr later and the bladders removed and weighed the same as the rats. There was no significant loss of weight in either the rats or mice with any treatment.

Lethality studies. Preliminary experiments determined that 500 mg/kg of CPA given i.v. very slowly killed approximately 50 per cent of the animals within 2 weeks. Those that died during the first 24 hr after CPA administration were not included in this study. This single dose of CPA was used throughout these experiments and the number of mice surviving at 14 days when the CPA was either given alone or in combination with NAC was recorded. Swiss Webster male mice, obtained from Curd Caviary, Los Angeles, CA, were used in all these experiments and they weighed between 18 and 20 g at the outset. The NAC was administered orally 30 min before the i.v. CPA.

Chemical measurement of free alkylating agent and total CPA

In vitro *conversion.* Male mice that had phenobarbital in their drinking water (500 mg/liter) for a mini-

mum of 10 days were used. The animals were sacrificed by cervical dislocation, livers removed and homogenized in KCl and centrifuged at 15,000 *g* in a refrigerated centrifuge for 15 min. The cell-free supernatant containing NADPH, glucose 6 phosphate (G-6-P) and G-6-P dehydrogenase was used for measurement of conversion. When NAC was administered *in vivo* it was given intragastrically 30 min prior to sacrifice. The NAC *in vitro* was added 5 min before the CPA and the preparation was incubated 15 min at 37° after the addition of CPA. The reaction was stopped by putting the tubes in an ice bath. The Morita [11] modifications of the method of Friedman and Boger [12] were used to determine alkylating activity by colormetrically determining the addition product of 4-(*p*-nitrobenzyl)-pyridine (NBP) with the alkylating agent.

In vivo *conversion.* Blood sera samples obtained from the retro-orbital sinus of mice given CPA or CPA plus NAC 30 min prior were treated according to the method of Berenbaum *et al.* [13] and the NBP reaction developed as described above.

Immunological studies

Graft vs host reaction. The local graft vs host reaction originally described by Levine [14] and Ford *et al* [15] and as utilized in our laboratory [16] was used. Briefly, hybrid rats obtained from Fisher–Wister parents were given parental (Wistar) spleen cells in the paw. The animals were sacrificed; after 1 week the popliteal nodes were removed and weighed. The dose of spleen cells was approximately 50×10^6/paw, which was adequate to give a popliteal node enlargement to approximately 150 mg. Rats were obtained from Hilltop Labs., Chatsworth, CA.

Response to sheep red cells

Plaque test. The Kappler modification [17] of the Mishell and Dutton [18] technique with Falcon Multiwell plates with 24 wells/plate was used. Prior to introduction of spleen cells from sensitized and drug-treated mice, 0.25 ml of 1.2% clear agarose was added into the wells to obtain a level base in each well so that when the plates were subsequently read, nearly all plaques would be in one focal plane upon magnification. The sheep cells originally used for sensitizing the mice were used in the plaque test. A 0.5% agarose solution in balanced salt solution (BSS) contained 0.1 ml of 10% sheep red blood cells (SRBC) and 0.1 ml of spleen cells in BSS with 10^5 to 10^6 cells/ml. This was overlayed on the clear agarose and allowed to gel. The plates were then incubated 1 hr at 37°. After the incubation, a 10% guinea pig complement solution was added and the mixture incubated another 2 hr at 37°. The plates were then removed from the incubator and read or stored overnight in the cold and read on a magnifying viewer independently by two viewers. The values of the two viewers were averaged. Periodic microscopic examinations were made to determine the presence of a lymphoid cell in the center of the plaque. Only direct (19S) plaques were assayed from mice immunized 4 days prior to sacrifice with SRBC. The animals were dosed between 1 and 1.5 hr after receiving 10^8 SRBC. Each spleen was examined at four dilutions from 10^4 to 10^5 spleen cells/well in duplicate in order to obtain more accurate plaque readings. Linear plaque number vs cell concentrations in each series had to be obtained for acceptable data. The best duplicate consistent with the dilution was used to evaluate the plaques/10^6 spleen cells.

Stimulation of human lymphocytes. The method of Berenbaum *et al.* [13] for PHA stimulation of lymphocytes in human blood was used. Retro-orbital blood samples from mice which had received CPA or CPA + NAC were obtained and the sera harvested. Human blood obtained from healthy volunteers was diluted 1:10 with RPMI buffer. The mouse sera had to be diluted 1:1 with RPMI prior to use or non-specific inhibition was obtained. All dilutions were made in 50% mouse sera. The mixture containing human cells, mouse sera and PHA was incubated at 37° for 96 hr before the addition of 1 μCi of tritiated thymidine. The mixture was incubated for an additional 2 hr at 37°, then filtered through glass filter discs, put in phosphor and counted on a liquid scintillator. Statistical values were obtained using the method of Litchfield and Wilcoxon [19].

RESULTS

Effect of NAC on CPA toxicity

Hemorrhagic cystitis. In Table 1, the results of two series of experiments in rats and mice are shown. The rats used were approximately three times as sensitive to CPA as was the mouse to hemorrhagic cystitis based upon the dosage required to obtain consistent injury. The time of evaluation in both species was

Table 1. Effect of NAC on CPA-induced hemorrhagic cystitis*

Species	Treatment	Bladder weight		Water content (mg)
		Wet mg ± S. E. M. (*n*)	Dry mg ± S. E. M. (*n*)	
Rat	CPA (100 mg/kg)	111 ± 5 (34)	16 ± 0.5	111.0
	CPA (100 mg/kg) + NAC (400 mg/kg)	73 ± 5 (11)†	12 ± 0.5†	61.0
Mouse	CPA (350 mg/kg)	34.96 ± 3.3 (5)	4.98 ± 0.28	29.9
	CPA (350 mg/kg) + NAC (1400 mg/kg)	20.18 ± 2.1 (15)†	3.94 ± 0.32†	16.2

* Female rats and male mice within limited respective weight ranges were used. For the CPA-treated animals and CPA- and NAC-treated animals, both wet and dry weights of bladders were compared statistically. The average untreated rat bladder weighs 68 mg and the mouse bladder 22 mg.

† $P < 0.05$.

Table 2. Effect of NAC on CPA 14-day lethality in mice*

Drug (mg/kg)		Dead/No. treated	% Death
CPA (500)		34/53	64
CPA (500)	+ NAC (2000)	4/22	18†
	+ NAC (1500)	2/8	25
	+ NAC (1000)	3/6	50
	+ NAC (500)	2/6	33

* The NAC-treated groups were compared to those receiving only CPA. The majority of mice died between days 4 and 10 after dosage. The cages were examined daily to remove the dead animals.
† $P < 0.05$.

also different; the rat manifested the hemorrhagic cystitis consistently in 24 hr, while the mouse needed 48 hr for reproducible results. Despite the obvious difference between the two species, a definite increase in bladder weight from control (bladder wet weight rat average 68 mg and mouse average 22 mg) was observed when CPA was given to the animals. Not only was there an obvious increase in water content but grossly the bladders were obviously hemorrhagic. Histological sections were not taken, but the animals receiving only CPA showed petechial or gross hemorrhages while this was rarely observed in the NAC-treated animals. A single administration of NAC at a 4:1 ratio 0.5 hr prior to CPA was capable of significantly ($P < 0.05$) preventing the edematous reaction and the bladderrs appeared perfectly normal.

Lethality induced by CPA. A single dose of 500 mg/kg of CPA slowly injected intravenously caused a 64 per cent death of mice within 2 weeks. Mice that died within 24 hr after the CPA were not counted so that we only observed the more chronic effects of CPA. In Table 2, the data for CPA alone and with 4:1 NAC indicated that a single dose of NAC given orally 30 min prior to the i.v. CPA was capable of significantly ($P < 0.05$) reducing the lethality of CPA.

Effect on circulating leukocytes. When the 64 per cent lethal dose of CPA (see section Lethality Induced by CPA above) was given to the mice and the peripheral white count was followed by obtaining retro-orbital blood samples, a profound leukopenia was observed on day 4 after CPA dosage. This was followed by a recovery and an overshoot leukocytosis in the remaining surviving mice. In Table 3, it can be seen that the single dose of 2000 mg/kg of NAC did not quantitatively affect the leukopenia and secondary leukocytosis. The difference in leukocytosis between the two groups was not significant because the variability in the CPA group was too large due to so few surviving mice.

Effect of NAC on production of CPA-derived alkylating agent

Conversion in vitro. In Table 4, the data are presented for the formation of free HN_2 from CPA as measured by the NBP reaction. If one adds CPA, either 1 or 2 μmoles, to a liver preparation from phenobarbital-treated mice, a good conversion of approximately 50 per cent is obtained at 15-min incubation. The presence of NAC (4 or 8 μm) at four times the CPA concentration did not affect the rate of conversion of CPA to NBP reacting product. To determine whether there was need to process the NAC to obtain an effect, some mice were dosed 30 min prior to sacrifice with NAC (1 g/kg p.o.) and then the livers were examined for their ability to convert CPA to free alkylating agent. As can be seen in Table 4, there were no observable differences in the three groups.

Conversion in vivo. The blood levels, measured at 15, 30 and 60 min after administration of different dosages of CPA given i.p., were studied. It was observed that the amount of free alkylating agent decreases markedly in hr 1, with the highest ratio of free to total alkylating agent at 30 min. Between 70 and 90 per cent of the measurable alkylating agent at 30 min is free. Using only the 320 mg/kg dose and measuring only at 30 min in Table 5, the effect of

Table 3. Effect of NAC on peripheral WBC depression by CPA*

Day	No drugs	White blood cells/mm^3 ± S. E. M.	
		CPA (500 mg/kg)	CPA (500 mg/kg) + NAC (2000 mg/kg)
0	8,875 ± 667		
2	10,960 ± 1,512	5,167 ± 1,314	4,338 ± 954
4	7,290 ± 863	162 ± 39	121 ± 24
7	6,540 ± 1,426	12,750 ± 4,015	10,862 ± 2,088
9	6,300 ± 1,245	28,366 ± 8,180	17,000 ± 1,865
11	6,740 ± 550	37,675 ± 4,500	13,400 ± 1,956

* Blood was obtained from the retro-orbital sinus in heparinized micropipettes. Initially each group contained eight mice and all but two of the CPA-alone animals died by the end of this experiment.

Table 4. Effect of NAC *in vivo* and *in vitro* on liver conversion of CPA*

Treatment	*n*	μm HN_2 Equiv./g liver/hr ± S. E. M.
CPA alone	23	7.24 ± 0.36
CPA + NAC (4:1) *in vitro*	25	7.08 ± 0.39
CPA + NAC (1 g/kg) *in vivo*	13	6.92 ± 0.38

* Supernatants from mouse liver homogenate equivalent to 100 mg were incubated with 1 or 2 μmoles CPA, and 4 or 8 μmoles NAC was added *in vitro*. Supernatants from NAC-treated animals were used for measurement of alteration *in vivo* of CPA metabolism *in vitro*. No significant differences were obtained as determined by Student's *t*-test.

NAC at the same 4:1 ratio (1280 mg/kg) does not decrease either the amount of free alkylating agent or the total amount of available circulating CPA.

Effect on immune system

Local graft vs host reaction in the rat. When Fisher–Wistar hybrid rats are given Wistar spleen cells in the footpad, a popliteal node enlargement is obtained which is used as the measurement of the local graft vs host reaction. In Table 6, a popliteal node weight of 172 mg was obtained in rats not receiving any drugs. Daily dosage of CPA from 1 to 10 mg/kg caused a significant graded inhibition of this popliteal node hyperplasia. When NAC at 100 mg/kg was administered prior to the daily dose of CPA, there was no significant difference between the two groups of rats with regard to the inhibition of the popliteal node enlargement.

Plaque-forming cells. In Table 7, the plaques obtained from the spleens of mice given SRBC are reported. This is from one representative experiment and it has been repeated at least five times. The number of plaques obtained in controls on different days varies. In no case using this schedule of dosage of CPA with or without NAC did the drug-treated animals have greater than 10 per cent of the number of plaques of the control treated mice. In none of the experiments did NAC increase the inhibitory action of 50 mg/kg of CPA in the 4:1 dose ratio used.

PHA stimulation of human lymphocytes. Mice were given CPA at 320 mg/kg and 30 min later were bled for sera. A comparable series of mice was given 1280 mg/kg of NAC prior to CPA and then bled 30 min after the CPA. Several dilutions of the mouse sera were added to the diluted human whole blood to which PHA had been added. After 96 hr of incubation, tritiated thymidine was added and the cells were allowed to incubate another 2 hr. In Table 8, the data for the incorporation of [^{3}H]T indicate that the sera from mice given CPA are completely inhibitory to the PHA stimulation but if one dilutes out the sera 1:16 with mouse sera approximately 50 per cent recovery is attained. In comparison, series of sera from the NAC-pretreated mice gave almost identical results.

When this is plotted using probits according to the method of Litchfield and Wilcoxin [19], the ID_{50} (dose needed to inhibit 50 per cent of [^{3}H]T uptake by cells) concentration of blood-free HN_2 equivalent material needed is 3 nM. If one adds HN_2 in the form of mechlorethamine to mouse sera and then dilutes the same way as with the CPA experiment *in vivo*, the ID_{50} is 9 nM, which is not signficantly different from the sera levels in CPA-treated mice.

DISCUSSION

The spectrum of tests one could perform to determine efficacy relative to toxicity is endless. Our

Table 6. Effect of NAC on CPA inhibition of local graft vs host reaction*

Treatment		Popliteal node wt [mg ± S. E. M. (*n*)]
No drug treatment		173 ± 15 (24)
CPA	(1 mg/kg × 5)	131 ± 9 (14)†
	(3 mg/kg × 5)	94 ± 12 (24)†
	(10 mg/kg × 5)	22 ± 3 (23)†
CPA	(1 mg) + NAC (100 mg/kg)	117 ± 16 (4)†
	(3 mg) + NAC (100 mg/kg)	110 ± 6 (10)†
	(10 mg) + NAC (100 mg/kg)	33 ± 4 (12)†

* CPA was administered i.p. 1 hr after cell transfer and 0.5 hr after p.o. NAC. The animals were dosed daily every morning. There are no significant differences at equal CPA dosages between the two groups. All treated groups were compared to the non-treated control. The data expressed are from three independent experiments. † $P < 0.05$ relative no drug control.

Table 5. Effect of NAC formation *in vivo* of CPA-derived alkylating material*

Treatment	μm HN_2 Equiv./100 ml sera ± S. E. M.	
	Free	Total
CPA (320 mg/kg) at 30 min	56.7 ± 2.4 (16)	96.5 ± 4.9 (12)
CPA (320 mg/kg) + NAC (1210 mg/kg)	62.7 ± 5.6 (5)	92.6 ± 9.0 (5)

* Blood was obtained from the retro-orbital sinus in conscious mice 30 min after CPA administration i.p. NAC was given p.o. 30 min prior to the CPA. Mechlorethamine (HN_2) and CPA controls were used to determine the amount of free and total alkylating agent.

Table 7. Effect of NAC on CPA inhibition of plaque-forming cells against sheep red blood cells*

Treatment	Plaques/10^6 cells
No drug	1720
CPA (50 mg/kg × 1)	30
CPA (50 mg/kg) + NAC (200 mg/kg)	70

* Mice were sensitized with 0.1 ml of a 10% SRBC suspension i.v. plus 0.025 ml in each rear paw. Direct plaques were measured in duplicate over a 4-fold dilution range. The CPA was administered i.p. 1 hr after SRBC injection and 30 min after p.o. NAC. The animals were sacrificed 4 days after SRBC injection and spleens removed. The data represent the average of three individual mouse spleens/treatment.

approach was to use representative tests at comparable dose levels to obtain reasonable therapeutic guidelines. To obtain this end we used hemorrhagic cystitis and 14-day lethality with concomitant leucocyte counts to obtain an index to toxicity. It is recognized that sterility and alopecia, as well as many other indices *in vivo* and *in vitro*, can be used. For the comparison of blood levels the amount of free measurable and available alkylating agent had to be demonstrated. Relative to the amount of available alkylating agent, predominantly T cell and predominantly T-dependent B cell immune responses were measured. Proliferation of lymphoid cells *in vivo* and *in vitro* were evaluated as to the modifications of CPA inhibition by NAC. In some instances, blood levels and biological responses were performed in one animal from the series of animals to obtain more credibility for the effects observed.

In the first two tables (1 and 2), the effect of NAC on our putative toxicity is demonstrated. The numbers in Table 1 are actually an understatement of the data, since we did not subtract the normal bladder weights. If this is done, there is essentially no increase in bladder weights for the CPA plus NAC animals. Thus, one could say we obtained 100 per cent protection of hemorrhagic cystitis from NAC. These data are consistent with the data obtained by Primack [20], who studied NAC and CPA in monkeys and dogs, as well as with the data of Botta *et al.* [21], who evaluated NAC and CPA at different dosage regimens in rats. Brincker [22] studied the effect of cysteine in CPA toxicity as did Garattini *et al.* [10] and Connors [23, 24], and they all arrived at the conclusion that inhibition of lethality was done at the expense of efficacy. NAC protection of lethality was shown by Kline *et al.* [25] when they described an increase in the therapeutic efficacy of isophosphamide (a congener of CPA) against murine L1210 leukemia in the presence of NAC. Thus, the data presented in Tables 1 and 2 represent confirmation of the protective effects of NAC on CPA-induced toxicity.

The obvious next question is whether this is due to the fact that the NAC causes a decrease in the formation of distribution of the metabolically active compound. Cyclophosphamide is biologically altered so that a number of potentially active nitrogen mustard alkylating agents are found [26]. It is believed that these alkylating agents are responsible for antitumor and immunosuppressive activity of CPA [27]. The conversion of CPA to the active metabolites can be done *in vivo* or *in vitro*, and this is conventionally measured by the colored addition product to NBP as described in Methods. Using liver supernatant containing primarily microsomes which have been fortified with nutrients, the conversion to nitrogen mustard equivalents based on the NBP reaction appears to be unaffected whether we used an animal who had been dosed by NAC or the NAC was present in the solution in significant quantities. In the data of Table 5, one must accept that, *in vivo*, neither the total amount of available alkylating agent, which is the sum of free alkylating agents and non-metabolized CPA, nor the converted CPA, which is free alkylating agent, is different 30 min after CPA + NAC dosage from what it is when CPA is given alone using the NBP method. Thus, one can conclude that the formation of active metabolite is unaffected by NAC.

It is possible that the chemicals being measured as alkylating agents are not really representative of the CPA biological activity. To obtain information about this, we examined proliferation and immunological reactions which were inhibited by CPA.

Probably one of the more complicated biological reactions we performed was the local graft vs host reaction (GvHR) in the rat. In this reaction, the method of evaluation is the proliferation response of lymphoid tissue to a histoincompatible stimulus which is believed to involve primary T cells [28]. Immunosuppressive drugs assayed with this technique can be interpreted to be either immunosuppressive

Table 8. Effect of NAC in CPA inhibition of human lymphocyte stimulation by PHA*

Treatment	Dilution	Net cpm/ml whole blood	% Inhibition
PHA with sera	1:2	1.03×10^4	
PHA with CPA sera	1:2	0	100
PHA with CPA sera	1:4	720	93
PHA with CPA sera	1:16	4570	54
PHA with CPA + NAC sera	1:2	0	100
PHA with CPA + NAC sera	1:4	1000	90
PHA with CPA + NAC sera	1:16	4410	56

* Normal human donors were used for a lymphocyte source. The CPA sera were similar to those shown in Table 5 and diluted with mouse sera from non-treated animals. All tubes had 1 μCi [^{3}H]T added 2 hr before removal of cells and counting. Triplicate samples were used for all treatments and the averages are reported. This is a representative experiment which has been repeated at least twice.

by preventing the transferred T cell from elaborating or reacting against the new host or by preventing the host from responding to the stimulus of the T cell. Although we did not dissect the mechanism of CPA inhibition, in a dose response curve (see Table 6) it is obvious that NAC did not alter the decrease in popliteal node size in the GvHR when gradually increasing doses of CPA are administered. It should be noted that in this experiment, rather than the usual 4:1 NAC:CPA ratio, we used between 10:1 and 100:1 without altering the CPA effect.

The formation of antibodies to SRBC is considered to be a T-dependent B cell response to the SRBC antigen. Measurements of lymphocyte hemolytic activity were profoundly inhibited by CPA at lower doses. When NAC was administered, there was no decrease in the CPA inhibition of these SRBC antibody responses. Since we did not use a T-independent antigen such as S III pneumococcal polysaccharide, we cannot say that we have any selective activity by CPA on a population of cells. The specific population of cells which is sensitive to CPA is still unsettled and these experiments do not support any position.

The stimulation of lymphocytes by the plant mitogen, phytohemagglutinin, is believed to be primarily on the T cell population. Using sera containing known amounts of biologically obtained alkylating agents, we used the bioassay technique of PHA-stimulated human lymphocytes to determine the action of NAC on the efficacy of circulating CPA-derived alkylating agents to inhibit PHA-induced proliferation. In this relatively simple lymphoproliferation system *in vitro*, the efficacy of CPA-alkylating metabolite to inhibit this proliferation was not affected by NAC.

These data and interpretations lead one to ask why some CPA effects are altered and others are not. Recent studies on the metabolism of CPA have indicated that a number of potential nitrogen mustards are formed from CPA [29]. In addition to these nitrogen mustards, a body of information is developing that acrolein is a metabolic by-product [30]. When analogues of CPA are incubated with rat liver microsomes and the products analyzed by mass spectra, analogues of acrolein are observed. Acrolein and other CPA-derived aldehydes were studied for irritation, and it was shown that NAC successfully inhibited the paw edema induced by acrolein and chloracetaldehyde but not that induced by mechlorethamine [31]. This set of experiments supports the thesis that the potential tissue irritant is an irritating aldehyde, probably acrolein, derived from CPA metabolism and not the nitrogen mustard. NAC because of its stability is present long enough to react with acrolein so that tissue irritant concentration is avoided. Since the acrolein is more reactive than the mustards produced, the mustards are not affected and there is no significant decrease in concentration of efficacy.

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