Partial suppressive effect of melatonin on indomethacin-induced renal injury in rat

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Intramuscular injection of a single high dose of indomethacin (20 mg/kg) in fasted rats produced renal injury. The results showed increases in the level of lipid peroxidation and cholesterol, and activity of acid phosphatase and alkaline phosphatase in the kidney. Also, the renal contents of both reduced glutathione and activity of total adenosine triphosphatase were decreased by the toxicant. In serum, indomethacin increased activity of lactate dehydrogenase and acid phosphatase, and levels of creatinine and inorganic phosphorus. Paradoxically, administration of melatonin (0.75 mg/rat/day) alone for 7 days decreased significantly the activity of lipid peroxidation and acid phosphatase, and increased, but not significantly, the level of reduced glutathione in the kidney. Also, serum level of creatinine tended to decrease, but not significantly.

Pretreatment with melatonin prevented the increase by subsequently administered indomethacin in the renal activity of lipid peroxidation and acid phosphatase. However, this pretreatment regimen partially suppressed the adverse changes in the remaining analyzed cytotoxic parameters induced by indomethacin in both serum and kidney. These results indicate that oral administration of melatonin at a low dose level exerted moderate antioxidant action, thereby it protected against some of the renal detrimental effects produced by indomethacin.

Keywords: Indomethacin, Melatonin, Rat, Renal injury

All the nonsteroidal anti-inflammatory drugs (NSAIDs), including indomethacin, can cause renal injury that is associated with various levels of functional impairment1-2. Studies on mechanisms implicated active oxygen and lipid peroxidation in pathogenesis of indomethacin-induced oxidative tissue damage3-4.

On the other hand, melatonin has been shown to be an effective antioxidant and free radical scavenger, therefore its cytoprotective action against different agents that produce cell damage has received great attention5-6. Currently, there is a dearth of information on melatonin’s ability to protect kidney from indomethacin intoxication. Present study was therefore planned to examine the potential role of melatonin in prevention of oxidative renal damage induced by injection of a single high dose of indomethacin in the rats.

Materials and Methods

Adult male white rats weighing about 190-220 g were used. The animals were initially divided into two groups (12 rats per group). One group of animals was left without any treatment to serve as control, and the other group received melatonin orally at a dose of 0.75 mg/rat/day for 7 consecutive days. On the 7th day, all the rats were deprived of food but had free access to water, and each group of animals was subdivided into two sub-groups (6 rats per sub-group). In respect to control rats, one sub-group continued to serve as control, i.e. had no treatment, and the other one injected intramuscularly with a toxic dose of indomethacin (20 mg/kg of body weight). On the other hand, among melatonin-treated rats, one sub-group of rats received melatonin alone, while the other an identical toxic dose of indomethacin. After 5 hr of injection of indomethacin7, all the used animals were sacrificed by decapitation, and blood samples were collected and centrifuged for separation of sera. The animals were then dissected and the kidneys removed and stored immediately in the deep freezer. Later, kidneys were weighed, homogenized in known volume of distilled water, and used for the biochemical investigations.

Renal content of reduced glutathione (GSH) was measured after acidic precipitation of protein by a colour-producing reaction of GSH with 5,5'-dithiobis-2-nitrobenzoic acid according to the method of Prins and Loose8. Lipid peroxidation in kidney homogenates was evaluated following the method of Ohkawa et al.7, by measuring the formation of thiobarbituric acid reactive substances (TBARS).
Serum activity of lactate dehydrogenase (LDH) was measured by the kinetic method of Jeserich. Serum and renal activity of acid phosphatase (ACP), and renal activity of alkaline phosphatase (ALP) were measured colorimetrically by the procedure of Kind and King, in which the enzyme-substrate reaction took place at pH 10 for ALP and 4.9 for ACP. Renal activity of total adenosine triphosphatase (T-ATPase) was evaluated by the method of Dixon et al., in which the enzyme was incubated with the substrate ATP and the liberated inorganic phosphorus was measured by the method of Fiske and Subbarow. The latter method was also applied for estimation of serum level of inorganic phosphorus. The concentration of total lipid in kidney was determined by the method of Frings, while total protein content in both serum and kidney was measured by the method of Gornall et al. Level of serum creatinine was measured as described by Hawk and Summerson.

All results were given as the means ± SE. Comparison between groups was statistically evaluated by one-way analysis of variance (ANOVA) and least significant difference test (LSD), considering P value equal to or below 0.05 as a minimum level of significance.

### Results and Discussion

Free radicals are believed to be involved in the pathogenesis of toxicity of many chemicals like indomethacin, and the role of melatonin in lowering free radical damage is well documented. In the present study, treatment with indomethacin in rats produced renal oxidative damage as reflected by marked increase in lipid peroxidation and decrease in reduced GSH in the kidney tissue (Table 1). Renal activity of ACP, the index of the lysosomal activity, was also enhanced. These findings are in line with published data on NSAIDs treatment which reported increased gastric, testicular, and renal oxidative stress in rats treated with indomethacin or piroxicam. In view of the fact that lipid peroxidation can cause structural and functional membrane changes, indomethacin-induced renal oxidative damage produced decreases in the activity of T-ATPase, total protein, and total lipid; and increase in the accumulation of ALP in the kidney of rats (Table 1). Concurrently, raised levels of creatinine and inorganic phosphorus, and a tendency for total protein content to lower in the serum were observed (Table 2). Similarly, previous studies demonstrated that indomethacin could induce glomerular and tubular changes in kidney of rat.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (C)</th>
<th>Melatonin (MT)</th>
<th>Indomethacin (IM)</th>
<th>Melatonin &amp; Indomethacin (MT&amp;IM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/g)</td>
<td>40.79±1.124 (n=6)</td>
<td>36.16±1.289 &lt;0.01 (n=6)</td>
<td>47.02±0.852 &lt;0.001 (n=6)</td>
<td>43.17±0.566 &lt;0.001 (n=6)</td>
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<tr>
<td>GSH (mg/g)</td>
<td>1.48±0.101 (n=6)</td>
<td>1.69±0.082 &lt;0.05 (n=6)</td>
<td>0.95±0.081 &lt;0.001 (n=6)</td>
<td>1.20±0.061 &lt;0.001 (n=6)</td>
</tr>
<tr>
<td>ACP (U/g)</td>
<td>1.01±0.011 (n=6)</td>
<td>0.92±0.007 &lt;0.01 (n=6)</td>
<td>1.13±0.018 &lt;0.001 (n=6)</td>
<td>1.01±0.020 &lt;0.001 (n=6)</td>
</tr>
<tr>
<td>ALP (U/g)</td>
<td>3.13±0.078 (n=6)</td>
<td>3.25±0.054 &lt;0.05 (n=6)</td>
<td>4.88±0.259 &lt;0.001 (n=6)</td>
<td>3.71±0.089 &lt;0.001 (n=6)</td>
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<tr>
<td>T-ATPase</td>
<td>63.60±0.299 (n=6)</td>
<td>64.47±0.410 &lt;0.05 (n=6)</td>
<td>57.49±0.394 &lt;0.001 (n=6)</td>
<td>60.12±0.568 &lt;0.001 (n=6)</td>
</tr>
<tr>
<td>TP (g/100g)</td>
<td>19.79±0.642 (n=6)</td>
<td>17.44±0.501 &lt;0.05 (n=6)</td>
<td>17.40±0.760 &lt;0.001 (n=6)</td>
<td>17.09±0.431 &lt;0.05 (n=6)</td>
</tr>
<tr>
<td>TL (g/100g)</td>
<td>2.83±0.025 (n=6)</td>
<td>2.76±0.007 &lt;0.001 (n=6)</td>
<td>2.63±0.011 &lt;0.001 (n=6)</td>
<td>2.59±0.013 &lt;0.001 (n=6)</td>
</tr>
<tr>
<td>TCh (mg/100g)</td>
<td>14.63±0.017 (n=6)</td>
<td>14.36±0.077 &lt;0.001 (n=6)</td>
<td>18.54±0.379 &lt;0.001 (n=6)</td>
<td>16.46±0.555 &lt;0.001 (n=6)</td>
</tr>
</tbody>
</table>

*p values: *<0.05; **<0.01; ***<0.001

TBARS: thiobarbituric acid reactive substances; GSH: glutathione; ACP: acid phosphatase; ALP: alkaline phosphatase; T-ATPase: total adenosine triphosphatase; TP: total protein; TL: total lipid; TCh: total cholesterol

### Table 1 — Effect of 7 days pretreatment with melatonin on a single high dose of indomethacin-induced biochemical changes in kidney of rat

[Values are mean ± SE. Number of observations are given in parentheses]
dysfunctions which result in the production of some renal adverse effects such as uremia, enzymuria, and a decrease in serum albumin\textsuperscript{18,19}. Current findings, in accordance with previous observations\textsuperscript{16,20}, also demonstrated that indomethacin, as a cytotoxic agent, was found to produce marked elevation in the serum activity of both LDH and ACP.

On the other hand, present study showed that administration of melatonin alone for 7 days moderately reduced lipid peroxidation and increased, but not significantly, the level of GSH in the kidney of rats (Table 1). This is in agreement with Gonca \textit{et al}\textsuperscript{21}. Melatonin treatment also caused mild decrease in the renal activity of ACP and a tendency to lower serum level of creatinine. However, pretreatment with melatonin prevented increased renal activity of lipid peroxidation and ACP produced by subsequently injected indomethacin, as compared with those in the control and indomethacin-treated groups. With respect to the results of the control group, prior administration of melatonin partially suppressed indomethacin-decreased renal levels of reduced GSH and activity of T-ATPase (Table 1). However, this suppressive effect of melatonin on the renal contents of GSH and activity of T-ATPase in pretreated group seemed to be of significant value when compared with corresponding results in indomethacin-treated group. Increased renal activity of ALP and serum level of inorganic phosphorus in rats treated with indomethacin alone were partially prevented by prior administration of melatonin, comparing with those in the control animals. However, the mean values of these parameters were significantly lower than those in the group of indomethacin (Tables 1 and 2). In addition, indomethacin-elevated serum levels of LDH, ACP, and creatinine were decreased slightly by prior administration of melatonin, and the remaining values were still significantly higher than those in the control group (Table 2).

Attenuation of the antioxidative efficacy of melatonin against indomethacin-induced renal injury in the current study could derive support from several published data describing that melatonin under certain circumstances has no emphasized antioxidant effect against some toxic compounds\textsuperscript{22-23}. For instance, melatonin did not able to modify either the basal production of lipid peroxidation or indomethacin-induced elevation of this parameter in the testis of rats exposed to similar experimental conditions\textsuperscript{23}. In addition, intragastric administration of melatonin failed to affect gastric lesions produced by ethanol and acified aspirin\textsuperscript{24}. However, the discrepancy that exists between the different findings associating the antioxidant action of melatonin against the oxidative damaging effects of various toxicants could be attributed to several factors, e.g. used dose of melatonin and/or toxicant, route of administration, duration of treatment, tissue sensitivity, nature of study (\textit{in vivo} or \textit{in vitro}), and mechanisms of action of both melatonin and used toxic compound\textsuperscript{21-23}. In view of this, suppression of melatonin's ability to protect completely against indomethacin-induced

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|}
\hline
Variables & Control (C) & Melatonin (MT) & Indomethacin (IM) & Melatonin & \textit{p}^d \\
& (n=5) & (n=6) & (n=6) & & \\
\hline
LDH (U/L) & 549±82.79 & 578±67.50 & 1129±100.13 & 1032±36.55 & <0.001 \\
(n=4) & (n=6) & (n=4) & (n=3) & & \\
ACP (U/L) & 9.63±0.296 & 9.18±0.353 & 12.19±0.226 & 11.78±0.435 & <0.001 \\
(n=6) & (n=6) & (n=6) & (n=6) & & \\
Cr (mg/100ml) & 1.48±0.192 & 1.21±0.161 & 2.63±0.329 & 2.46±0.271 & 0.001 \\
(n=6) & (n=6) & (n=6) & (n=6) & & \\
Pi (mg/100ml) & 8.16±0.152 & 7.70±0.095 & 9.71±0.139 & 9.25±0.039 & <0.001 \\
(n=6) & (n=6) & (n=6) & (n=6) & & \\
TP (g/100ml) & 7.70±0.301 & 6.18±0.227 & 6.05±0.500 & 6.11±0.302 & >0.05 \\
(n=4) & (n=4) & (n=4) & (n=3) & & \\
\hline
\end{tabular}
\caption{Effect of 7 days pretreatment with melatonin on a single high dose of indomethacin-induced biochemical changes in serum of rat}
\textit{Values are mean±SE. Number of observations are given in parentheses.}
\end{table}

\textsuperscript{1}Significantly different from C; \textsuperscript{2}Significantly different from MT; \textsuperscript{3}Significantly different from IM
\textit{P} values: \textsuperscript{a} <0.05; \textsuperscript{b} <0.01; \textsuperscript{c} <0.001
\textit{d} By analysis of variance

LDH: lactate dehydrogenase; ACP: acid phosphatase; Cr: creatinine; Pi: inorganic phosphorus; TP: total protein
renal injury in the present study was likely attributable in part to the lowered used dose and route of administration of melatonin, the intragastric inhalation. This suggestion gained support from DeMuro et al. who reported that oral administration of melatonin may show decreased bioavailability, either due to poor absorption, large first-pass hepatic metabolism or both.

Indomethacin treatment decreased the total protein content in the serum and various tissues of the animals, probably due to an inhibitory action on protein synthesis and/or induction of tissue damage. Indomethacin also enhanced the lipolysis of membrane phospholipid. Similarly, both in vivo and in vitro treatment with melatonin evoked inhibition of protein synthesis and some lipid components in the serum. Moreover, decline in the endogenous secretion of melatonin with aging paradoxically accompanied by increased visceral fat content in the rats. In agreement with these observations, present results exhibited decreases in the renal content of total protein and total lipid of rats treated with either indomethacin or melatonin (Table 1). However, the same treatments also lowered the serum level of total protein, but not significantly (Table 2). As melatonin and indomethacin, separately, exerted lowering effect on the content of total protein and total lipid, treatment with both, as described in the pretreated group, resulted in suppressing the concentration of these parameters. Although both indomethacin and melatonin treatment alone could exert similar changes in the total protein and total lipid, the mechanisms of action may be different; at least some of indomethacin action could be related to its peroxidative damaging effect.

Current study demonstrated that melatonin pretreatment was found to reduce partially indomethacin-increased renal cholesterol level in the rats, comparing with the control group (Table 1). However, This melatonin effect seemed to be of significant value when results of the pretreated group was compared with those in the indomethacin-treated group. On the other hand, melatonin treatment alone did not affect renal cholesterol content, as compared with the control group. Supporting these observations, Chan and Tang have reported that melatonin participates in the homeostatic regulation of cholesterol metabolism, and may afford a protective action by exerting a cholesterol-lowering effect, which was manifested primarily following induction of cholesterolemia.

In summary, present study indicates that oral administration of melatonin alone moderately lowered activity of renal oxidative damage. In indomethacin-treated rats, pretreatment with melatonin thus prevented some of the renal injury induced by the toxicant. Decreased protection afforded by antioxidant melatonin against indomethacin-induced renal adverse effects may be due, in part, to lowered level of used dose and the route of administration.

References
HEMEIDA et al.: EFFECT OF MELATONIN ON INDOMETHACIN INDUCED RENAL INJURY

17 Tappel A L, Lipid peroxidation damage to cell components, Fed Proc, 32 (1973) 1870.