Preliminary Chronic Toxicity Study of Copper Aspirinate

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Introduction

Following the observation that copper complexes of many ligands had antiinflammatory and antiulcer activity [1], the copper complex of aspirin, $Cu(II)_2$-(acetylsalicylate)$_4$, was selected for acute and chronic toxicity evaluations in preparation for Phase I clinical studies in Man. The oral $LD_{50/7}$ values for male and female rats were found by probit analysis to be 895 ± 222 and 977 ± 297 milligrams per kilogram of body weight (mg/kg) respectively. Based upon these data a Food and Drug Administration approved protocol was obtained for chronic toxicity study of this complex. This study involves the treatment of male and female rats and dogs with intragastric doses of 0, 25, 50, or 100 mg/kg $Cu(II)_2$(acetylsalicylate)$_4$ for three months followed by three months without treatment. Two animals from each group are to be sacrificed every month and tissues removed for pathologic evaluation as well as copper and zinc analyses. This presentation reports results of our preliminary study using 100 mg/kg of $Cu(II)_2$(acetylsalicylate)$_4$.

Experimental

Two groups of ten male rats were given either 0 or 100 mg/kg $Cu(II)_2$(acetylsalicylate)$_4$ orally five days a week for three months. Body weights were obtained weekly. Treatment ceased at the end of the third month. The remaining animals were continued without treatment for an additional two months.

At the end of each month two rats were anesthetized with carbon dioxide and exsanguinated via the aorta before tissues were taken for atomic absorption analysis. Samples of liver, kidney, spleen, lung, heart, stomach, small and large intestine, testes, skin, fur, brain, and blood were prepared for atomic absorption analysis using an Instrumentation Laboratory 157 spectrophotometer. Blood plasma was analyzed after 3:1 dilution with deionized distilled water. Solid tissues were frozen and stored prior to atomic absorption analysis. After thawing tissues were wet-ashed in concentrated nitric acid at 90 °C. Perchloric acid was added to the nitric acid digests to decolorize. Appropriately diluted solutions were analyzed using atomic absorption spectrophotometric methods, which have been shown to be interference free [2].

At the end of each month two rats were also sacrificed to obtain tissues for pathological examination. For this purpose animals were anesthetized with Nembutol and killed by intracardial perfusion with normal saline followed by neutral buffered 10% Formalin. General necropsy was performed on each animal. Tissues sampled included liver, kidney, spleen, lung, heart, stomach, small intestine, testes, skin, brain, and eye. All tissues were further fixed in 10% buffered formalin, dehydrated with graded ethanol, cleared with xylene, and embedded with paraffin. Five to six micron thick sections were cut and stained with hematoxylin and eosin. Sections of liver and kidney were also stained with Masson's Trichrome and Wilder's reticulum stains to detect any fibrous changes. All stained tissue sections were examined at the light microscopic level.

Results and Discussion

As shown in Fig. 1, there were no significant differences in growth of treated and non-treated rats except during the last month, one month after treatment had been discontinued. The significance of this difference is unclear since only one of the two remaining rats lost weight.

Data presented in Figs. 2 and 3 show that treatment with $Cu(II)_2$(acetylsalicylate)$_4$ did not affect either plasma zinc or copper levels. Data presented in Table I show that treatment with $Cu(II)_2$(acetylsalicylate)$_4$ did *not* cause accumulation of copper in

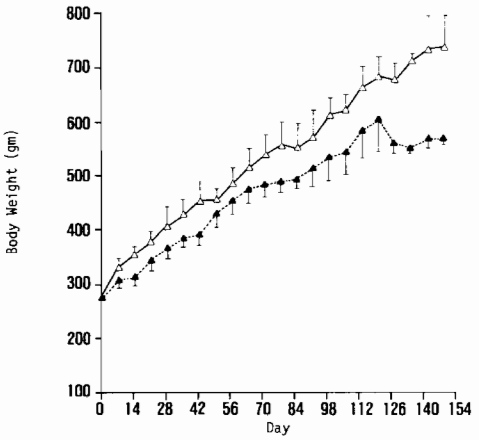

Fig. 1. Growth curves for control (△) and Copper Aspirinate treated (▲) rats.

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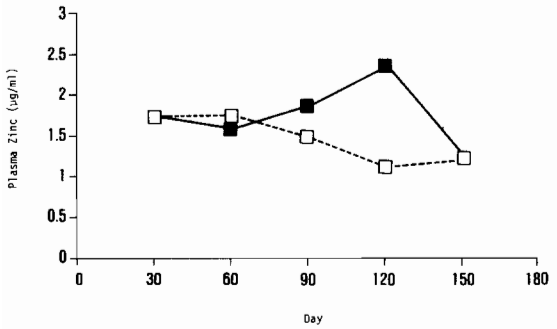

Fig. 2. Plasma zinc concentrations in control (■) and Copper Aspirinate treated (□) rats.

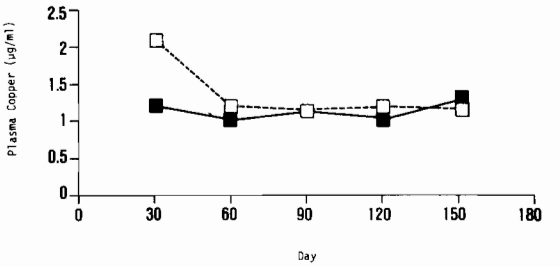

Fig. 3. Plasma copper concentrations in control (■) and Copper Aspirinate treated (□) rats.

TABLE I. Tissue Copper and Zinc Concentrations (µg/g ± SD) for Each Sacrifice Time Period (1 to 5 months) for Both Non-treated and Copper Aspirinate-treated Animals.

Brain:Cu			Brain:Zn	
Month	Control	Treated	Control	Treated
1	2.03 ± 0.22	2.14 ± 1.29	10.03 ± 1.91	8.95 ± 1.88
2	1.58 ± 0.11	5.27 ± 4.65	14.66 ± 8.31	4.05 ± 2.37
3	2.23 ± 0.39	2.61 ± 0.20	8.14 ± 2.75	9.49 ± 1.86
4	2.55 ± 0.06	2.43 ± 0.94	10.24 ± 3.17	13.96 ± 5.29
5	2.19 ± 0.41	9.53 ± 11.08	14.54 ± 4.50	6.28 ± 3.99
Liver:Cu			**Liver:Zn**	
Month	Control	Treated	Control	Treated
1	3.16 ± 0.48	3.47 ± 0.62	19.26 ± 3.02	7.60 ± 4.94
2	3.58 ± 1.16	3.76 ± 0.57	61.09 ± 53.53	14.84 ± 1.92
3	12.10 ± 8.62	4.11 ± 1.35	56.38 ± 39.27	12.89 ± 0.43
4	3.89 ± 0.22	2.97 ± 0.44	21.28 ± 3.82	27.08 ± 9.16
5	4.76 ± 2.07	1.55 ± 1.80	22.64 ± 2.26	18.99 ± 5.92
Kidney:Cu			**Kidney:Zn**	
Month	Control	Treated	Control	Treated
1	5.13 ± 2.44	13.99 ± 5.27	17.22 ± 3.12	16.54 ± 5.94
2	8.92 ± 0.42	17.71 ± 7.37	34.35 ± 13.50	13.24 ± 0.48
3	9.24 ± 5.51	7.67 ± 2.95	17.37 ± 9.17	15.32 ± 0.16
4	9.16 ± 0.63	6.86 ± 0.47	19.55 ± 6.64	23.05 ± 0.36
5	9.07 ± 1.07	2.79 ± 1.72	22.34 ± 4.35	16.24 ± 5.77
Lung:Cu			**Lung:Zn**	
Month	Control	Treated	Control	Treated
1	1.86 ± 0.09	5.60 ± 5.66	16.66 ± 1.75	12.61 ± 0.03
2	1.18 ± 0.24	1.83 ± 0.34	16.94 ± 12.42	11.40 ± 1.11
3	1.35 ± 0.23	1.79 ± 0.20	8.97 ± 3.14	13.15 ± 4.45
4	1.74 ± 0.42	1.18 ± 0.06	14.28 ± 1.41	17.10 ± 10.69
5	1.80 ± 0.53	2.40 ± 1.45	18.51 ± 7.18	10.81 ± 4.13
Spleen:Cu			**Spleen:Zn**	
Month	Control	Treated	Control	Treated
1	1.08 ± 0.27	3.92 ± 3.25	12.73 ± 6.99	12.91 ± 3.92
2	0.92 ± 0.12	1.29 ± 0.30	29.19 ± 24.04	17.47 ± 19.56
3	1.42 ± 0.13	1.38 ± 0.34	7.40 ± 5.26	12.24 ± 7.04
4	5.24 ± 5.19	0.72 ± 0.30	14.89 ± 4.52	22.13 ± 13.32
5	15.86 ± 17.35	9.26 ± 6.92	45.29 ± 55.19	23.51 ± 22.25

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TABLE I. *(continued)*

Stomach:Cu			Stomach:Zn	
Month	Control	Treated	Control	Treated
1	1.78 ± 0.04	2.00	16.79 ± 5.42	18.18
2	1.63 ± 0.62	2.01 ± 0.63	26.85 ± 10.69	13.27 ± 1.27
3	5.53 ± 4.87	4.18 ± 3.28	13.51 ± 8.86	9.37 ± 7.03
4	1.68 ± 0.31	1.64 ± 0.29	13.50 ± 1.10	21.37 ± 4.63
5	1.62 ± 0.50	2.20 ± 0.07	16.93 ± 3.53	14.02 ± 0.86
Heart:Cu			Heart:Zn	
Month	Control	Treated	Control	Treated
1	5.81 ± 1.99	36.46 ± 44.10	15.08 ± 0.37	12.72 ± 2.30
2	3.68 ± 0.32	4.18 ± 0.69	17.94 ± 10.65	13.64 ± 8.29
3	4.55 ± 0.15	5.52 ± 0.04	10.58 ± 3.63	11.11 ± 4.32
4	4.59 ± 0.64	4.11 ± 0.95	11.73 ± 6.50	16.52 ± 7.92
5	4.37 ± 0.89	3.02 ± 1.18	12.54 ± 6.32	8.77 ± 4.48
Small Intestine:Cu			Small Intestine:Zn	
Month	Control	Treated	Control	Treated
1	1.99 ± 0.54	4.51 ± 3.84	22.56 ± 6.12	29.39 ± 17.49
2	1.06 ± 0.05	1.58 ± 0.20	19.30 ± 4.39	14.63 ± 5.93
3	1.65 ± 0.21	1.95 ± 0.11	13.66 ± 2.04	14.12 ± 0.31
4	3.99 ± 2.96	1.64 ± 0.52	13.27 ± 0.77	26.64 ± 12.10
5	1.51 ± 0.57	1.52 ± 0.37	16.13 ± 2.93	14.37 ± 3.78
Large Intestine:Cu			Large Intestine:Zn	
Month	Control	Treated	Control	Treated
1	2.04 ± 0.34	7.10 ± 3.51	15.49 ± 6.60	37.22 ± 22.93
2	1.40 ± 0.26	1.76 ± 0.28	20.16 ± 4.65	13.89 ± 0.35
3	1.51 ± 0.99	2.52 ± 0.50	16.56 ± 0.37	16.09 ± 0.72
4	2.46 ± 0.08	1.64 ± 0.04	19.19 ± 2.71	20.79 ± 7.82
5	1.51 ± 1.10	1.63 ± 0.01	24.35 ± 8.79	13.30 ± 2.76
Testes:Cu			Testes:Zn	
Month	Control	Treated	Control	Treated
1	1.71 ± 0.21	5.15 ± 4.18	23.87 ± 6.43	16.63 ± 2.97
2	1.58 ± 0.60	1.58 ± 0.03	29.19 ± 13.33	9.96 ± 7.47
3	1.65 ± 0.30	1.66 ± 0.07	16.82 ± 3.95	13.44 ± 2.38
4	1.65 ± 0.04	3.92 ± 2.92	17.38 ± 1.56	33.12 ± 18.97
5	1.56 ± 0.18	1.78 ± 0.27	19.02 ± 1.70	16.40 ± 0.09
Skin:Cu			Skin:Zn	
Month	Control	Treated	Control	Treated
1	0.59 ± 0.01	1.05 ± 0.09	14.70 ± 10.29	3.74 ± 0.90
2	1.41 ± 0.54	2.85 ± 2.05	21.73 ± 10.47	23.55 ± 23.58
3	0.76 ± 0.07	1.08 ± 0.38	6.56 ± 1.87	10.26 ± 3.49
4	1.61 ± 1.24	0.80 ± 0.03	9.45 ± 6.75	15.36 ± 2.64
5	0.88 ± 0.03	0.76 ± 0.04	9.82 ± 1.76	3.45 ± 0.90
Fur:Cu			Fur:Zn	
Month	Control	Treated	Control	Treated
1	5.37	5.51 ± 5.10	73.48	88.13 ± 13.17
2	7.63	2.99 ± 2.96	128.91	33.28 ± 41.45
3	7.84 ± 0.95	8.41 ± 0.80	79.74 ± 14.49	56.09 ± 67.87
4	5.81 ± 0.22	8.17 ± 0.27	49.51 ± 6.14	132.38 ± 54.43
5	8.47 ± 1.05	8.74 ± 0.73	155.38 ± 40.78	91.79 ± 5.52

brain, liver, kidney, lung, spleen, stomach, heart, small or large intestine, testes, skin, or fur. Nor was there any observable interference with zinc levels in these tissues. These results are consistent with the hypothesis that normal animals excrete ingested copper in excess of homeostatic requirements. The 100 mg/kg dose of $Cu(II)_2(acetylsalicylate)_4$ represented a daily intake of 15 mg of copper per kg at the outset and about 7.5 mg/kg during the third month of treatment, since body weights were nearly twice as much at the beginning of the third month of treatment compared to their initial weights.

With the exception of the liver there was no remarkable histopathology observed in the tissue examined. Livers from copper aspirinate-treated animals showed an increase of phagocytic Kupffer cells in the hepatic sinusoids. An increase in Kupffer cells was most evident in animals treated with copper aspirinate for 3 months. This change was observable one month after treatment but was significantly less two months after treatment.

The precise significance of the increase in Kupffer cell activity is still obscure. Such changes may be secondary to chronic hepatocytic injury or irritation which may be too minute to be detected by light microscopy or is a direct effect of copper aspirinate on the reticuloendothelial system. Further investigation, particularly on the liver, by both light and electron microscopy is warranted.

One purpose of a chronic toxicity study is to produce toxicity at a high dose. A second purpose is to show that the observed toxicity does not occur at the projected therapeutic dose or that there is toxicity remission when treatment is discontinued. The latter point has been demonstrated in this preliminary study using a dose which is twenty times the projected therapeutic dose. We hope to be able to do the complete chronic toxicity study to confirm remission following cessation of treatment with 100 mg/kg and, more importantly, determine whether or not the number of liver macrophages increases at lower doses of 50, 25, and 5 mg/kg.

Acknowledgement

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