Toxicology Reports 6 (2019) 389–394

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Comparative effect of indomethacin (IndoM) on the enzymes of carbohydrate metabolism, brush border membrane and oxidative stress in the kidney, small intestine and liver of rats

Sheeba Khan[a], Faiz Noor Khan Yusufi[b], Ahad Noor Khan Yusufi[a,*]

[a] Department of Biochemistry, Faculty of Life Sciences, Aligarh Muslim University, India
[b] Department of Statistics and Operations Research, Faculty of Science, Aligarh Muslim University, Aligarh, 202002, U.P., India

ARTICLE INFO

Keywords:
Indomethacin
Carbohydrate metabolism
Oxidative stress
Kidney
Intestine
Liver
Toxicity

ABSTRACT

Indomethacin (IndoM) has prominent anti-inflammatory and analgesic-antipyretic properties. However, high incidence and severity of side-effects on the structure and functions of the kidney, liver and intestine limits its clinical use. The present study tested the hypothesis that IndoM causes multi-organ toxicity by inducing oxidative stress that alters the structure of various cellular membranes, metabolism and hence functions. The effect of IndoM was determined on the enzymes of carbohydrate metabolism, brush border membrane (BBM) and oxidative stress in the rat kideny, liver and intestine to understand the mechanism of IndoM induced toxicity. Adult male Wister rats were given IndoM (20 mg/kg) intra-peritoneally in sodium bicarbonate twice a day for 3 d. The body weights of the rats were recorded before and after experimental procedure. IndoM administration significantly increased blood urea nitrogen, serum creatinine, cholesterol and alkaline phosphatase but inorganic phosphate indicating IndoM induced renal, hepatic and intestinal toxicity. Activity of lactate dehydrogenase along with glucose-6- and fructose-1, 6-bis phosphatase, glucose-6-phosphate dehydrogenase and NADP-malic enzyme increased but malate dehydrogenase decreased in all tissues. Lipid peroxidation (LPO) significantly increased whereas the antioxidant enzymes decreased in all rat tissues studied. The results indicate that IndoM administration caused severe damage to kidney, liver and intestine by icreasing LPO, suppressing antioxidant enzymes and inhibiting oxidative metablolism. The energy dependence was shifted to anaerobic glycolysis due to mitochondrial damage supported by increased gluconeogenesis to provide more glucose to meet energy requirements.

1. Introduction

The non-steroidal anti-inflammatory drugs (NSAIDs) including indomethacin (IndoM) are extensively used in the management of acute and chronic pain. It was introduced in 1963 for the treatment of rheumatoid arthritis, degenerative joint diseases, gout, acute musculoskeletal disorders, inflammation and edema [1]. IndoM has prominent anti-inflammatory and analgesic-antipyretic properties. However, high incidence and severity of side-effects has limited its clinical usage. IndoM produces multisystem lesions in various tissues including kidney, liver, intestine, brain, lungs, spleen, blood vessels and glands causing degeneration, necrosis and erosion in many experimental animal species [2–7,1–10]. Morphologic studies have reported that IndoM caused ultra-structural alterations in the kidney including tubular degeneration, swollen mitochondria, dilated Golgi complex and endoplasmic reticulum, nephritic syndrome associated with interstitial nephropathy and papillary necrosis leading to acute renal failure [7,11–13]. Prenatal maternal IndoM use also showed a potential cause

Abbreviations: ACPase, Acid phosphatase an enzyme; ALP, Alkaline phosphatase an enzyme; ANOVA, Analysis of variance statistical tool; ATP, Adenosine 5'-triphosphate energy currency; BBM, Brush border membrane intestinal membrane; BBMV, Brush border membrane vesicles; BUN, Blood urea nitrogen blood parameter; GGTase, γ-Glutammyl transferase an enzyme; G6Pase, Glucose-6-phosphatase an enzyme; G6PDH, Glucose-6-phosphate dehydrogenase an enzyme; HK, Hexokinase an enzyme; HMP, Hexose monophosphate; LDH, Lactate dehydrogenase an enzyme; LAP, Leucine amino peptidase, an enzyme; LPO, Lipid peroxidation; MDH, Malate dehydrogenase an enzyme; ME, Malic enzyme an enzyme; NADPH, Nicotinamide adenine dinucleotide phosphate (reduced) reducing equivalent; NADP +, Nicotinamide adenine dinucleotide phosphate; Pi, Inorganic phosphate; ROS, Reactive oxygen species; SOD, Superoxide dismutase, an enzyme; SH, Sulfhydryl groups; TCA cycle, Tri-carboxylic acid cycle

* Corresponding author at: Department of Biochemistry, Faculty of Life Sciences, A.M.U, Aligarh, 202002, India.
E-mail address: yusufi@lycos.com (A.N.K. Yusufi).

https://doi.org/10.1016/j.toxrep.2019.04.010
Received 28 March 2018; Received in revised form 18 April 2019; Accepted 28 April 2019
Available online 04 May 2019

of renal dysfunction including glomerular injury, renal insufficiency and renal failure death in the newborn infant [14–17]. Wide spread necrosis especially in stomach, ulceration in the small intestine and mitochondrial damage and coagulative necrosis of the liver were also reported by IndoM and other NSAIDS [5,10,18–20].

The mechanism by which long-term exposure of IndoM causes multi-organ toxicity is not well understood and has not been completely elucidated. Inhibition of prostaglandins synthesis and cellular respiration, DNA damage and increase in oxidative stress are considered to be involved in the pathogenesis of IndoM induced toxicity [6,21,8,5,9]. Reactive oxygen species (ROS) and oxidative stress are considered as the most important mechanism of toxicity caused by various drugs and chemicals [22–241–28]. We now hypothesized that IndoM exposure induces oxidative stress that causes damage to various cellular membranes including mitochondria and perturbs antioxidant defense mechanism leading to alterations in cellular metabolic functions of various rat tissues.

To address the above hypothesis, the effect of IndoM was examined on toxicity parameters in blood and on biomarker enzymes of brush border membrane (BBM), carbohydrate metabolism and parameters of oxidative stress in the rat renal cortex and medulla and in the liver and small intestine obtained from same animals under similar experimental conditions.

The results of the present study showed that IndoM significantly increased serum creatinine, cholesterol, ALP activity and BUN indicating IndoM induced renal and liver toxicity. IndoM markedly altered the enzymes of carbohydrate metabolism, BBM, mitochondria and lysosomes and perturbed antioxidant defense mechanism in renal cortex and medulla and liver, and intestine although differently in different rat tissues. We conclude that IndoM caused severe damage to various rat tissues by inducing oxidative stress as reflected by increased LPO and decreased activity of the antioxidant defense enzymes.

2. Materials and methods

2.1. Materials

Chemicals: Indomethacin (IndoM) was purchased from Sisco Research Laboratory Mumbai, India. Sucrose, p-nitro phenyl phosphate, NADH and NADP + were purchased from Sigma Chemical Co. St Louis, MO, USA. All other chemicals used were of analytical grade and were purchased from standard suppliers.

Animals: Adult albino rats (Wistar strain) were purchased from local animal supplier, Aligarh (UP), India.

Diet: The standard rat pellet diet was purchased from Aashirwad Industries, Chandigarh, India.

2.2. Experimental design

Adult Wistar rats weighing 150–200 g were used in the study and were conditioned for one week on a standard rat diet and water ad libitum before the start of the experiment. All animals were kept under conditions that prevented them from experiencing unnecessary pain according to the guide lines of institutions ethical committee approved by the Ministry of Environment and Forests, Government of India. Two groups of rats (10 rats per group) in each experiment entered the study. Indomethacin (IndoM; 20 mg/kg body weight) was given to IndoM-treated rats intra-peritoneally in sodium bicarbonate twice a day for 3 days whereas the control rats received the same amount of vehicle in the same manner for 3 days. This dose was chosen by preliminary experiments that caused minimal death to the animals during the experiments and that is also used by other investigators [7,21,8]. After completion of experimental procedure, the rats were sacrificed under light ether anesthesia. Blood was collected and the liver, kidney and intestine were removed and kept in ice-cold buffered saline for further analyses. All samples were processed simultaneously under similar experimental conditions to avoid any variations as described in methods [26]. Body weights of the rats were recorded at the start and end of the experimental procedure.

2.3. Preparation of homogenates

The tissue homogenates were prepared by standard methods as described in our previous studies [26,29]. Briefly, the intestines were washed by flushing them with ice-cold buffered saline (1 mM Tris-HCl, 9 g/l of NaCl, pH 7.4) and entire mucosa was collected by a glass slide. A 6.5% homogenate of this mucosa was prepared in 50 mM mannitol, pH-7.0, in a glass/Teflon homogenizer. It was further homogenized in an Ultra Turex homogenizer at high speed for three strokes of 30 s each with an interval of 30 s in between each strokes and centrifuged at 2000 g for 10 min to remove the cell debris. The kidneys were decapsulated and the cortex was carefully separated from medulla and both were homogenized in 50 mM mannitol buffer to obtain 10% (w/v) homogenate. A 10% liver homogenate was also prepared in 10 mM Tris-HCl buffer, pH 7.5. The homogenates were centrifuged at 2000 *g* at 4 °C for 10 min and the supernatants thus obtained was saved in aliquots and stored at -20 °C for assaying the enzymes of carbohydrate metabolism, anti-oxidant system and for the estimation of total-SH and LPO as described in the Methods [26,30].

2.4. Preparation of brush border membrane vesicles (BBMV)

The intestinal BBMV was prepared as described by Farooq et al (2004; [18]) by CaCl2 precipitation and differential centrifugation method. Mucosa scraped from 4 to 5 washed intestines was used for each BBM preparation. Briefly, the mucosal scrapings were collected in a beaker containing 50 mM mannitol, 5 mM Tris-HCl, and pH 7.5. The mucosal homogenate was diluted with the above mentioned Tris-mannitol buffer (15 ml/g tissue) and further homogenized using Ultra-Turex T25 homogenizer with three pulses of 30 s each with 30 s interval between each pulse. CaCl2 was added to the homogenate to a final concentration of 10 mM and stirred for 20 min on ice. This homogenate was then centrifuged at 2000 *g* for15 min and the supernatant was re-centrifuged at 35,000 *g* for 20 min. The pellet was resuspended in a small volume (1–2 ml) of 50 mM sodium maleate buffer, pH 6.8, with four complete passes by a loose fitting Dounce homogenizer (Thomson PA, Wheaton IL, USA) in a 15 ml Corex glass tube and centrifuged against 35,000 *g* for 20 min. The outer white fluffy layer of the pellet is resuspended in sodium maleate buffer. Aliquots of homogenates and BBM thus prepared were saved and stored at –20C until further analysis.

The kidney BBMV were prepared from cortical homogenates by MgCl2 precipitation and differential centrifugation method [31] similar to intestinal BBMV except that the final BBMV preparation was re-suspended in 300 mM mannitol, pH-7.4 as described earlier [31]. Each sample of BBM was prepared by pooling tissues from two to three rats.

2.5. Serum parameters

Various biochemical parameters in the serum were determined according to methods described earlier [26,32]. Serum was mixed with 3% tri-chloroacetic acid in a ratio 1: 3, left for 10 min and centrifuged at 2000 x g for 10 min. The inorganic phosphate and creatinine were determined in the supernatant whereas the precipitate was used to determine total phospholipids. Blood Urea Nitrogen (BUN) and cholesterol were determined directly in serum samples as described by Banday et al. [23]. Glucose in the serum was estimated by o-toluidene method using kit from Span diagnostics (Mumbai, India).

2.6. Enzyme assays

The activities of BBM enzymes, alkaline phosphatase (ALP), leucine

amino peptidase (LAP), and γ- glutammyl transferase (GGTase) in the homogenates and BBM vesicles and lysosome enzyme, acid phosphatase (ACP) in the homogenates were determined by colorimetric methods [33]. The activities of carbohydrate metabolism enzymes, LDH, MDH, G6PDH and NADP-malic enzyme (ME), were determined in a spectrophotometer by measuring the extinction changes at 340 nm [32,34]. The other enzymes, glucose-6-phosphatase (G6Pase), fructose-1, 6-bisphospatase (FBPase) and hexokinase (HK) were determined by the methods described in our previous studies [32]. The activities of antioxidant enzymes, superoxide dismutase (SOD) and catalase and Lipid peroxidation (LPO) and total SH-groups were determined as described by Priyamvada et al. [30].. Protein concentration was determined by the modified method of Lowry et al [35] as described by Yusufi et al. [31].

2.7. Statistical analyses

All data are expressed as means ± for at least 5 separate preparations. Statistical evaluation was conducted by group *t*-test and by one-way ANOVA using SPSS 7.5 software for validity of the results obtained. A probability level of $p < 0.05$ was selected as indicating statistical significance.

3. Results

3.1. Effect of indomethacin (IndoM) on body weight and serum parameters

The effect of IndoM was determined on body weight and various serum parameters and on the enzymes of carbohydrate metabolism, brush border membrane (BBM) and oxidative stress in different rat tissues. Apparently, there was no significant difference in food and water intake between control and treated rats. However, IndoM caused a small decrease in the body weights (Table 1). IndoM exposure resulted in significant increase in serum creatinine (+22%) and blood urea nitrogen (BUN, +43%), serum cholesterol (+30%), phospholipids (+47%) and the activity of serum alkaline phosphatase (ALP, +29%) indicating IndoM induced nephrotoxicity and hepatic toxicity as reported earlier [2,5]. Serum Pi (−11%), however, decreased by IndoM administration (Table 2).

3.2. Effect of indomethacin (IndoM) on enzymes of brush border membrane (BBM) and lysosome in different tissues

The effect of IndoM was determined on BBM and lysosomal biomarker enzymes in the renal cortical and medullary, liver and intestinal homogenates and in the isolated BBM vesicles from renal cortex and intestinal mucosa to assess the integrity of these organelles by IndoM exposure. The results summarized in Table 3 demonstrate that IndoM differentially altered the activities of various enzymes in the homogenates of different rat tissues. The activity of ALP markedly decreased in all tissues (-11% to -44%). The activity of GGT significantly lowered in the renal cortex and medulla (-22%), liver (-38%) and intestine (-30%). IndoM exposure resulted in marked decrease of LAP in the liver (-42%), intestine (-33%) and renal medulla (-27%) and cortex (-23%).

IndoM also altered lysosomal enzyme, acid phosphatase (ACP) differentially in different tissues. The activity of sucrase, a marker of intestinal BBM, also significantly decreased (-21%) in the mucosal homogenate (Table 3).

Similar to homogenates, the activities of ALP, GGT and LAP significantly decreased in cortical BBM by IndoM exposure. In accordance with the alterations in mucosal homogenate, IndoM caused significant decline of ALP, GGT, LAP and sucrase activities in the BBM, isolated from small intestine (Table 4).

Table 1
Effect of indomethacin (IndoM) on body weight (grams) of rats.

Groups	Before treatment	After treatment	% Change
Control	160.00 ± 11.23	167.00 ± 9.80	(+4%)
Indomethacin	156.00 ± 9.23	148.26 ± 6.31	(−5%)

Results are mean ± SEM of eight different preparations.
Values in parentheses represent percentage change from controls.

3.3. Effect of indomethacin (IndoM) on enzymes of carbohydrate metabolism

Studies of cellular energetic have revealed important roles of metabolic pathways in determining cell fate and response to injury. Insights from 2017 into the mechanisms underlying these pathways might identify therapeutic targets to minimize injury and promote repair [36]. The effect of IndoM was determined on the enzymes involved in various pathways of carbohydrate metabolism in different rat tissues (Tables 5 and 6) to ascertain any effect on energy metabolism. IndoM administration caused significant increase in hexokinase (glycolysis) activity from (+5% to +13%) whereas the activity declined in the intestine (-16%). However, LDH activity, a marker of anaerobic glycolysis significantly increased in all tissues, renal cortex (+34%) and medulla (+27%), liver (+24%) and intestine (+30%). In contrast, IndoM significantly decreased the activity of MDH, a TCA cycle enzyme in the renal cortex (−42%), renal medulla (−10%), liver (−36%), and intestine (−39%) (Table 5).

The effect of IndoM was also determined on the activities of enzymes involved in gluconeogenesis and hexose monophosphate (HMP) – shunt pathway (Table 6). The enzymes of these pathways were differentially altered by IndoM in different tissues. The activities of both G6Pase and FBPase significantly increased in the renal tissues, liver and intestine. The maximum increase of G6Pase and FBPase was observed in renal cortex (+93%) and in intestine (+60%), respectively. The activity of G6PDH increased in all tissues, renal cortex (+31%), renal medulla (+21%), liver (+33%) and intestine (+24%). Moreover, ME activity also increased in all tissues (Table 6).

3.4. Effect of indomethacin (IndoM) on the antioxidant parameters

It is evident that reactive oxygen species (ROS) generated by various toxins are important mediators of cell injury and pathogenesis of various diseases especially in the kidney and other tissues [37]. A major cellular defense against ROS is provided by superoxide dismutase (SOD), catalase and some other enzymes. To ascertain the role of antioxidant system in IndoM induced toxicity, the effect of IndoM was examined on enzymatic and non-enzymatic antioxidant parameters. IndoM administration caused significant decrease in SOD and catalase activities in almost all tissues (Table 7). However the decrease in SOD and/ or catalase activity was appeared to be tissue specific. The decrease in antioxidant enzyme activities was associated with significant elevated lipid peroxidation (LPO) measured in terms of malondialdehyde (MDA).

4. Discussion

IndoM along with other NSAIDS are commonly used as analgesic and anti-pyretic and anti-inflammatory drugs [1]. However, repeated long-term use of IndoM resulted in multi-organ toxicities in humans and experimental animals [3,5–7,9,10]. The kidney, liver and small intestine were found to be most notable targets of IndoM toxicity [2–4,6,7,19,20]. Morphological studies have shown that IndoM exposure produced significant changes in the structure of kidney, gastric and intestinal mucosa and the liver. The renal proximal tubule, small intestine particularly their BBM, mitochondria, endoplasmic reticulum, Golgi and lysosomes were shown to be major IndoM targets affecting their functions [6,11,12,21,7,8,13]. Despite over-the-counter status of

Table 2
Effect of indomethacin (IndoM) on the various serum parameters.

Groups	BUN	Creatinine (mg/ dL)	Cholesterol (mg/ dL)	Phospholipids (μg/ mL)	Inorganic Phosphate (μmol/mL)	ALP (KAunits)
Control	38.88 ± 1.80	0.09 ± 0.07	90.96 ± 2.50	104.40 ± 3.19	7.49 ± 6.18	35.02 ± 1.54
IndoM	55.61 ± 1.30* (+43%)	0.11 ± 0.03* (+22%)	118.33 ± 6.58* (+30%)	153.97 ± 9.84* (+47%)	6.69 ± 0.39 (−11%)	45.07 ± 0.23* (+29%)

Results are mean ± SEM of eight different preparations.
Values in parentheses represent percentage change from controls.
* Significantly different at $P < 0.05$ from controls.

NSAIDS there are no long-term studies that document the safety of these drugs. We now hypothesized that IndoM causes multi-organ toxicity by inducing oxidative stress and suppressing antioxidant defense system thereby altering the structure and metabolic functions of the kidney, small intestine and liver. The present investigation was undertaken to determine the multifaceted adverse effects of IndoM on certain serum parameters and on various enzymes of carbohydrate metabolism, BBM, lysosomes, and oxidative stress to understand the mechanism of IndoM induced toxic and other adverse effects in rat renal cortex and medulla, and small intestine and liver.

IndoM administration significantly increased serum creatinine, BUN, serum cholesterol, phospholipids and alkaline phosphatase but decreased Pi indicating IndoM induced nephrotoxicity and hepatic toxicity as observed earlier by morphological studies [2,3,5,10,18]. Since the BBM of intestinal mucosa and renal proximal tubules are the major functional sites [26,32,38] and the primary target of IndoM, the integrity of BBM was evaluated by the status of specific biomarker enzymes in tissue homogenates and in isolated BBM vesicles. IndoM induced marked reductions in the activities of several BBM enzymes, ALP, GGTase, LAP and sucrase (a specific marker of mucosal BBM) in the homogenates of renal cortex and medulla and intestine (Table 3) and in BBM preparations isolated from renal cortex and small intestine (Table 4) showed that IndoM has caused alterations in the architecture of BBM especially to that of renal proximal tubules and mucosal BBM from small intestine as observed earlier with other toxicants [22,26,29,38]. When BBM is damaged by toxic insult including IndoM, these enzymes are usually dissociated from damaged BBM and lost in the lumen along with fragmented microvilli and sometimes are excreted into the urine [23–25,1–27,38]. IndoM elicited decrease in the activity of acid phosphatase (ACP), a marker enzyme for lysosomes, indicate that lysosomes along with BBM were also significantly damaged by IndoM exposure.

The kidney performs a number of essential functions including the clearance of endogenous waste products, control of volume status, maintenance of electrolyte and acid-base balance, and endocrine function whereas intestine epithelium and liver play crucial role in the digestion and absorption of nutrients and recognition of food-derived signals. It is well established that many cellular functions including transport of ions and solutes especially by renal and/or mucosal BBM required energy as ATP which is provided by various metabolic pathways [24–2613,39]. The effect of IndoM on energy and nutrition metabolism has not been studied in detail in many tissues. A preliminary study, however, showed IndoM induced mitochondrial dysfunction and decreased rates of oxygen consumption along with increased rates of glycolysis [40]. Although we have not determined the actual rates of any pathway, however, the present results showed that the activities of various enzymes involved in glycolysis, TCA cycle, gluconeogenesis and HMP shunt pathway were significantly affected by IndoM administration in different rat tissues. IndoM significantly increased the activity of LDH (glycolysis) but decreased MDH (TCA cycle) in the cortex, medulla and liver and intestine alike. A marked increase in LDH activity and profound decrease in MDH activity indicate that due to the damage caused to mitochondria by IndoM the energy dependence in all the tissues studied has shifted from oxidative metabolism to anaerobic glycolysis as reported by Jacob et al. [40] and also observed for other drugs and toxicant [23–25,1–28,41,42]. Since anaerobic glycolysis has become the sole source of energy hence more glucose would be needed to meet energy requirements for the survival of the tissues. This supported by the fact that the enzymes of gluconeogenesis, namely, G-6-Pase and FBPase were increased in IndoM-treated compared to control rats that can produce more glucose as a compensatory cellular response to toxic insult. It appears that serum glucose was also decreased by IndoM exposure due to its over utilization by glycolysis. IndoM also decreased serum Pi which is required for the production of glycolytic metabolites and ATP. It implies that IndoM affected both Pi and ATP metabolism that would result in lower ATP production and hence decreased energy dependent functions of the kidney, small intestine and liver.

In addition to enzymes of glycolysis and gluconeogenesis, the activity of G6PDH (HMP-shunt pathway) was also significantly increased in all the tissues but the activity of NADP-malic enzyme (ME) increased only in the liver and intestine. These enzymes increase the production of NADPH that is involved in lipogenesis and anti-oxidant defense mechanism. Thus increased NADPH may have increased lipid

Table 3
Effect of indomethacin (IndoM) on brush border membrane enzymes in different tissue homogenates.

Groups	ALP (μmol/ mg protein/ h)	GGTase (μmol/ mg protein/ h)	LAP (μmol/ mg protein/ h)	ACP (μmol/ mg protein/ h)	Sucrase (μmol/ mg protein/ h)
Cortex					
Control	2.07 ± 0.05	49.50 ± 2.10	7.04 ± 0.04	2.74 ± 0.14	
IndoM	1.15 ± 0.06* (−44%)	38.80 ± 1.74* (−22%)	5.42 ± 0.07* (−23%)	1.95 ± 0.27* (−29%)	
Medulla					
Control	3.10 ± 0.02	52.40 ± 0.32	1.13 ± 0.02	1.92 ± 0.07	
IndoM	2.02 ± 0.01* (−35%)	40.90 ± 1.16* (−22%)	0.82 ± 0.04* (−27%)	1.64 ± 0.14 (−15%)	
Liver					
Control	1.90 ± 0.06	1.68 ± 0.01	0.11 ± 0.03	1.13 ± 0.05	
IndoM	1.70 ± 0.05 (−11%)	1.04 ± 0.04* (−38%)	0.07 ± 0.02* (−36%)	0.58 ± 0.02* (−49%)	
Intestine					
Control	5.90 ± 0.20	7.52 ± 0.04	3.35 ± 0.23	9.74 ± 0.93	22.90 ± 0.92
IndoM	4.40 ± 0.03* (−25%)	5.30 ± 0.02* (−30%)	2.25 ± 0.24* (−33%)	7.81 ± 0.68* (−20%)	18.10 ± 0.32* (−21%)

Results are mean ± SEM of eight different preparations.
Values in parentheses represent percentage change from controls.
* Significantly different at $P < 0.05$ from controls.

Table 4
Effect of indomethacin (IndoM) on BBM Vesicles isolated from renal cortex and small intestine.

Groups	Renal Cortex			Small Intestine			
	ALP (μmol/ mg protein/ h)	GGTase (μmol/ mg protein/ h)	LAP (μmol/ mg protein/ h)	ALP (μmol/ mg protein/ h)	GGTase (μmol/ mg protein/ h)	LAP (μmol/ mg protein/ h)	Sucrase (μmol/ mg protein/ h)
Control	10.40 ± 0.07	524.00 ± 3.20	48.16 ± 3.57	11.20 ± 0.16	40.06 ± 0.62	24.70 ± 0.24	249.68 ± 20.23
IndoM	6.42 ± 0.24* (−38%)	409.23 ± 1.26* (−22%)	27.85 ± 0.91* (−42%)	18.71 ± 0.42* (−67%)	30.52 ± 2.43* (−24%)	16.04 ± 0.45* (−35%)	168.00 ± 19.40* (−33%)

Results are mean ± SEM of eight different preparations.
Values in parentheses represent percentage change from controls.
* Significantly different at $P < 0.05$ from controls.

Table 5
Effect of indomethacin (IndoM) on metabolic enzymes on different tissue homogenates.

Tissues	Hexokinase (μmol/ mg protein/ h)	LDH (μmol/ mg protein/ h)	MDH (μmol/ mg protein/ h)
Cortex			
Control	30.76 ± 1.24	6.56 ± 0.19	40.80 ± 0.79
IndoM	32.29 ± 0.67 (+5%)	8.82 ± 0.95* (+34%)	23.57 ± 0.35* (−42%)
Medulla			
Control	20.89 ± 0.65	10.24 ± 0.23	21.10 ± 0.26
IndoM	23.59 ± 0.66 (+13%)	12.96 ± 0.12* (+27%)	19.00 ± 0.33 (−10%)
Liver			
Control	6.97 ± 0.33	18.80 ± 0.32	2.09 ± 0.63
IndoM	7.85 ± 0.32 (+13%)	23.38 ± 0.62* (+24%)	1.33 ± 0.22* (−36%)
Intestine			
Control	157.98 ± 4.80	8.05 ± 0.71	17.12 ± 0.72
IndoM	132.20 ± 3.20 (−16%)	10.50 ± 0.53* (+30%)	10.50 ± 0.44* (−39%)

Results are mean ± SEM of eight different preparations.
Values in parentheses represent percentage change from controls.
* Significantly different at $P < 0.05$ from controls.

Table 6
Effect of indomethacin (IndoM) on metabolic enzymes on different tissue homogenates.

Tissues	G6Pase (μmol/ mg protein/ h)	FBPase (μmol/ mg protein/ h)	G6PDH (μmol/ mg protein/ h)	ME (μmol/ mg protein/ h)
Cortex				
Control	0.31 ± 0.04	0.16 ± 0.02	2.02 ± 0.45	3.14 ± 0.01
IndoM	0.60 ± 0.07* (+93%)	0.19 ± 0.01* (+19%)	2.65 ± 0.60* (+31%)	2.90 ± 0.20 (−8%)
Medulla				
Control	0.21 ± 0.01	0.12 ± 0.003	3.56 ± 0.27	1.84 ± 0.23
IndoM	0.28 ± 0.03* (+33%)	0.14 ± 0.007 (+17%)	4.29 ± 0.42* (+21%)	1.99 ± 0.22 (+8%)
Liver				
Control	0.08 ± 0.02	0.30 ± 0.01	7.52 ± 0.54	3.05 ± 0.13
IndoM	0.11 ± 0.01* (+38%)	0.35 ± 0.02 (+17%)	10.01 ± 0.43* (+33%)	4.01 ± 0.23* (+31%)
Intestine				
Control	3.37 ± 0.25	1.30 ± 0.87	36.60 ± 0.54	2.90 ± 0.14
IndoM	5.45 ± 0.34* (+62%)	2.08 ± 0.33* (+60%)	45.55 ± 0.65* (+24%)	3.58 ± 0.23* (+23%)

Results are mean ± SEM of eight different preparations.
Values in parentheses represent percentage change from controls.
* Significantly different at $P < 0.05$ from controls.

Table 7
Effect of indomethacin (IndoM) on enzymatic and non-enzymatic antioxidant parameters in different tissue homogenates.

Tissues	LPO (nmole/ g tissue)	SOD (units/ mg protein)	Catalase (μmol/ mg protein/ min)
Cortex			
Control	201.53 ± 10.57	36.86 ± 5.16	220.86 ± 11.87
IndoM	293.58 ± 6.40* (+46%)	15.24 ± 3.23* (−59%)	113.84 ± 13.78* (−48%)
Medulla			
Control	131.70 ± 11.55	22.92 ± 0.56	130.26 ± 8.42
IndoM	180.68 ± 10.23* (+37%)	15.25 ± 0.43* (−33%)	90.55 ± 3.26* (−30%)
Liver			
Control	168.16 ± 10.20	80.90 ± 2.84	42.88 ± 3.26
IndoM	212.02 ± 8.43* (+26%)	65.42 ± 1.96* (−19%)	33.75 ± 2.62* (−21%)
Intestine			
Control	87.20 ± 6.75	8.06 ± 0.23	10.15 ± 0.56
IndoM	110.60 ± 7.04* (+27%)	5.63 ± 0.14* (−30%)	6.84 ± 0.43* (−33%)

Results are mean ± SEM of eight different preparations.
Values in parentheses represent percentage change from controls.
* Significantly different at $P < 0.05$ from control.

biosynthesis and might be responsible for higher blood cholesterol and phospholipid levels. The cholesterol and phospholipids are essential membrane components and are required to facilitate repair and regeneration of various cellular membranes damaged by IndoM as reported earlier for other toxicants [23,41]. Recent studies of cellular energetic have revealed important roles of metabolic pathways in determining cell fate and response to injury. Insights from 2017 into the mechanisms underlying these pathways might identify therapeutic targets to minimize injury and promote repair [36,42].

The mechanism by which IndoM causes multi-organ toxicity remains unclear. It has been shown to inhibit prostaglandins synthesis, cellular respiration and decrease antioxidant parameters in whole kidney extract and intestine [6,21,8]. Reactive oxygen species (ROS) are considered as one of the important mechanisms of toxicity caused by various drugs and chemicals [22–241–28]. IndoM caused perturbation in antioxidant defense system in renal cortex and medulla and in small intestine and liver as reflected by increased LPO (indicator of tissue injury) and suppressed activity of SOD and catalase in above tissues. The effect of IndoM seems to be tissue specific as all the parameters studied altered to greater extent in renal cortex and medulla than in the intestine and least in the liver. Thus the differential effects of IndoM in different tissues can be attributed to differential impact of IndoM or its bio-derivatives and/or on the oxygen tension, oxidant/ antioxidant ratio or occurrence of specific metabolic pathways in those tissues. Thus, IndoM induced suppression of antioxidant defense mechanism appears to be the major cause of tissue injury that disrupted cellular structures and altered metabolic activities and hence may result in lower tissue functions.

In summary, the present findings clearly demonstrate that IndoM administration produces nephrotoxicity, hepatic and intestinal toxicity and causes profound structural damage to renal and mucosal BBM, mitochondria and lysosomes as reflected by significant alterations in their specific biomarker parameters. The enzymes of carbohydrate metabolism, BBM, mitochondria, lysosomes and antioxidant defense

mechanism appeared to be severely affected similarly in renal cortex and medulla and the liver and intestine by IndoM administration. IndoM caused alterations in energy metabolism and energy dependence appeared to be shifted from aerobic to anaerobic glycolysis as indicated by marked increase in LDH and decrease in MDH activity due to IndoM induced mitochondrial damage. We conclude that IndoM exerts it nephrotoxic, hepato-toxic and other deleterious effects at least in part by increasing free radical generation/oxidative stress that causes damage to cellular membrane structures and intracellular organelles and eventually affecting energy metabolism leading to decrease in overall functioning of various tissues/organs. The results of the present study provide useful information of clinical importance in drug induced toxicity.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

Research grant (5821/2001-BMS) from Indian Council of Medical Research (ICMR), New Delhi to ANKY. Financial support to the department from University Grant Commission (UGC-DRF), Department of Science and Technology (DST-FIST), is also gratefully acknowledged.

References

[1] J.G. Hardman, L.E. Limbird, A.G. Gilman, Goodman and Gilman's The Pharmacological Basic of Therapeutics, 10th international edition, The McGraw-Hill Companies Inc, New York, 2001, p. 2148.
[2] M. Falzon, P.H. Whiting, S.W. Ewen, A.S. Milton, M.D. Burke, Comparative effects of indomethacin on hepatic enzymes and histology and on serum indices of liver and kidney function in the rat, Br. J. Exp. Pathol. 66 (5) (1985) 527.
[3] M.E. Fracasso, L. Cuzzolin, P. Del Soldato, R. Leone, G.P. Velo, G. Benoni, Multisystem toxicity of indomethacin: effects on kidney, liver and intestine in the rat, Inflamm. Res. 22 (3) (1987) 310–313.
[4] P.H. Whiting, N. Barnard, A. Neilsch, J.G. Simpson, M.D. Burke, Interactions between cyclosporin A, indomethacin and 16, 16-dimethyl prostaglandin E2: effects on renal, hepatic and gastrointestinal toxicity in the rat, Br. J. Exp. Pathol. 68 (6) (1987) p.777.
[5] M.A. Slagle, Featured CME topic: pain management, Southern Med. J. 94 (2001) 771–774.
[6] J. Basivireddy, A. Vasudevan, M. Jacob, K.A. Balasubramanian, Indomethacin-induced mitochondrial dysfunction and oxidative stress in villus enterocytes, Biochem. Pharmacol. 64 (2) (2002) 339–349.
[7] J. Basivireddy, M. Jacob, A.B. Pulimood, K.A. Balasubramanian, Indomethacin-induced renal damage: role of oxygen free radicals, Biochem. Pharmacol. 67 (3) (2004) 587–599.
[8] J. Basivireddy, M. Jacob, K.A. Balasubramanian, Indomethacin induces free radical-mediated changes in renal brush border membranes, Arch. Toxicol. 79 (8) (2005) 441–450.
[9] P. Ejaz, K. Bhojani, V.R. Joshi, NSAIDs and kidney, JAPI 52 (2004) 632–639.
[10] V.O. Taiwo, O.L. Conteh, The rodenticidal effect of indomethacin: pathogenesis and pathology, Vet. Arh. 78 (2) (2008) 167–178.
[11] A. Whelton, C.W. Hamilton, Nonsteroidal anti-inflammatory drugs: effects on kidney function, J. Clin. Pharmacol. 31 (7) (1991) 588–598.
[12] W.M. Bennett, W.L. Henrich, J.S. Stoff, The renal effects of nonsteroidal anti-inflammatory drugs: summary and recommendations, Am. J. Kidney Dis. 28 (1) (1996) S56–S62.
[13] M.A. Perazella, Renal vulnerability to drug toxicity, Clin. J. Am. Soc. Nephrol. 4 (7) (2009) 1275–1283.
[14] J.M. Gloor, D.G. Muchant, L.L. Norling, Prenatal maternal indomethacin use resulting in prolonged neonatal renal insufficiency, J. Perinatol. 13 (6) (1993) 425–427.
[15] B. Lantz, P. Cochat, J.L. Bouchet, M. Fischbach, Short-term niflumic-acid-induced acute renal failure in children, Nephrol. Dial. Transplant. 9 (9) (1994) 1234–1239.
[16] B.J. Van der Heijden, C. Carlus, F. Narcy, F. Bavoux, A.L. Delezoide, M.C. Gubler, Persistent anuria, neonatal death, and renal microcystic lesions after prenatal exposure to indomethacin, Am. J. Obstet. Gynecol. 171 (3) (1994) 617–623.
[17] A.L. Kent, L.E. Maxwell, M.E. Koina, M.C. Falk, D. Willenborg, J.E. Dahlstrom, Renal glomeruli and tubular injury following indomethacin, ibuprofen, and gentamicin exposure in a neonatal rat model, Pediatr. Res. 62 (3) (2007) 307.
[18] M.O. Abatan, I. Lateef, V.O. Taiwo, Toxic effects of non-steroidal anti- inflammatory agents in rats, Afr. J. Biomed. Res. 9 (3) (2006).
[19] S. Sabiu, T. Garuba, T. Sunmonu, E. Ajani, A. Sulyman, I. Nurain, A. Balogun, Indomethacin-induced gastric ulceration in rats: protective roles of Spondias mombin and Ficus exasperata, Toxicol. Rep. 2 (2015) 261–267.
[20] Y. Cherkas, M.K. McMillian, D. Amaratunga, N. Raghavan, J.C. Sasaki, ABC gene-ranking for prediction of drug-induced cholestasis in rats, Toxicol. Rep. 3 (2016) 252–261.
[21] J. Basivireddy, M. Jacob, P. Ramamoorthy, A.B. Pulimood, K.A. Balasubramanian, Indomethacin-induced free radical-mediated changes in the intestinal brush border membranes, Biochem. Pharmacol. 65 (4) (2003) 683–695.
[22] N. Farooq, S. Priyamvada, F. Khan, A.N.K. Yusufi, Time dependent effect of gentamicin on enzymes of carbohydrate metabolism and terminal digestion in rat intestine, Hum. Exp. Toxicol. 26 (7) (2007) 587–593.
[23] A.A. Banday, S. Priyamvada, N. Farooq, A.N.K. Yusufi, F. Khan, Effect of uranyl nitrate on enzymes of carbohydrate metabolism and brush border membrane in different kidney tissues, Food Chem. Toxicol. 46 (2008) 2080–2088.
[24] S.A. Khan, S. Priyamvada, S. Khan, M.W. Khan, A.N.K. Yusufi, Protective effect of green tea extract on gentamicin-induced nephrotoxicity and oxidative stress in kidney and other rat tissues, Pharmacol. Res. 59 (2009) 254–262.
[25] S.A. Khan, S. Priyamvada, M.W. Khan, S. Khan, N. Farooq, A.N.K. Yusufi, Studies on the protective effect of green tea against Cisplatin induced nephrotoxicity, Pharmacol. Res. 60 (2009) 382–391.
[26] S. Khan, S. Priyamvada, S.A. Khan, M.W. Khan, N. Farooq, F. Khan, A.N.K. Yusufi, Effect of trichloroethylene (TCE) toxicity on the enzymes of carbohydrate metabolism, BBM, and oxidative stress in kidney and other rat tissues, Food Chem. Toxicol. 47 (2009) 1562–1568.
[27] F. Shahid, S. Rizwan, M.W. Khan, S.A. Khan, A. Naqshbandi, A.N.K. Yusufi, Studies on the effect of sodium arsenate on the enzymes of carbohydrate metabolism, brush border membrane, and oxidative stress in the rat kidney, Environ. Toxicol. Pharmacol. 37 (2) (2014) 592–599.
[28] S. Khan, S. Priyamvada, S.A. Khan, W. Khan, A.N.K. Yusufi, Studies on hexachlorobenzene (HCB) induced toxicity and oxidative damage in the kidney and other rat tissues, Int. J. Drug Metab. Toxicol. 1 (1) (2017) 001–009.
[29] N. Farooq, S. Priyamvada, N.A. Arivarasu, S. Salim, F. Khan, A.N.K. Yusufi, Influence of Ramadan-type fasting on enzymes of carbohydrate metabolism and brush border membrane in small intestine and liver of rat used as a model, Br. J. Nutr. 96 (6) (2006) 1087–1094.
[30] S. Priyamvada, M. Priyadarshini, N.A. Arivarasu, N. Farooq, S. Khan, S.A. Khan, M.W. Khan, A.N.K. Yusufi, Studies on the protective effect of dietary fish oil on gentamicin-induced nephrotoxicity and oxidative damage in rat kidney, Prostaglandins Leukot. Essent. Fatty Acids 78 (2008) 369–381.
[31] A.N.K. Yusufi, N. Murayama, S.M. Gapstur, M. Szczepanska-Konkel, T.P. Dousa, Differential properties of brush-border membrane vesicles from early and late proximal tubules of rat kidney, Biochim. Biophys. Acta (BBA)-Biomembr. 1191 (1) (1994) 117–132.
[32] S. Salim, N. Farooq, S. Priyamvada, M. Asghar, S.J. Khundmiri, S. Khan, F. Khan, A.N.K. Yusufi, Influence of Ramadan-type fasting on carbohydrate metabolism, brush border membrane enzymes and phosphate transport in rat kidney used as a model, Br. J. Nutr. 98 (2007) 984–990.
[33] S.J. Khundmiri, M. Asghar, F. Khan, S. Salim, A.N.K. Yusufi, Effect of reversible and irreversible ischemia on marker enzymes of BBM from renal cortical PT subpopulations, Am. J. Physiol. 273 (1997) F849–F856.
[34] S.J. Khundmiri, M. Asghar, F. Khan, S. Salim, A.N.K. Yusufi, Effect of ischemia and reperfusion on enzymes of carbohydrate metabolism in rat kidney, J. Nephrol. 17 (2004) 1–7.
[35] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randal, Protein measurement with Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[36] T.J. Rabelink, P. Carmeliet, Renal metabolism in 2017: glycolytic adaptation and progression of kidney disease, Nat. Rev. Nephrol. 14 (2018) 75–76.
[37] P.D. Walker, Y. Barri, S.V. Shah, Oxidant mechanisms on gentamycin nephrotoxicity, Ren. Fail. 21 (1999) 433–442.
[38] S.A. Khan, S. Priyamvada, S. Khan, M.W. Khan, A.N.K. Yusufi, Green tea consumption ameliorates intestinal and hepatic -Toxicity induced by long-term administration of cisplatin, Int. J. Drug Metab. Toxicol. 2 (1) (2018) 001–009.
[39] M.W. Khan, S. Priyamvada, S.A. Khan, S. Khan, A. Naqshbandi, A.N.K. Yusufi, Protective effect of w 3 polyunsaturated fatty acids (PUFAs) on sodium-nitroprusside-induced nephrotoxicity and oxidative damage in rat kidney, Hum. Exp. Toxicol. 31 (10) (2012) 1035–1049.
[40] M. Jacob, I. Bjarnason, R.J. Simpson, Effects of indomethacin on energy metabolism in rat Jejunal tissue in vivo, Clin. Sci. 102 (5) (2002) 541–546.
[41] S. Priyamvada, S.A. Khan, M.W. Khan, S. Khan, N. Farooq, F. Khan, A.N.K. Yusufi, Studies on the protective effect of dietary fish oil on uranyl-nitrate-induced nephrotoxicity and oxidative damage in rat kidney, Prostaglandins Leukot. Essent. Fatty Acids 82 (2010) 35–44.
[42] R. Lan, H. Geng, P.K. Singha, P. Saikumar, E.P. Bottinger, J.M. Weinberg, M.A. Venkatachalam, Mitochondrial pathology and glycolytic shift during proximal tubule atrophy after ischemic AKI, J. Am. Soc. Nephrol. 27 (11) (2016) 3356–3367.