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

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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 kidney, 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 increasing LPO, suppressing antioxidant enzymes and inhibiting oxidative metabolism. 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 [2–7,11–13]. 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...
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 Ashirwad 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, 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 UltraTurex T25 homogenizer with three pulses of 30 s each with 30 s interval between each pulse. CaCl2 was added to the homogenate to a concentration of 10 mM and stirred for 20 min on ice. This homogenate was then centrifuged at 2000 g for 15 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 Cortex 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 −20°C 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 resuspended 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
Values in parentheses represent percentage change from controls.

The 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 a 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).

The effect of IndoM was determined 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).

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-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 the most notable targets of IndoM toxicity [2-4,6,7,9,20]. 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 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%) |
| IndoM | 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.
NSAIDS there are no long-term studies that document the change in 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 functions. Thus increased NADPH may have increased lipid 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 G6Pase and FBPase was 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.

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 2**

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

| Groups   | BUN (mg/dL) | Creatinine (mg/dL) | Cholesterol (mg/dL) | Phospholipids (μg/mL) | Inorganic Phosphate (μmol/mL) | ALP (Kunit) |
|----------|-------------|--------------------|---------------------|-----------------------|-----------------------------|------------|
| 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| 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%)|

**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   | 2.07 ± 0.05               | 49.50 ± 2.10                  | 7.04 ± 0.04               | 2.74 ± 0.14              |                               |
| Control  | 1.15 ± 0.06 (–44%)        | 38.80 ± 1.74 (–22%)          | 5.42 ± 0.07 (–23%)       | 1.95 ± 0.27 (–29%)       |                               |
| Medulla  | 3.10 ± 0.02               | 52.40 ± 0.32                  | 1.13 ± 0.02               | 1.92 ± 0.07              |                               |
| Control  | 2.02 ± 0.01 (–35%)        | 40.90 ± 1.16 (–22%)          | 0.82 ± 0.04 (–27%)       | 1.64 ± 0.14 (–15%)       |                               |
| Liver    | 1.90 ± 0.06               | 1.68 ± 0.01                   | 0.11 ± 0.03               | 1.13 ± 0.05              |                               |
| Control  | 1.70 ± 0.05 (–11%)        | 1.04 ± 0.04 (–38%)           | 0.07 ± 0.02 (–36%)       | 0.58 ± 0.02 (–49%)       |                               |
| Intestine| 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.
Values in parentheses represent percentage change from controls.

Results are mean ± SEM of eight different preparations.

* 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          | Small Intestine | Renal Cortex |
|-----------------|-----------------|--------------|
|                 | 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) |
| Control         | 11.20 ± 0.16     | 48.16 ± 3.57  | 4.06 ± 0.62      | 24.70 ± 0.24              | 249.68 ± 20.23             |
| IndoM           | 18.71 ± 0.42     | 27.85 ± 0.91  | 30.52 ± 2.43     | 16.04 ± 0.45              | 168.00 ± 19.40             |

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              | 23.57 ± 0.35             |
| IndoM Control    | 32.29 ± 0.67 ( +5%)            | 8.82 ± 0.95 ( +34%)      | 21.10 ± 0.26 ( +10%)     |
| Medulla Control  | 20.89 ± 0.60 ( +13%)           | 10.24 ± 0.23 ( +27%)     | 19.00 ± 0.33 ( −30%)     |
| IndoM Control    | 23.59 ± 0.66 ( +13%)           | 12.96 ± 0.12 ( +24%)     | 13.3 ± 0.22 ( +36%)      |
| Liver Control    | 6.97 ± 0.33                   | 18.80 ± 0.32 ( +24%)     | 2.09 ± 0.63 ( −24%)      |
| IndoM Control    | 7.85 ± 0.32 ( +13%)           | 23.38 ± 0.62 ( +24%)     | 1.33 ± 0.22 ( −30%)      |
| Intestine Control| 157.98 ± 4.80                 | 8.05 ± 0.71 ( +60%)      | 17.12 ± 0.72 ( −30%)     |
| IndoM Control    | 132.20 ± 3.20                 | 10.50 ± 0.53 ( +60%)     | 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 ( +93%)         | 0.16 ± 0.02                 | 2.02 ± 0.45 ( +27%)       | 3.14 ± 0.01 ( +31%)    |
| IndoM Control    | 0.26 ± 0.05 ( +53%)         | 0.14 ± 0.04 ( +17%)         | 4.29 ± 0.42 ( +21%)       | 1.99 ± 0.22 ( +31%)    |
| Medulla Control  | 0.21 ± 0.01 ( +93%)         | 0.12 ± 0.00 ( +19%)         | 3.56 ± 0.27 ( +31%)       | 1.84 ± 0.23 ( +31%)    |
| IndoM Control    | 0.11 ± 0.01 ( +38%)         | 0.35 ± 0.02 ( +17%)         | 10.01 ± 0.43 ( +33%)      | 4.01 ± 0.23 ( +31%)    |
| Liver Control    | 0.08 ± 0.02                 | 0.30 ± 0.01                 | 7.52 ± 0.54 ( +33%)       | 3.05 ± 0.13 ( +31%)    |
| IndoM Control    | 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 ( +62%)         | 1.30 ± 0.87                 | 36.60 ± 0.54 ( +60%)      | 2.90 ± 0.14 ( +24%)    |
| IndoM Control    | 5.45 ± 0.34 ( +62%)         | 2.08 ± 0.33 ( +60%)         | 45.55 ± 0.65 ( +60%)      | 3.58 ± 0.23 ( +24%)    |

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   | 210.53 ± 10.57        | 36.86 ± 5.16            | 220.86 ± 11.87                   |
| IndoM Control    | 293.58 ± 6.40 ( +46%) | 15.24 ± 3.23 ( +33%)    | 113.84 ± 13.78 ( −46%)           |
| Medulla Control  | 131.70 ± 11.55        | 22.92 ± 0.56 ( +37%)    | 130.26 ± 8.42 ( −30%)            |
| IndoM Control    | 180.68 ± 10.23 ( +37%)| 15.25 ± 0.43 ( +37%)    | 90.55 ± 3.26 ( −33%)             |
| Liver Control    | 168.16 ± 10.20        | 80.90 ± 2.84 ( +26%)    | 42.88 ± 3.26 ( −21%)             |
| IndoM Control    | 212.02 ± 8.43 ( +26%) | 65.42 ± 1.96 ( +26%)    | 33.75 ± 2.62 ( −21%)             |
| Intestine Control| 87.20 ± 6.75          | 8.06 ± 0.23 ( +27%)     | 10.15 ± 0.56 ( −20%)             |
| IndoM Control    | 110.60 ± 7.04 ( +27%) | 5.63 ± 0.14 ( +27%)     | 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.

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 [22–24–28]. 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 its nephrotic, 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.

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