The influence of single application of paracetamol and/or N-acetylcysteine on rats in subchronic exposition to trichloroethylene vapours. II. Effect on hepatic glutathione level

Wpływ pojedynczej dawki paracetamolu i/lub N-acetylocysteiny na szczury przewlekle eksponowane na trichloroetylen. II. Wpływ na wątrobowy poziom glutationu

Danuta Plewka [1(d, f)], *Andrzej Plewka* [2 (a, d, f)], *Joanna Kowalówka-Zawieja* [3 (b ,c)], *Barbara Zielińska-Psuja* [3 (a, b)], *Jędrzej Przystanowicz* [3 (c, e)]

[1] *Department of Histology, Medical University of Silesia, Katowice*
Head of Department: Ryszard Wiaderkiewicz MD, PhD, associated professor
[2] *Department of Proteomics, Medical University of Silesia, Sosnowiec*
Head of Department: Prof. Andrzej Plewka MD PhD
[3] *Department of Toxicology, Medical University, Poznań*
Head of Department: Prof. Jadwiga Jodynis-Liebert PhD

(a) *Idea*
(b) *Compilation of grant application form*
(c) *Collection of material for research*
(d) *Laboratory tests*
(e) *Statistics*
(f) *Working on text and references*

Abstract

Background: Feature of modern existing hazards both environmental and occupational is cumulative exposure often leading to unexpected response of the organism resulting, among other things, in interactions with cytochrome P450 system involved in biotransformation of trichloroethylene and paracetamol. Hepatotoxity of paracetamol is closely connected with hepatic glutathione level. „In therapy of acute paracetamol poisoning application of N-acetylcysteine as a factor, which protects GSH level in cells, is recommended."

Materials and method: Tests were performed on rats which were treated with trichloroethylene, paracetamol and/or N-acetylcysteine. In rat liver total level of glutathione was determined i.e. reduced and oxidized form.

Results: Paracetamol just after completion of the exposure affected the glutathione level. Trichloroethylene throughout the period of observation stimulated growth of glutathione level in liver. N-acetylcysteine didn't have any influence on the level of investigated tripeptyde.

Conclusions: N-acetylcysteine removes negative effect of paracetamol especially when it's applied with 2-hour delay. After exposure for trichloethylene immediate application of N-acetylcysteine caused noticeable lowering of glutathione level. Cumulative exposure for three xenobiotics had positive influence for glutathione level in rat liver.

Keywords: glutathione, liver, trichloroethylene, paracetamol, N-acetylcysteine

Streszczenie

Wstęp: Cechą współcześnie występujących zagrożeń, zarówno środowiskowych jak i zawodowych jest narażenie łączne, wielokrotnie prowadzące do nieprzewidzianej odpowiedzi biologicznej organizmu, wynikają-

Nadesłano: 28.02.2012
Zatwierdzono do druku: 26.03.2012

cej między innymi z oddziaływań na układ cytochromu P450 biorący udział w biotransformacji trichloroetylenu i paracetamolu. Hepatotoksyczność paracetamolu jest między innymi ściśle związana z wątrobowym poziomem glutationu. W terapii zatruć ostrych paracetamolem zalecane jest podawanie N-acetylocysteiny jako czynnika ochraniającego poziom GSH w komórkach.

Materiał i metody: Badania wykonano na szczurach, które traktowano trichloroetylenem, paracetamolem i/lub N-acetylocysteiną. W wątrobie szczura oznaczano poziom całkowity glutationu, tj. formę zredukowaną i utlenioną.

Wyniki: Paracetamol tuż po zakończeniu ekspozycji negatywnie wpływał na poziom glutationu. Trichloroetylen przez cały czas obserwacji stymulował wzrost poziomu glutationu w wątrobie. N-acetylocysteina nie miała żadnego wpływu na poziom badanego tripeptydu.

Wnioski: N-acetylocysteina usuwała negatywny wpływ paracetamolu, szczególnie wtedy, kiedy podano ją z 2-godzinnym opóźnieniem. Po narażeniu na trichloroetylen natychmiastowe podanie N-acetylocysteiny niosło za sobą wyraźnie obniżenie poziomu glutationu. Narażenie łączne na trzy oceniane ksenobiotyki

Słowa kluczowe: glutation, wątroba, trichloroetylen, paracetamol, N-acetylocysteina

Introduction

In recent years glutathione is one of the most widely examined molecules present in human body cells. It is attributed unusual and unique properties in scientific literature [1]. This compound is involved in maintaining an appropriate oxidation-reduction potential in the cell [2] which is of great importance for intracellular metabolism [3]. In eukaryotic cells a reduced form of glutathione (GSH) is predominant.

Paracetamol hepatotoxicity is among other factors closely connected with hepatic level of glutathione [4]. The role of glutathione in the maintenance of hepatic cells integrity has been known for many years [5, 6]. Availability of this tripeptide to form complexes with reactive chemical compounds is frequently a critical factor in toxicity of numerous foreign compounds. It is even suggested that GSH level can have great significance in determination of the body detoxication abilities. In the therapy of severe paracetamol poisoning, N-acetylcysteine (NAC) administration is recommended, as an agent protecting GSH level in cells. What is more, in animal studied it was demonstrated that NAC drastically decreases animals death rate following paracetamol overdose [7–9].

Paracetamol is a commonly used medication which, if applied in therapeutic doses, is safe, whereas in case of an overdose it can damage the liver through a toxic metabolite N-acetyl-benzoquinoneimine. This metabolite is inactivated mainly through conjugation with glutathione (GSH) [10]. After APAP ingestion in doses exceeding therapeutic dose, hepatic detoxication ability is rapidly saturated and by-products are accumulated. It is important to note that reactive metabolites formed during trichloroethylene and paracetamol alterations can synergically increase depletion of endogenous glutathione supplies.

Therefore, the aim of this study is to evaluate the effect of N-acetylcysteine (NAC) as an agent protecting GSH level in hepatocytes. Among other factors, NAC drastically decreases animals death rate after paracetamol overdose [7] and is effective in the treatment of acute acetaminophen poisoning [8, 9].

Materials and methods

Animals

The examinations were conducted on male Wistar rats with body mass 280–300 g. The animals were kept separately in plastic cages throughout the examination in controlled culture conditions with constant air humidity (60%), constant temperature (22 ± 2° C) and 12 hour cycle day/night. The animals were fed on Murigan type standard granulated fodder, with unlimited water access.

This research was approved by the Local Bioethics Committee of The Medical University in Poznań.

Experiment outline

The animals were divided into groups, 6 in each. They were administered xenobiotics separately and collectively according to the following regimen:

1. The control group
2. APAP – 250 mg/kg m.c.
3. TRI – 50 mg/m^3
4. NAC – 150 mg/kg m.c.
5. TRI 50 mg/m^3 + NAC (0 h) 150 mg/kg m.c.
6. TRI 50 mg/m^3 + NAC (2 h) 150 mg/kg m.c.
7. APAP – 250 mg/kg m.c. + TRI 50 mg/m^3
8. APAP – 250 mg/kg m.c. + NAC (0 h) 150 mg/kg m.c.
9. APAP – 250 mg/kg m.c. + NAC (2 h) 150 mg/kg m.c.
10. APAP – 250 mg/kg m.c. + TRI 50 mg/m^3 + NAC (0 h) 150 mg/kg m.c.
11. APAP – 250 mg/kg m.c. + TRI 50 mg/m^3 + NAC (2 h) 150 mg/kg m.c.

The control group were the animals not exposed to the mentioned xenobiotics. Experimental group animals were exposed to TRI vapours through inhalation route in the dynamic toxicological chamber in concentration 50 mg/m^3 of air for the following 7 days, 6 hours daily. Exposure to TRI lasted between 9.00 and 15.00. On the last day of exposure, on 9.00 were administered APAP by stomach tube. NAC was administered along with examined xenobiotics right after the exposure (0 h) or 2 hours following their application (2 h).

Determination of glutathione levels

The total level of glutathione in the rat liver was determined by the method of Adams et al. [11]. Liver samples about 50 mg tissue. were homogenized in 10 mM/dm3 DNTB dissolved in 100 mM phosphate buffer (pH 7.5) containing 5 mM/dm^3 EDTA (buffer A). The resultant suspension was diluted with 10 volumes of buffer A. After centrifuging (2000 g, 5 min) 0.1 cm3 of supernatant was mixed with 0.1 cm^3 of a 5 mM/dm^3 solution of DNTB in buffer A and 0.1 cm^3 of glutathione reductase (5 U/cm^3 in buffer A). The control sample contained buffer A instead of the supernatant.

The reaction was triggered by adding 0.7 cm^3 of 0.3 mmole/dm^3 NADPH dissolved in buffer A. Change in absorption at 412 nm for a period of 6 min (Hitachi U-3210 spectrophotometer) was measured. The level of glutathione was determined using a calibration curve (ΔA/6 min) which was plotted for known levels of glutathione. The results were expressed in mg of glutathione per gram liver tissue.

Statistical analysis

One- and two-way ANOVA followed by the Dunnett test were used for comparison amongst the multiple treated groups and the control. Results represent the means ± S.D., n = 6. The statistically significant differences at $p < 0.05$ were marked using a star symbol "*".

Results

Exposure to APAP resulted in glutathione decrease (approximately 80% of control value) immediately after completion of the experiment (Table I), Inhibitory effect of paracetamol lasted till 12 hour of the experiment. Since 24 hour, there was a tendency to increase tripeptide concentration. After 5 days glutathione concentration significantly exceeded control level.

If rats were treated with TRI, the effect of glutathione stimulation would be already demonstrated after 4 hours (Table I). Such condition lasted till 48 hour since the completion of the experiment, to return to control level after 5 days.

Treatment with N-acetylcysteine had no effect on glutathione level (Table I). Throughout the experiment there was a tendency to a subtle decrease of this level.

Exposure to combined APAP and TRI dose initially led to a distinct decrease of glutathione level just after 4 hours of the experiment (80% of control value) which increased with time (55% in 12 hours after the experiment completion). Since then, the concentration of this tripeptide increased, reaching 135% of control value by the end of the experiment (Table I).

Table I. Effect of studied xenobiotics on level of glutathione in liver of rat
Tabela I. Wpływ badanych ksenobiotyków na poziom wątrobowego glutationu u szczura wyrażonego w mg/g tkanki wątrobowej

Xenobiotic	Time after exposition [w hours]				
	4	12	24	48	120
	The control = 1,218 ± 0,159				
APAP	0,936 ± 0,063	0,867 ± 0,054*	1,039 ± 0,077	1,273 ± 0,038	1,462 ± 0,105*
TRI	1,627 ± 0,075*	1,698 ± 0,058*	1.790 ± 0,144*	1,571 ± 0,054*	1,352 ± 0,086
NAC	1,103 ± 0,094	1,522 ± 0,147	1,166 ± 0,118	0,989 ± 0,129	1,074 ± 0,159
APAP + TRI	0,963 ± 0,093	0,687 ± 0,045*	1,339 ± 0,177	1,473 ± 0,038	1,662 ± 0,125*
APAP + NAC [0 h]	0,896 ± 0,081	1,117 ± 0,119	1,169 ± 0,165	1,202 ± 0,072	1,163 ± 0,146
APAP + NAC [2 h]	1,626 ± 0,180	1,163 ± 0,131	1,144 ± 0,186	1,393 ± 0,019	1,543 ± 0,235
TRI + NAC [0 h]	0,647 ± 0,094*	0,670 ± 0,039*	0,844 ± 0,110	1,076 ± 0,085	1,257 ± 0.179
TRI + NAC [2 h]	1,722 ± 0,210	1,027 ± 0,101	1,127 ± 0,208	1,148 ± 0,063	0,814 ± 0,071*
APAP + TRI + NAC [0 h]	1,608 ± 0,240	1,539 ± 0,121	1,574 ± 0,098	1,248 ± 0,033	1,034 ± 0,145
APAP + TRI + NAC [2 h]	1.050 ± 0,202	1,633 ± 0,125*	1,025 ± 0,152	1,017 ± 0,049	1,113 ± 0,162

If rats were administered APAP combined with N-acetylcysteine, there would be a tendency to a mild decrease in glutathione concentration (75% of control value in 4 hour of the experiment) which faded and control level was maintained till 120 hour (Table I).

If N-acetylcysteine was administered 2 hours after paracetamol treatment, a different course of glutathione concentration changes would be revealed. Through the first hours of the experiment glutathione level tended to increase, however, after 12 hours the concentration of this peptide returned to control level and its repeated increase after 5 days (Table I).

Combined administration of TRI and N-acetylcysteine LED to a marked decrease of glutathione concentration in initial stage of the experiment (approximately 55–60% till 24 hour – Table I). In 48 hour of the experiment the control level of glutathione concentration was already revealed, which remained unchanged till the experiment completion.

However, if N-acetylcysteine was administered 2 hours following exposure to TRI, the effect would be the opposite. After 4 hours of the experiment, an increased glutathione level was found and till 48 hour of the experiment the control level was revealed, which after 5 days decreased to 65% of control value (Table I).

Exposure to combined, simultaneous administration of all three xenobiotics had no significant effect on glutathione concentration in final stages of the experiment (Table I). In the first two stages of the experiment a slight tendency to stimulation was found.

However, if N-acetylcysteine was administered with delay, with the exception of 12 hour where GSH level increase was observed, in the remaining stages a tendency to a subtle decrease in tripeptide concentration was found (Table I).

Discussion

The examined possibilities of increasing GSH level in cells include: administration of exogenous GSH and its derivatives, increase of GSH biosynthesis by administration of non toxic cysteine precursors and increase in the rate of GSSG reduction process.

The reactions leading to GSH cellular concentration decrease are, among others, formation of S-conjugates with toxic electrophilic exogenous and endogenous substances. Biological significance of these alterations lies in an increase of the substance hydrophilicity, so that they can be successfully excreted by the urinary system. However, a decrease in toxicity not always occurs, occasionally it can even intensify (12–14). Such is the case with an increased incidence of neoplasms, located in renal proximal tubules in individuals exposed to prolonged action of trichloroethylene [15–17].

In case of paracetamol detoxication, microsomal metabolism of this medication with P450 cytochrome involved, leads to extremely toxic N-acetyl-p-benzoquinoneimine [10]. In GSH deficiency this toxic metabolite causes DNA and protein arylation, modification of cellular thiolic groups, increase in oxidation reactions and finally necrosis of hepatic cells. With sufficient supply of GSH, N-acetyl-p-benzoquinoneimine is conjugated, which yields non toxic, mercapturic acid, excreted with urine. In this case xenobiotic biotransformation with GSH involvement is the actual detoxication process.

If monooxygenase system dependent on P450 cytochrome was decreased to 120 hour, the level of examined tripeptide throughout the experiment maintained the level characteristic for control.

We suggest that with the used APAP dose in these studies, appeared already advanced lipid peroxidation, combined with paracetamol-induced liver damage mechanism. These alterations are independent of alternative mechanism which causes hepatotoxicity through intermediate product of APAP bioactivation, which is unfavorable since a protective effect of GSH fails in this case [6]. Therefore, it appears that GSH level decrease as a result of hepatic GSH binding with APAP electrophilic intermediate product is an important factor determining hepatotoxicity of this xenobiotic and is probably the major path of its detoxication [4]. APAP in higher doses is a hepatotoxic substance. It is bioactivated by a set of cytochromes P450 to toxic N-acetyl-p-benzoquinone imine, which finally leads to liver damage unless it is immediately conjugated with GSH [18, 19].

As we have demonstrated, in a single dose of 250 mg/kg – APAP had a stimulating effect on P450 cytochrome level, but in a double dose it was markedly stimulated [20] which may be the evidence that rats are relatively resistant to this compound [5]. That high resistance partially is due to the fact that intermediate metabolite (NAPQI) is effectively conjugated with GSH.

Evaluation of GSH level alterations showed that tripeptide level is induced with combined exposure. It is in accordance with the study of Zhao and Shichi [21]. We demonstrated, therefore, a protective effect of NAC on glutathione level following exposure to APAP, which facilitates NAPQI detoxication through conjugation with glutathione. Very interesting observations concerned the behaviour of glutathione level with protective agent administration. After simultaneous administration of NAC till 24 hour the GSH level was lower than control and only in 120 hour it began to exceed this level. Delayed NAC administration usually led also to

a negative effect on GSH level. Our results indicate the need to continue these studies.

Reduced glutathione functions as a reductive agent in metabolism of numerous peroxides. This reaction is catalyzed by glutathione peroxidases [22]. Relatively high reduction of intracellular GSH level may lead to oxidative stress [23, 24]. On the other hand, monoelectrone paracetamol reduction by P450 cytochrome leads to the formation of reactive oxygen forms, which results in excessive loss of thiolic groups and finally causes hepatotoxic effect [25, 26].

N-acetylcysteine removes negative effect of paracetamol especially when it is applied applicated with 2-hour delay. After exposure for trichloethylene only immediate application of N-acetylcysteine caused noticeable lowering of glutathione level. Cumulative exposure for three xenobiotics had positive influence for glutathione level in rat liver.

The study was supported by a grant from the State Committee for Scientific Research (KBN – 4 P05D 021 15)

References

1. Winiarska K.: Glutation: niezwykłe funkcje pospolitego peptydu. Post Biochem 2000; 46: 318–326.
2. Sies H.: Glutathione and its role in cellular functions. Free Radic Biol Med 1999; 27: 916–921.
3. De Nicola M., Gualandi G., Alfonsi A., et. al.: Different fates of intracellular glutathione determine different modalities of apoptotic nuclear vesiculation. Biochem Pharmacol 2006; 72: 1405-1416.
4. Kuralay F., Akarca U.S., Ozutemitz O., et. al.: Possible role of glutathione in prevention of acetaminophen-induced hepatotoxicity enhanced by fish oil in male Wistar rats. J Toxicol Environm Health 1998 Part A; 53: 223-229.
5. Amimoto T., Matsura T., Koyama S.-Y., et. al.: Acetaminophen-induced hepatic injury in mice: The role of lipid peroxidation and effects of pretreatment with coenzyme Q10 and ?-tocopherol. Free Radic Biol Med 1995; 19: 169-176.
6. Comporti M., Maellaro E., Del Bello B., et. al.: Glutathione depletion: its effects on other antioxidant systems and hepatocellular damage. Xenobiotica 1991; 21: 1067-1076.
7. Tran A., Treluyer J.M., Rey E., et. al.: Protective effect of stiripentol on acetaminophen-induced hepatotoxicity in rat. Toxicol Appl Pharmacol 2001; 170: 145-152.
8. Woo O.F., Mueller P.D., Olson K.R., et. al.: Shorter duration of oral N-acetylcysteine therapy for acute acetaminophen overdose. Ann Emerg Med 2000; 35: 363-368.
9. Buckley N.A., Whyte I.M., O'Connell D.L., et. al.: Oral or intravenous N-acetycysteine: Which is the treatment of choice for acetaminophen (paracetamol) poisoning? J Toxicol Clin Toxicol 1999; 37: 759-767.
10. Dahlin D.C., Miwa G.T., Lu A.Y., et. al.: N-acetyl-p-benzoquinone imine: A cytochrome P-450-mediated oxidation product of acetaminophen. Proc Natl Acad Sci USA 1984; 81: 1327-1331.
11. Adams J.D., Lauterburg B.H., Mitchell J.R.: Plasma glutathione and glutathione disulfide in the rat: regulation and response to oxidative stress. J Pharmacol Exp Ther 1983; 227: 745-754.
12. Bloemen L.J., Monster A.C., Kezic S., et. al.: Study on the cytochrome P-450– and glutathione-dependent biotransformation of trichloroethylene in humans. Int Arch Occup Environ Health 2001; 74: 102-108.
13. Lash L.H., Qian W., Putt D.A., et. al.: Renal and hepatic toxicity of trichloroethylene and its glutathione-derived metabolites in rats and mice: sex-, species-, and tissue-dependent differences. J Pharmacol Exp Ther 2001; 297: 155-164.
14. Lash L.H., Putt D,A., Huang P., et. al.: Modulation of hepatic and renal metabolism and toxicity of trichloroethylene and perchloroethylene by alterations in status of cytochrome P450 and glutatione. Toxicology 2007; 235: 11-26.
15. Henschler D., Vamvakas S., Lammert M., et. al.: Increased incidence of renal cell tumors in a cohort of cardboard workers exposed to trichloroethene. Arch Toxicol 1995; 69: 291-299.
16. Lash LH, Putt DA, Parker JC.: Metabolism and tissue distribution of orally administered trichloroethylene in male and female rats: identification of glutathione– and cytochrome P-450-derived metabolites in liver, kidney, blood, and urine. J Toxicol Environ Health A 2006; 69: 1285-1309.
17. Lash L.H., Lipscomb J.C., Putt D.A., et. al.: Glutathione conjugation of trichloroethylene in human liver and kidney: kinetics and individual variation. Drug Metab Dispos 1999; 27: 351-359.
18. Ryu B.K., Ahn B.O., Oh T.Y., et. al.: Studies on protective effect of DA-9601, Artemisia asiatica extract, on acetaminophen– and CCl4-induced liver damage in rats. Arch Pharm Res 1998; 21: 508-513.
19. Abdel Salam OM., Baiuomy A..R, El-Shenawy S.M., et. al.: Effect of pentoxifylline on hepatic injury caused in the rat by the administration of carbon tetrachloride or acetaminophen. Pharmacol Rep 2005; 57: 596-603.
20. Plewka A., Grażyna Nowaczyk G., Plewka D., et. al.: N-acethylcysteine effect on microsomal monooxygenase system and glutathione levels in rat after acute trichloroethylene intoxications. Acta Toxicol 2003; 11: 99-111.
21. Zhao C., Shichi H.: Prevention of acetaminophen-induced cataract by a combination of diallyl disulfide and N-acetylcysteine, J Ocul Pharmacol Ther 1998; 14: 345-355.
22. Halliwell B., Gutteridge J.M.C.: Protection against oxidants in biological systems: The superoxide theory of oxygen toxicity. In: Halliwell B., Gutteridge J.M.C., eds. Free radicals in biology and medicine, Oxford University Press, New York, 1989; pp. 86-187.
23. Cummings B.S., Parker J.C., Lash LH.: Role of cytochrome P450 and glutathione S-transferase alpha in the metabolism and cytotoxicity of trichloroethylene in rat kidney. Biochem Pharmacol 2000; 59: 531-543.
24. Bilska A., Krypczyk A., Włodek L.: Różne oblicza biologicznej roli glutationu. Post Hig Med Dosw 2007; 61: 438-453.
25. Moore M., Thor H., Moore G., et. al.: The toxicity of acetaminophen and N-acetyl-p-benzoquinone imine in isolated hepatocytes is associated with tiol depletion and increased cytosolic Ca2+. J Biol Chem 1985; 260: 13035-13040.
26. Corcoran G.B., Wong B.K. Role of glutathione in prevention of acetaminophen-induced hepatotoxicity by N-acetyl-L-cysteine in vivo: studies with N-acetyl-D-cysteine in mice. J Pharmacol Exp Ther 1986; 238: 54-61.

Address for correspondence:
Andrzej Plewka
Department of Proteomics
Medical University of Silesia,
ul. Ostrogórska 30, 41-200 Sosnowiec
tel./fax +48 32 364-14-40
e-mail: aplewka@sum.edu.pl