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Effect of Route of Trihalomethanes (THM) Administration on Renal Toxicity in Male Rat

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Abstract □ Single non-lethal doses of chloroform ($CHCl_3$), dichlorobromomethane ($CHCl_2Br$), dibromochloromethane ($CHClBr_2$), or bromoform ($CHBr_3$) were administered to male rats. Routes of exposure including single intraperitoneal (ip) and subcutaneous (sc) injection were used in order to permit comparison of severity of THM effects and renal toxicity was assessed at varied times following treatment. On an equimolar basis, sc administration of $CHCl_3$ (either 12 or 3 mmoles/kg) is more effective at increasing KW/BW than ip $CHCl_3$ treatment. Plasma urea nitrogen (BUN) following ip THM injections are markedly increased with all four THM at 24 hours post treatment. BUN response to $CHCl_2Br$ and $CHClBr_2$ treatment remains elevated at 48 hours post administration, but $CHCl_3$ and $CHBr_3$-effected BUN levels have essentially returned to those of vehicle control. THM sc treatment results in a BUN response similar to that seen following ip treatment, with only the time course being different. With the exception of $CHBr_3$, sc and ip-treatments appear to be equally effective in evoking absolute BUN elevations. These results suggest that THM administration induce renal toxicity dependent upon the route of exposure.

Keywords □ THM (Trihalomethanes), ip, sc, renal toxicity

Chlorine is currently the most widely used water supply disinfectant in the world. Its addition during water purification generates low-molecular weight halogenated hydrocarbon from natural humic precursors. The four principal resultant compound are trihalomethanes (THM): chloroform ($CHCl_3$), bromodichloromethane ($CHCl_2Br$), chlorodibromomethane ($CHClBr_2$), and bromoform ($CHBr_3$)[1]. The presence of these four compounds in public drinking water supplies has frequently been detected and suggests a possible health hazard should these aquatic contaminants reach sufficient levels to produce renal toxicity[2,3].

Chloroform, in addition to being an environmental contaminant, is also a widely used manufacturing solvent in Korean industry. Because of its present potential as an occupational health hazard and its past usage as a general anesthetic, it is a frequent subject of toxicologic research[4]. Indeed, chloroform and other closely related compounds have already been shown to be toxic to the mammalian kidney[5-7]. Not surprisingly, however, most previous experimentation has concentrated on toxic effects following exposure by inhalation. Only occasionally has chloroform's renotoxic effects following other routes of exposure been examined and these investigations have concentrated primarily on histopathology[8]. Except for these isolated studies of effects of extra-pulmonary chloroform exposure on the kidney, thorough screening for renal effects by other THM has been almost non-existent.

The experiments described in this study were performed to further characterize THM-effected renal toxicity. The investigations consisted of time course studies of two different dosing regimens. Routes of exposure included single intraperitoneal (ip) and subcutaneous (sc) administrations. Single ip administrations were selected because preliminary research had demonstrated THM-effected renal toxicity at 24 hours post treatment. Subcutaneous administrations were selected because reduced central nervous system (CNS) toxicity and lethality

was anticipated with these treatment regimens. Of these two routes of administration, less CNS toxicity was expected from sc THM treatment because the THM would be absorbed less rapidly than by ip injection. Additionally, since THM are known to be metabolized by mixed function oxidase enzyme systems (which are concentrated in the liver and kidney), the comparison of severity of THM effects upon renal function following ip (a route subjecting the THM to hepatic metabolism prior to transport to the rest of the body) and sc (a route permitting more of the parent THM substance to be transported to the kidney on the first circulatory pass) treatments was expected to provide valuable information concerning the importance of hepatic and renal metabolism in THM-induced renal toxicity.

EXPERIMENTAL METHODS

Animals and exposure regimens

Male Sprague-Dawley rats weighing 230 to 300 g were housed in polypropylene cages for at least four days prior to initiation of experimental protocol and remained there through time of sacrifice. Animals were fed Purina chow and given access to water *ad libitum*. Lighting in the animal room was automatically regulated by a timer to maintain a 12: 12 light-dark cycle.

Rats were administered doses of THM with a single ip injection in corn oil vehicle (5.0 ml/kg) or corn oil vehicle alone. In the case of $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$, the doses selected were those determined to be maximum non-lethal doses (3 mmol/kg, ip) in a preliminary 24 hr investigation. In the case of $CHCl_3$, the doses selected were 12 mmol/kg (maximum non-lethal) and/or 3 mmol/kg (equimolar to the other THM) to permit comparisons of relative THM potency. Response to THM treatment was examined at 8, 24, and 48 hrs.

In addition, rats were administered doses of THM with a single sc injection in corn oil vehicle (5.0 ml/kg) or corn oil vehicle alone. THM doses selected for study were either equal to those used for ip treatment (to permit comparison of severity of THM effects using different routes of exposure) or maximum non-lethal sc doses (12 mmol/kg $CHCl_3$ and 6 mmol/kg for the other THM) determined in preliminary 24 hr investigation.

Renal function assessment

Renal function was assessed by gross pathology and blood chemistry. After excision, the kidneys were examined visually for general appearance with particular attention being paid to features such as abnormal size, color, and the presence of surface blemishes. Only abnormalities in appearance were noted and included in results. Kidney weight/body weight ratios (KW/BW) were calculated by diving KW on day of sacrifice by BW on day of pretreatment and multiplied by 10^3. Pretreated BW rather than BW at the time of sacrifice was used in the KW/BW ratio calculation to prevent influence of THM-effected reductions in BW (essentially a non-renal toxic event) during the experiments. Therefore, all ratios were normalized by using day of pretreatment body weights in all calculations.

Blood chemistry was performed on plasma samples. Immediately after each animal's sacrifice by exsanguination, 8-10 ml of heparinized blood (containing 200 units sodium heparin) were transferred from a syringe to centrifuge tube. Tubes were spun at 900×g for 10 min. Plasma supernatant was removed and stored in a freezer for further analysis. Blood urea nitrogen analysis was done by Sigma procedure 59. 20 μl of plasma were added to tubes containing 5 ml of pre-mixed reagent composed of two parts BUN color reagent to three parts BUN acid reagent. The tubes were mixed and placed into a dry heat incubation block at 105℃ for 20 min. Tubes were then cooled to room temperature and their contents were measured at 535 nm using UV-VIS spectrophotometer.

Statistical analysis

The means and standard errors of means were calculated for all treatment groups. The data were then subjected to either one-way or two-way analysis of variance followed by Duncan's Multiple Range test to determine which means were significantly different from vehicle controls. In all cases, a p-value <0.05 was used to determine significance.

RESULTS AND DISCUSSION

There are essentially no differences in Kidney Weight/Pretreated Body Weight (KW/BW) ratios

Table I. Eight hour response of kidney weight/body weight ratios (KW/BW) to ip and sc trihalomethane administration (3 mmol/kg)

Treatment	ip	sc
Vehicle	7.25± 0.20 n=4	6.87± 0.21 n=3
$CHCl_3$	7.35± 0.23 n=4	6.24± 0.19 n=3
$CHCl_2Br$	7.25± 0.12 n=4	6.62± 0.26 n=3
$CHClBr_2$	7.15± 0.39 n=4	6.98± 0.38 n=3
$CHBr_3$	7.60± 0.39 n=4	6.70± 0.11 n=3

Eight hours prior to sacrifice by rapid exsanguination, rats were adminstered 3 mmol/kg in corn oil vehicle (5 ml/kg). Values represent the ratio of kidney weight /pretreated body weight $\times 10^3$.

between any of the 3 mmol/kg treatment groups at eight hours following either ip or sc administration (Table I). In addition, there are no observable qualitative changes in gross appearance of the kidneys by this time, suggesting that any changes in gross pathology of the kidney that occuring post sc or ip THM treatment at these doses requires a greater length of time for manifestation.

Because sc treatment results in less central nervous system toxicity than ip treatment, larger THM doses (6 mmol/kg $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$) are non-lethal and therefore are also examined in this study. Subcutaneous administration of either 12 mmol/kg $CHCl_3$ or 6 mmol/kg $CHClBr_2$ results in swollen, discolored kidneys and significantly increases KW/BW ratios by 24 hrs following treatment (Fig. 1). Treatment with lower doses of THM by sc route still fails to show any effect upon KW/BW ratios, as does ip treatment by any non-lethal THM doses. By 48 hrs post sc treatment (Fig. 2), even 3 mmol/kg of $CHCl_3$ and $CHClBr_2$ show blotched and swollen kidneys and increased KW/BW ratios, while ip treatment continues to result in ratios essentially those of vehicle alone. Consequently, on an equimolar basis, sc administration of $CHCl_3$ (either 12 or 3 mmol/kg) is more effective at increasing KW/BW than ip $CHCl_3$ treatment. These results, therefore, suggest that sc administration of THM is more effective at increasing KW/BW ratios (and presumably at inducing overall renal edema) than ip administration.

Plasma urea nitrogen response (BUN) to ip THM treatment (3 mmol/kg) is illustrated in Fig. 3. Only

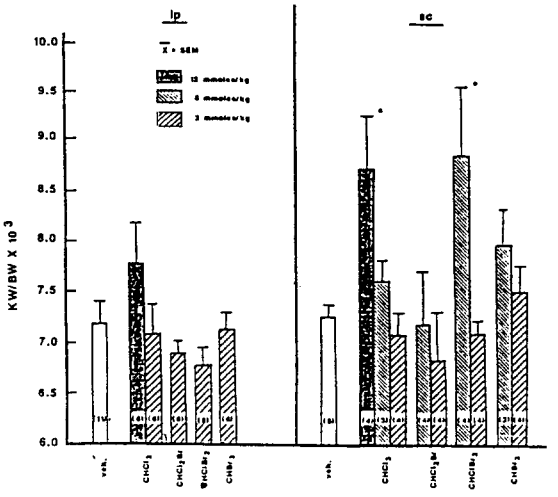

Fig. 1. Twenty-four hour response of kidney weight/body weight ratios (KW/BW) to ip and sc trihalomethane (THM) administration.
Rats were administered maximum non-lethal THM doses in corn oil vehicle (5 ml/kg) at 24 hrs prior to sacrifice by rapid exsanguination. Data is expressed as X± SEM of total kidney weight/pretreated body weight $\times 10^3$. *denotes significant difference from vehicle. $p<0.05$.

$CHCl_2Br$ shows a statistically significant effect at 8 hrs following treatment by decreasing BUN slightly. This decrease, however, is only transient and may well be related to a possible concomitant increase in glomerular filtration rate. In sharp contrast to the 8 hr data, BUNs are markedly increased at 24 hrs post THM treatment, with all four THMs effecting highly significant BUN elevations from vehicle control levels. BUN response to $CHCl_2Br$ and $CHClBr_2$ treatment remains elevated at 48 hrs post administration, but $CHCl_3$ and $CHBr_3$-effected BUN levels have essentially returned to those of vehicle control. These data indicate some degree of renal toxicity by 24 hrs following ip treatment of all four THMs, with $CHCl_3$ and $CHBr_3$-treated animals showing recovery by 48 hrs.

Fig. 4 represents BUN response following sc THM administration (3 mmol/kg). At 8 hrs post treatment, no effect is seen. At 24 hrs post treatment, only $CHClBr_2$ shows any significant effect. By 48 hrs, however, only $CHBr_3$, fails to significantly increase BUN. Apparently, sc treatment results in a BUN response similar to that seen following ip tratment, with only the time course being different.

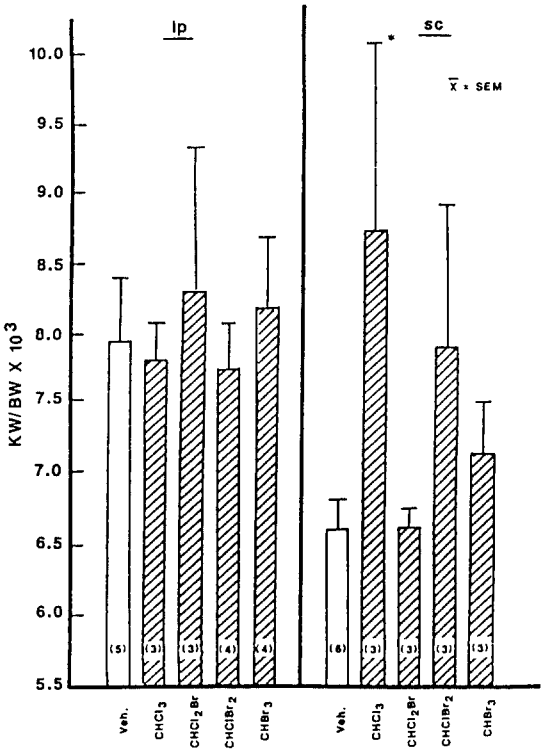

Fig. 2. Forty-eight hour response of kidney weight/body weight ratios (KW/BW) to ip and sc trihalomethane (THM) administration (3 mmol/kg).

Rats were administered THMs in corn oil vehicle (5 ml/kg) at 48 hrs prior to sacrifice by rapid exsanguination. Data is expressed as X± SEM of total kidney weight/pretreated body weight×10^3. *denotes significant difference from vehicle, $p<0.05$.

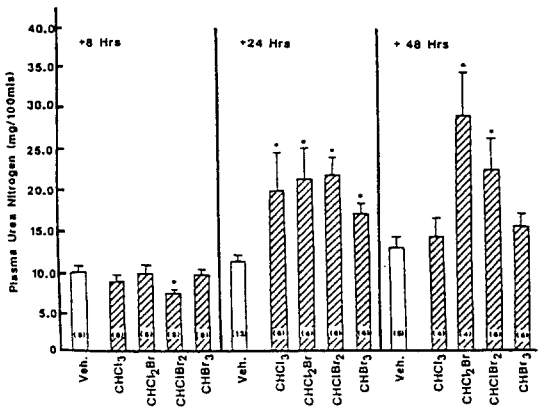

Fig. 3. Time course of plasma urea nitrogen response to ip trihalomethane (THM) administration (3 mmol/kg).

Rats were sacrificed by rapid exsanguination at 8, 24, or 48 hrs following administration of THMs in corn oil vehicle (5 ml/kg). *denotes significant difference from vehicle, $p<0.05$.

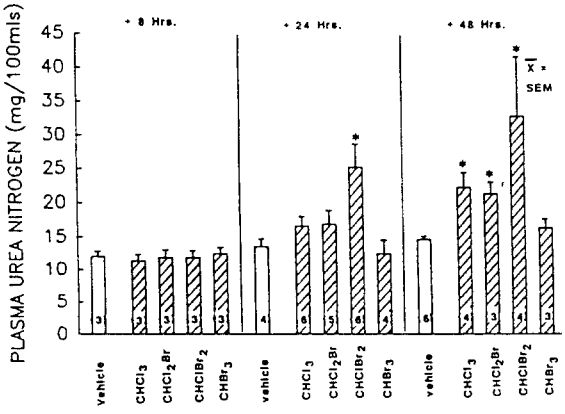

Fig. 4. Time course of plasma urea nitrogen response to sc trihaloethane (THM) administration (3 mmol/kg).

Rats were sacrificed by rapid exsanguination at 8, 24, or 48 hrs following administration of THMs in corn oil vehicle (5 ml/kg). *denotes significant difference from vehicle, $p<0.05$.

Manifestation of elevated BUN requires more time following sc treatment than it does following ip treatment. With the exception of $CHBr_3$, sc and ip-treatments appear to be equally effective in evoking absolute BUN elevations.

The greater time required for maximal sc response is not unexpected, since sc absorption is known to be slower than hepatic periportal absorption. Differences in response to the two routes of exposure may be related to endogenous metabolic differences between the two species.

THM-induced renal toxicity may result from more than one metabolic pathway of activation. For example, it is known that $CHCl_3$ (and therefore presumably other THM) are metabolized by cytochrome P-450 mixed function oxidase (MFO) systems to reactive, unstable electrophilic species. These reactive species are, in turn, either detoxified by conjugation with glutathione (GSH) and/or amino acids or attack cellular targets. It is known that both the liver and the kidney contain MFOs, with the majority of P-450 activity being located in the hepatic centrilobular region and the renal pro-

ximal tubule, the two areas showing greatest necrosis and/or dysfunction following THM exposure. If renal metabolic activation alone was responsible for THM renotoxicity, the sc route would promote the most severe renal toxicity (as shown if Fig. 1. and Fig. 2), since sc administration permits a greater amount of parent substance THM to directly reach the kidney. However, this assumption may not be true, since sc and ip-treatments appear to be equally toxic in terms of renotoxic BUN parameter.

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