Lipoperoxide levels in tissues of the Rat Administered CdCl₂ Under Various Ambient Temperatures

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Abstract To investigate how ambient temperatures affect hemorrhagic inflammations induced by CdCl₂ in the testis of rats, the lipoperoxide concentrations were measured in the testis and other organs (liver and kidney) of rats under three environmental conditions (temperature and relative humidity: 7°C and 50%, 20°C and 50%, 32°C and 60%). As the ambient temperature increased, lipoperoxide concentrations in all organs of rats treated with or without CdCl₂ also increased. In particular, lipoperoxide level in the testis of CdCl₂-intoxicated rats reared at 32°C was higher than those of rat reared at other temperatures, and the lipoperoxide levels in the testis of control animals also increased. Cd deposition in all tissues showed no association with the levels of ambient temperature. Cd contents were highest at the lowest temperature (7°C) in all tissues at both 24 h and 72 h after Cd administration. It appears that the lipoperoxide in testis was leveled up by the increase in temperature and not as a secondary effect of the increased lipid peroxidation *in vivo* induced by CdCl₂.

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We already reported that an intradermal injection of CdCl₂ induced various degrees of hemorrhagic inflammation within a short period of time accompanied by an increase in lipoperoxide in the testis of rats¹⁾. Furthermore, a single dose of vitamin E protected the testis from these inflammations²⁾. Based on these observations, we suggested that lipid peroxidation played an important role in inducing these inflammations in the testis.

MATSUI and NOMIYAMA³⁾ reported that the degree of inflammation induced by CdCl₂ in the testis of rats increased as the ambient temperature increased. It was reported that the injury in the testis by cadmium occurred only in animals such as mice and rats which have the scrotum outside of the body cavity, but not in those which have testis in their abdomen, such as frogs, pigeons, hens, and other fowls⁴⁾. This observation suggests that injury by CdCl₂ to the testis of rats should be easily influenced by ambient temperature.

In order to investigate how ambient temperature affects the inflammation in the testis, lipoperoxide concentration, which is considered to be a good parameter of inflammation induced by CdCl₂ in rat testis was determined in the testis and other organs of rats under three ambient temperatures.

Fig. 1. Changes of lipoperoxide concentrations in the tissues at various ambient temperatures. Values are expressed as mean $\pm$ standard error. $\square$ control ▨ CdCl₂-treated

Materials and Methods

Sixty male Wistar rats weighing around 200 g were divided into 3 groups; the first group was maintained in a room at 7°C and 50% relative humidity (R.H.); another at 20°C and 50% R.H.; and the remaining group at 32°C and 60% R.H. Rats were reared using a climatic chamber (Tabai Co. Ltd.). After rearing under those conditions for approximately 20 days for acclimatization, a single dose of CdCl₂ (5.0 mg/kg) was injected intradermally. All animals were allowed free access to food (Rearing diet F₂; Funabashi Farm) and were starved overnight (16 hrs) before they were killed. The rats were sacrificed 24 or 72 hours after the injection. Four addi-

tional experimental rats were reared under each condition but did not receive an injection. These 12 rats served as controls. The period of acclimatization and the temperatures were set in accordance with the reports by MATSUI and NOMIYAMA³⁾.

Organs were removed and immediately frozen using a thick aluminium board previously chilled below -80°C .

Lipoperoxide concentrations in tissues were assayed within 24 hours after death according to the thiobarbituric acid (TBA) method reported by OHKAWA *et al.*⁵⁾.

Assay for Cd was performed using a flame atomic absorption spectrophotometer (Perkin-Elmer 305) after the tissues were decomposed with HNO_3 . Data were analyzed by two-way or three-way analysis of variance using the least squares method.

Results and Discussion

Changes in lipoperoxide concentration in various tissues under various conditions are shown in Fig. 1. Table 1 shows the results of least squares analysis of variance with significance levels. Lipoperoxide levels in the tissues (liver, kidneys, and testis except testis-24 hrs) of rats increased as the ambient temperature increased, irrespective of CdCl_2 injection.

Table 1. Three-way least-squares analysis of variance for the lipoperoxide values in tissues

Source of variation	Tissues		
	Liver	Kidney	Testis
	M. S. (d. f.)	M. S. (d. f.)	M. S. (d. f.)
Temperature (T)	506021.3** (2)	169752.3** (2)	9846.1 (2)
Time (H)	68052.3** (1)	62.5 (1)	16893.3* (1)
Treatments (Tr)	20131.3* (1)	220.5 (1)	103962.7** (1)
Interaction (T) $\times$ (H)	24918.5** (2)	55891.2** (2)	5100.4 (2)
(T) $\times$ (Tr)	296.3 (2)	1079.1 (2)	663.2 (2)
(H) $\times$ (Tr)	3837.1 (1)	2510.4 (1)	27327.6** (1)
Error	4668.8 (52)	1048.3 (52)	5152.1 (52)

M. S. = mean square. (d. f.) = degrees of freedom. ** $P < 0.01$, * $P < 0.05$

The statistical analysis of data was performed for each tissues, because the differences in lipoperoxide values among tissues were significant. As for the changes in lipoperoxide in the liver, significant differences were observed firstly among the three experimental conditions (7°C , 50% R.H.; 20°C , 50% R.H.; 32°C , 60% R.H.), secondly between times of measurement after CdCl_2 treatment (24 hrs vs 72 hrs), and thirdly between treatments (CdCl_2 vs. control) at the level of 1%, 1% and 5% of probability respectively. In the kidneys, the difference among the ambient temperatures in lipoperoxide values was significant ($P < 0.01$). A multiple range test (Turkey's test) showed that the lipoperoxide levels at 32°C were significantly higher than those at 7°C and 20°C in the liver (24 hrs and 72 hrs) and kidneys (24 hrs). On the other hand, in the testis, a significant difference in lipoperoxide values between treatment

times was observed at the level of 5%. The lipoperoxide level in the testis was also remarkably increased by CdCl₂ treatment ($P < 0.01$). In particular, the lipoperoxide values in the testis of CdCl₂-treated rats sacrificed after 72 hrs were definitely higher than in controls under all three ambient conditions.

In the liver and the kidneys, lipoperoxide concentrations increased with temperature at both 24 and 72 hrs after CdCl₂ treatment, even in control rats. The lipoperoxide level in the testis tended to increase as temperature rose. As for three tissue weights (liver, kidney and testis), significant differences were not seen among three ambient temperatures. These evidences suggest that the acceleration of lipid peroxidation in all tissues as the ambient temperature rises is a biological reaction against extreme ambient temperatures.

Concerning the change in Cd content in the tissues, there was no tendency to increase the Cd level as ambient temperature changes. The transfer of Cd to tissues at a low temperature (7°C) was rather large in the liver. Similar observations were also reported by NOMIYAMA *et al.*⁽⁸⁾ Cd deposition increased with time in the liver ($P < 0.05$) and kidneys ($P < 0.01$), but decreased with time in the testis ($P < 0.01$) (Table 2).

Although the deposition of Cd in the testis was smaller than in the liver and kidneys, a remarkable increase in lipoperoxides was observed only in the testis. The testes are structurally different from other organs in having a 'blood-testis-barrier'

Table 2. Changes of Cd contents in the tissues of Cd treated rats

Temp. (°C)	hrs	Tissues		
		Liver ^{a)}	Kidney ^{a)}	Testis ^{a)}
7	24	31.53±0.89	5.67±0.16	0.24±0.003
	72	37.16±1.22	17.82±0.78	0.18±0.01
20	24	23.94±0.53	4.09±0.17	0.17±0.007
	72	28.16±0.56	18.11±0.43	0.09±0.002
32	24	26.36±0.46	4.77±0.15	0.24±0.005
	72	28.29±0.61	15.93±0.40	0.16±0.007

µg/g wet wt, Mean±SE.

Table 2-1. Two-way least-squares analysis of variance

Source of variation	Tissues		
	Liver	Kidney	Testis
	M. S. (d. f.)	M. S. (d. f.)	M. S. (d. f.)
Temperature (T)	255.42** (2)	10.67 (2)	0.024** (2)
Time (H)	141.07* (1)	1541.57** (1)	0.055** (1)
Interaction (T) × (H)	12.19 (2)	8.05 (2)	0.001 (2)
Error	24.35 (36)	6.29 (34)	0.002 (33)

M. S. = mean square. (d. f.) = degrees of freedom. ** $P < 0.01$, * $P < 0.05$

a) Cd was not detected in any tissues of control rats.

(BTB) to protect spermatogenesis from deleterious substances⁷⁾. For this reason, the concentration of Cd in the interstitial tissue is increased. This was confirmed by BERLIN and ULLBERG⁹⁾. It is not denied that this regional increase of Cd in the testis induces the remarkable increase in lipoperoxide value in the testis mentioned above.

Moreover, it has been reported⁹⁾ that rat testicular tissue contains abundantly unsaturated fatty acids such as docosapentaenoate, a member of the linoleate family, which is a source of lipid peroxidation. Recently, we observed that superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase activity levels in the testis acting as scavengers against free radicals which promote lipid peroxidation were smaller than in the liver¹⁰⁾. It is also considered that these physico-chemical differences among tissues cause acute and severe inflammation accompanied by an increase in lipid peroxidation in the testis.

It is seen from Fig. 1 that the lipid peroxidation in the testis induced by CdCl₂ is independent of ambient temperature, because the differences between the treated and control rats under three levels of temperature sacrificed after 72 hrs were almost equal, although the levels of lipoperoxide increased as the temperature rose. Therefore the increase in lipoperoxide concentrations in the testis of rats intoxicated by CdCl₂ proportional to ambient temperature is induced primarily by the increased rate of lipid peroxidation resulting from high temperature and is not a secondary effect of the lipid peroxidation induced by CdCl₂. It is considered therefore that high ambient temperature plays an important role in the increase of the inflammation observed in the testis of CdCl₂-treated rats.

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異なる環境温度下におけるカドミウム 中毒ラットの臓器中過酸化脂質値について

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カドミウムのひきおこすラットの精巣障害（出血性の炎症）に環境温度がどのような影響を及ぼすか検討するため、炎症のパラメーターと考えられている過酸化脂質の測定を臓器について行なった。

環境温度が高まると、 CdCl_2 の投与いかんにかかわらず、すべての臓器中（肝、腎、精巣）の過酸化脂質値が増加した。とくに高温下（ 32°C ）における CdCl_2 中毒ラットの精巣中過酸化脂質値は高値を示したが、コントロールラットの値も同様に高まっていた。

一方、カドミウム蓄積量は、すべての組織において、低温下（ 7°C ）で最も高かった。

以上の実験結果から、カドミウム中毒精巣で観察された過酸化脂質値の増加は、直接 CdCl_2 に起因するのではなく、環境温度の上昇によってひきおこされるものと推論される。この温度依存性のために、高温下においては、Cd による精巣炎症がいっそう増強されるものと考えられる。

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