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Antioxidant Effect of Taurine Against Lead-Induced Oxidative Stress

H. Gürer,[1] H. Özgünes,[1] E. Saygin,[2] N. Ercal[3]

[1] University of Hacettepe, Faculty of Pharmacy, Department of Toxicology, 06100 Ankara, Turkey
[2] Department of Chemistry, University of Missouri-Rolla, Rolla, Missouri 65409-0010, USA
[3] Department of Medicine, Washington University School of Medicine, Box 8046, Lipid Research, 660 S. Euclid Ave., St. Louis, Missouri 63110, USA

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Abstract. Oxidative stress is proposed as a molecular mechanism in lead toxicity, which suggests that antioxidants might play a role in the treatment of lead poisoning. The present study was designed to investigate whether taurine has a beneficial effect both on Chinese hamster ovary (CHO) cells and on Fisher 344 (F344) rats following lead exposure. Therefore, oxidative stress parameters (glutathione, malondialdehyde levels, catalase, and glucose-6-phosphate dehydrogenase [G6PD] activities) of lead-exposed CHO cells and F344 rats were determined following taurine treatment. Taurine was found to be effective in (1) increasing glutathione levels that had been diminished by lead; (2) reducing malondialdehyde levels, an end-product of lipid peroxidation; (3) decreasing catalase and erythrocyte G6PD activity, which had been increased by lead exposure; and (4) improving cell survival of CHO cells. However, taurine had no effect on blood and tissue lead levels when 1.1 g/kg/day taurine was administered to F344 rats for 7 days, following 5 weeks of lead exposure (2,000 ppm lead acetate). As a result, taurine seems to be capable of fortifying cells against lead-induced oxidative attack without decreasing lead levels. Therefore, administration of taurine, accompanied by a chelating agent, might increase its effectiveness in the treatment of lead poisoning.

Lead poisoning is a multifaceted problem. Disruption of a variety of biochemical processes, rather than a single mechanism, is responsible for the toxicity. Although chelating agents are currently available for the treatment of lead poisoning, they have been shown to have many side effects and to be incapable of alleviating some toxic effects of lead (Cory-Slechta *et al.* 1987; Mortensen and Walson 1993; Porru and Alessio 1996). Accumulated evidence of lead's capacity to induce oxidative stress suggests that antioxidants may have an important role in abating some hazards of lead. We hypothesize that usage of an antioxidant agent in the presence of a chelator will increase the efficacy of the lead poisoning treatment.

We have previously shown that N-acetylcysteine and lipoic acid, two well-known antioxidants, are effective in rebalancing the oxidant/antioxidant ratio in lead-exposed Chinese hamster ovary (CHO) cells and Fisher 344 (F344) rats (Ercal *et al.* 1996; Gurer *et al.* 1998, 1999a). Taurine, a sulfur-containing β-amino acid (Figure 1) is found in millimolar concentrations, especially in tissues that are excitable, rich in membranes, and generate oxidants (Jacobsen and Smith 1968; Wright *et al.* 1986). The sulfonate group in taurine is a strong acid that makes it completely zwitterionic over the physiological pH range (Huxtable 1992). A number of functions have been shown for taurine, such as maintaining calcium homeostasis, osmoregulation, removal of hypochlorous acid, and stabilizing the membranes (Gaull *et al.* 1985; Wright *et al.* 1986; Huxtable 1992). Some of the recent data indicate that taurine can act as a direct antioxidant by scavenging reactive oxygen species (ROS) (Cozzi *et al.* 1995; Redmond *et al.* 1996) and/or as an indirect antioxidant by preventing changes in membrane permeability due to oxidant injury (Nakashima *et al.* 1982; Banks *et al.* 1992; Gordon *et al.* 1992). However, some contradictory data have also been reported (Aruoma *et al.* 1988; Shi *et al.* 1997).

In the present study, the main goal was to investigate the effects of taurine on lead-induced oxidative stress in *in vivo* and *in vitro* systems. CHO cells were used as the *in vitro* model, while brain, kidney, and liver from lead-exposed F344 rats were analyzed as the *in vivo* model. To achieve the above goals, measurements of malondialdehyde (MDA) levels, as an indicator of lipid peroxidation; glutathione (GSH) levels, representing the thiol status of the cells; catalase (CAT) activity, as an important component of antioxidant defense system of the cells; and glucose-6-phosphate dehydrogenase (G6PD) activity, as an important reducing equivalent provider for erythrocytes were made. The possible protective effect of taurine against the cytotoxic effect of lead was examined in CHO cells. Lead concentrations from tissue and blood samples of F344 rats were further analyzed to evaluate whether taurine lowers lead levels in blood and tissues.

Correspondence to: N. Ercal; *email:* nercal@umr.edu

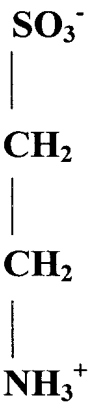

Fig. 1. Chemical structure of taurine at pH 7.4

Materials and Methods

Materials

The N-(1-pyrenyl)-maleimide, 1,1,3,3-tetramethoxypropane, and 2-vinyl pyridine were purchased from Aldrich (Milwaukee, WI). All other chemicals were purchased from Sigma (St. Louis, MO). HPLC-grade reagents were used in GSH and MDA analysis.

Cell Culture Studies

CHO cells were propagated in Ham's F12 culture media supplemented with 10% fetal calf serum and 0.5% glutamine. Cells were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

Colony Formation Assays: Exponentially growing cells were collected after trypsinization and centrifuged at 1,000 g for 5 min. The resulting cell pellets were resuspended in fresh media and counted on a hemocytometer. Between 100–2,000 cells were plated into small (60-mm) petri dishes and incubated for 4 h to allow cell attachment. Cells were then exposed to gradually increasing concentrations of lead as lead acetate (0 to 500 μM) for an additional 6 h. Following lead treatment cells were washed and covered with 5 ml of fresh media either plain or containing 10 mM taurine and incubated for 7–10 days. At the end of incubation period, resulting cell colonies were stained with methylene blue and counted. Results reported from colony formation assays represent at least five separate experiments performed in triplicates. The colony efficiency (CE) was calculated as:

$$\text{CE} = \frac{\text{Colonies counted}}{\text{Cells seeded}} \times 100$$

A cell survival curve was constructed by plotting the surviving fraction (number of colonies counted, divided by the number of cells seeded, times the colony efficiency of the control) from the "lead only" and "lead + taurine" groups versus the lead concentration.

Oxidative Stress Studies: CHO cells (5×10^5 cells/flask) were plated into flasks and incubated for 4 h to facilitate attachment. Three groups were prepared. The "control group" was incubated in the basic media for 24 h. The "lead only" group was incubated in 500 μM lead acetate containing media for 20 h. At the end of this period, lead-containing media were discarded and cells were incubated for additional 4 h in plain media. Cells from the "lead + taurine" group were exposed to 10 mM taurine containing media for 4 h following exposure to lead for 20 h. All solutions were freshly prepared prior to use. At the end of the incubation period, cells were trypsinized, resuspended in fresh media, and homogenized, then analyzed for oxidative stress parameters.

Animal Studies

All experiments were performed with F344 male rats weighing 75–100 g. The animals were housed in stainless steel cages in a temperature-controlled room (22°C) with a 12 h light:dark cycle. They were fed with standard rat chow (Purina rat chow). The animals were randomized into three groups. Group I (n = 10) served as the control and was given only standard rat chow and water for 6 weeks. Group II (n = 10) received 2,000 ppm lead acetate in drinking water for 5 weeks and received plain water only during the 6th week. Group III (n = 5) received 2,000 ppm lead acetate in drinking water for 5 weeks and, during the 6th week, received 1.1 g/kg/day taurine in drinking water.

At the end of the 6th week, after overnight fasting, the animals were anesthetized with metofane, and blood samples were collected via intracardiac puncture using heparin as an anticoagulant. Plasma and the buffy coat were removed by centrifugation for 10 min at 3,000 rpm. The red blood cells (RBCs) were washed three times with an equal volume of cold saline. The RBCs samples were maintained at −70°C for MDA assays (not longer than 7 days) and at 4°C for CAT assay (not longer than 4 days) (Aebi 1984). The tissue samples were also collected after sacrificing and kept at −70°C until analyzed.

GSH Determination: GSH levels were determined using the method developed by Winters et al. (1995). Briefly, tissue samples were homogenized in serine-borate buffer (100 mM Tris-HCl, 10 mM boric acid, 5 mM L-serine, 1 mM DETAPAC, pH 7.4) and derivatized with 1.0 mM *N*-(1-pyrenyl)maleimide (NPM) in acetonitrile. The samples were incubated for 5 min, then acidified with 1:6 HCl:H_2O. The derivatized samples were filtered through a 0.2-μm acrodisc and injected onto a 3-μm C_{18} column in a reverse-phase HPLC system. The GSH assays were performed with a Shimadzu HPLC system comprised of a model LC-10A pump, a model RF-535 spectrofluorometer (330 nm excitation, 380 nm emission), and a model SCL-10A system controller. The column (Astec, Whippany, NJ; 100 × 4.6 mm) was packed with 3-μm pore size C_{18} packing material. Quantification of the peaks was performed with a Shimadzu model CR601 Chromatopac. The mobile phase consisted of 35% water, 65% acetonitrile, with 1 ml/L acetic acid and 1 ml/L phosphoric acid. The samples were eluted isocratically at a flow rate of 0.5 ml/min. The results were given as nmol/mg protein where protein concentrations were determined by using Bradford method (Bradford 1976).

MDA Determination: The samples were analyzed according to the Draper method (Draper *et al.* 1993) with the following modifications: Tissue samples were homogenized in serine-borate buffer; 0.250 ml of homogenate was added to 0.650 ml of 5% trichloroacetic acid and 0.100 ml of 500 ppm butylated hydroxytoluene in methanol. The sample was heated in a boiling water bath for 30 min, followed by cooling on ice and centrifugation. The supernatant was reacted 1:1 (v/v) with saturated 2-thiobarbituric acid. The sample was again heated for 30 min followed by a second cooling on ice. One-half milliliter of the sample was extracted with 1.0 ml n-butanol and centrifuged to facilitate phase separation. The supernatant was then filtered through a 0.45-μm acrodisc and injected onto a reverse-phase 3-μm C_{18} column, 250 × 4.6 mm. The mobile phase used was 30% acetonitrile, 69.4% phosphate buffer (5 mM, pH = 7.0), and 0.6% tetrahydrofuran. The reaction mixture was eluted from the column isocratically at a flow rate of 0.75 ml/min.

CAT Activity Determination: The activity of tissue CAT was determined spectrophotometrically (Beers 1952), which measured the dis-

appearance of 10 mM H_2O_2 at 240 nm. The equation used to determine the reduction of H_2O_2 was:

$$A_{60} = A_0 e^{-kt}$$

where k = the rate constant dependent on CAT activity.

G6PD Activity Determination: Determination of G6PD activity was performed by using a spectrophotometer, as detailed in Tietz (1986), where glucose-6-phospate and $NADP^+$ were used as substrates. The enzyme activity is determined by measurement of the rate of increase (at 340 nm) in NADPH concentration.

Protein Level Determination: Protein levels were spectrophotometrically estimated by the method of Bradford (1976) using concentrated Coomassie Blue (Bio-Rad). Absorbance of samples was measured at 595 nm.

Hemoglobin Measurement: Hemoglobin contents of the erythrocyte samples were measured spectrophotometrically, as detailed by Tietz (1986).

Tissue and Blood Lead Levels: Lead levels were assayed by atomic absorption spectroscopy (Varian SpectrAA) by the CDC-certified analytical laboratory at the Springfield-Greene County Department of Public Health, Springfield, MO.

Statistical Analysis

The nonparametric Mann-Whitney U test was used to analyze the significance of the differences between control and experimental groups.

Results

Cell Culture Studies

Colony Formation: Figure 2 represents the survival curve, generated by plotting the survival fractions of cells treated with lead in the presence or absence of taurine against increasing concentrations of lead. Incubation of CHO cells with lead inhibited colony formation in a concentration-dependent manner. The inhibitory effect of 500 μM lead acetate on survival fractions of CHO cells was negated by 10 mM taurine administration.

GSH Levels: Lead exposure significantly diminished GSH levels of CHO cells. GSH content of lead-exposed cells was notably increased in the taurine-supplemented group (Table 1).

MDA Levels: MDA levels of cells from control and treated groups are shown in Table 1. MDA levels were raised twofold by lead exposure. Incubation with taurine in lead-treated groups significantly decreased MDA levels.

CAT Activity: Lead exposure induced a significant increase in CAT activity of CHO cells. Increased CAT activity in lead-exposed cells was slightly decreased by further incubating the cells with taurine, although the results were not statistically significant (Table 1).

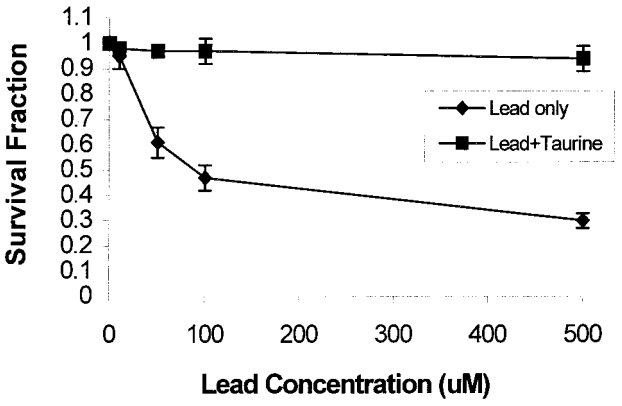

Fig. 2. Survival curve of CHO cells exposed to lead in the presence or absence of taurine

Animal Studies

GSH Levels: Lead exposure resulted in significantly reduced RBCs (Table 2) and brain (Table 3) GSH levels. Taurine administration, following lead exposure, was effective in increasing the diminished GSH levels both in RBCs and the brain (Tables 2 and 3). On the other hand, GSH levels increased in the kidneys of lead-exposed animals and were significantly higher in taurine-treated lead-exposed animals (Table 3).

MDA Levels: MDA levels were significantly elevated in RBCs (Table 2) brain and kidney (Table 3) of lead-exposed rats. Taurine administration markedly lowered MDA levels in all three tissues.

CAT Activity: Lead exposure caused an increase in CAT activity both in RBCs (Table 2) and kidney tissues (Table 3) of F344 rats. The enzyme activity reverted to control levels both in RBCs and kidney specimens of taurine-treated animals. Brain CAT activity was undetectable.

G6PD Activity: G6PD activity was measured in RBCs of the control, lead-exposed and taurine-treated F344 rats. Enzyme activity was considerably elevated following lead exposure. However, taurine treatment for 7 days significantly lowered the G6PD activity (Table 2).

Blood and Tissue Lead Levels: Table 4 shows the blood and tissue lead levels of control, lead-exposed and taurine-treated rats. Blood, brain and kidney lead levels were profoundly elevated after lead exposure. No significant effect of taurine on blood and tissue lead levels was observed following taurine administration.

Discussion

Our previous studies (Ercal *et al.* 1996; Gurer *et al.* 1998, 1999a) as well as the present one support the hypothesis indicated by several other groups (Ribarov and Bochev 1982; Ito *et al.* 1985; Monterio *et al.* 1985, 1991; Lawton and Donaldson 1991; Sandhir *et al.* 1994; Solliway *et al.* 1996) that

Table 1. Selective oxidative stress parameters of lead-exposed CHO cells in the presence or absence of taurine

	GSH (nmol/mg protein)	MDA (nmol/100 mg protein)	CAT (U/mg protein)
Control	43 ± 2.7	22.7 ± 2.7	16.7 ± 5.0
Lead only	32 ± 1.4*	45.5 ± 6.0*	26.2 ± 3.3#
Lead + taurine	52.4 ± 2.7**	18.7 ± 4.5**	21.5 ± 7.5

* $p < 0.01$, compared to the corresponding value of control group.
# $p < 0.05$, compared to the corresponding value of control group.
** $p < 0.05$, compared to the corresponding value of lead group.

Table 2. Selective oxidative stress parameters of erythrocytes from lead-exposed F344 rats

	GSH (nmol/g Hb)	MDA (nmol/g Hb)	CAT (U/g Hb)	G6PD (U/g Hb)
Control	25 ± 3	40 ± 7	98 ± 33	22 ± 1
Lead only	17 ± 2**	62 ± 20*	167 ± 14**	29 ± 6*
Lead + taurine	21 ± 1§	36 ± 7§	93 ± 23§	24 ± 1§§

* $p < 0.005$, compared to the corresponding value of control group.
** $p < 0.0005$, compared to the corresponding value of control group.
§ $p < 0.005$, compared to the corresponding value of lead group.
§§ $p < 0.05$, compared to the corresponding value of lead group.

Table 3. Oxidative stress related parameters from brains and kidneys of F344 rats

	Brain		Kidney		
	GSH (nmol/mg protein)	MDA (nmol/100 mg protein)	GSH (nmol/mg protein)	MDA (nmol/100 mg protein)	CAT (U/mg prot.)
Control	18.4 ± 1.4	14.6 ± 1.8	0.07 ± 0.04	7.2 ± 1.2	0.15 ± 0.03
Lead only	16.1 ± 1.7*	21.4 ± 0.7*	0.47 ± 0.1*	10.3 ± 0.3*	0.24 ± 0.03*
Lead + taurine	20.2 ± 1.0**	14.8 ± 2.8**	0.74 ± 0.3**	8.8 ± 0.9**	0.21 ± 0.01***

* $p < 0.005$, compared to the corresponding value of control group.
** $p < 0.01$, compared to the corresponding value of lead group.
*** $p < 0.05$, compared to the corresponding value of lead group.

Table 4. Blood and tissue lead concentrations from F344 rats

	Blood Lead Concentrations (μg/dl)	Brain Lead Concentrations (ppm)	Kidney Lead Concentrations (ppm)	Liver Lead Concentrations (ppm)
Control	0.43 ± 0.5	0.08 ± 0.1	0.20 ± 0.4	0.08 ± 0.08
Lead	36.4 ± 4.4*	1.30 ± 0.6**	10.8 ± 1.6**	1.3 ± 0.6**
Lead + taurine	33.8 ± 2.0	1.75 ± 0.5	13.3 ± 1.7	1.5 ± 0.5

* $p < 0.0005$, compared to the corresponding value of control group.
** $p < 0.005$, compared to the corresponding value of control group.

lead-induced oxidative stress could be, in part, responsible for lead-induced toxicity. In the present study, lead is shown to alter the oxidative stress-related parameters both in F344 rats *in vivo* and CHO cells *in vitro*. Decreased cell survival in lead-exposed CHO cells was accompanied by increased lipid peroxidation and altered antioxidant defense systems, suggesting oxidative stress as a possible mechanism for lead-induced damage. Furthermore, in one of our earlier studies, treatment of lead-exposed CHO cells with superoxide dismutase + CAT was found to reverse MDA and GSH levels back to control levels indicating that part of this injury may be mediated by ROS (Gurer *et al.* 1999a). The possible curicial role of oxidative stress in the pathophysiology of lead toxicity brought up the idea that induced damage could be relieved in part by antioxidants. We previously suggested some thiol antioxidants such as N-acetylcysteine (NAC), lipoic acid, and captopril to restore the impaired prooxidant/antioxidant balance in lead toxicity (Ercal *et al.* 1996; Gurer *et al.* 1998, 1999a, 1999b). The antioxidant activity of succimer, a potent chelating agent with two free thiol groups, was also investigated in our lab (Ercal *et al.* 1996; Gurer *et al.* 1998). Both NAC and lipoic acid as known antioxidants and succimer as a chelator were found to bolster the cells against deleterious effects of lead. Captopril was also found to have beneficial effects to some extent.

Although the mechanism of antioxidant activity in lead toxicity has not been fully elucidated yet, the free sulfhydryl group in their structure seems to play an alternate ROS scavenger role. On the other hand, taurine, a sulfur-containing β-amino acid, is also suggested as having a protective effect against oxidative damage induced by compounds such as carbon tetrachloride (Nakashima *et al.* 1982), bleomycin and paraquat (Gordon *et al.* 1992) and ethanol (Kerai *et al.* 1999). The following mechanisms have been proposed for the antioxidant effects of taurine: (1) As a direct antioxidant, taurine would quench and detoxify some reactive intermediates such as hypochlorous acid generated by myeloperoxidase (Wright *et al.* 1986; Huxtable 1992; Timbrell *et al.* 1995), nitric oxide (Redmond *et al.* 1996), and H_2O_2 (Cozzi *et al.* 1995). Taurine was also reported to have scavenged $HO^{\bullet}$, but this scavenging activity has been shown to be weak compared to that of glucose and mannitol (Aruoma *et al.* 1988). (2) As an indirect antioxidant, taurine may protect cells via intercalating into the membrane and stabilizing it (Nakashima *et al.* 1982; Gordon *et al.* 1992; Timbrell *et al.* 1995). The membrane-protective effect of taurine is suggested to be related to an action on permeability to ions and water (Wright *et al.* 1986; Timbrell *et al.* 1995).

In the light of this knowledge, the current study was undertaken to investigate whether taurine mitigates lead-induced oxidative damage in *in vivo* and *in vitro* models. The effects of taurine on MDA, as an endpoint indicator of lipid peroxidation, GSH, CAT, and G6PD activities as components of the antioxidant defense system were evaluated in lead-exposed CHO cells and F344 rats.

Lipid peroxidation, which was increased by lead exposure both in CHO cells and F344 rats, was found to be decreased following taurine supplementation. Lipid peroxidation is a chain reaction where ROSs are involved. Therefore, a molecule can inhibit the reactions by quenching ROSs. Although several studies (Cozzi *et al.* 1995; Redmond *et al.* 1996) reported that taurine scavenges ROSs, this finding has not been supported by several other groups (Aruoma *et al.* 1988; Shi *et al.* 1997). Taurine is known to be a powerful scavenger of hypochlorous acid (HOCl) (Thomas *et al.* 1986). The reaction of taurine with this oxidant yields a taurine chloramine, which is a known toxin for α_1-antiproteinase; however HOCl does not seem to be involved in the lipid peroxidation process induced by lead. Therefore, the membrane-stabilizing effect of taurine and the resulting decline of membrane susceptibility to lipid peroxide formation seems more likely to happen. Furthermore, taurine-supplemented CHO cells demonstrated an increase in viability (as determined by the colony formation assay), along with a decrease in lipid peroxidation. This would also indicate that oxidative damage might contribute to the cell damage that occurred.

Further evidence of the efficacy of taurine in abating lead-induced oxidative stress includes the increase in GSH content and decreases in both CAT and G6PD activities in lead-treated cells or animals after taurine supplementation. Taurine does not have a known stimulating effect on GSH biosynthesis. On the other hand, GSH is known to react with and detoxify reactive species where its cellular levels are expected to decrease. The increases in MDA content in lead-exposed cells and tissues suggest that lead-stimulated lipid peroxidation results in the formation of aldehydic and reactive by-products, which, in turn, decrease GSH content. Therefore, it seems plausible that taurine can improve the antioxidant defense system via inhibiting the lipid peroxidation process, thereby mitigating the consumption of GSH. The same mechanism, rather than a direct effect on the enzymes, could also explain the beneficial effects of taurine on CAT and G6PD activities.

Taurine was shown to form less stable metal complexes with various transition metals, such as Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Fe^{2+}, Mn^{2+}, Mg^{2+}, than do other amino acids. Direct interaction between taurine and metal ions is mainly attributed to the electric association between metal cations and the sulfonate anion or to the interaction between metal ions and the nitrogen's unshared pair of electrons (Wright *et al.* 1986). The present study also explored whether taurine is capable of removing lead from some target tissues. Lead levels in blood, brain, kidney or liver specimens of rats from "lead" and "lead + taurine" groups were detected and compared. Taurine was shown to have no effect on tissue lead levels, indicating that its antioxidant effect shown in the present study is not a consequence of removal of lead from target tissues.

In conclusion, oxidative stress seems to contribute to lead-induced damage, even in animals with moderate blood lead levels (36.4 μg/dl). Therefore, including an antioxidant in the treatment protocol might increase the effectiveness of the treatment of lead poisoning. Taurine, as an amino acid with no known toxic effects to humans, seems to have a promising antioxidant effect against oxidative damage induced by lead. Although the present data is not designed to designate a singular molecular mechanism for the antioxidant effect of taurine, it seems likely that taurine would increase the efficacy of the treatment when administered together with a chelator. Otherwise, it would partially abate the toxic effects of lead in patients with low blood lead levels ($<$ 45 μg/dl) who are presently left untreated.

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