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EVOLUTION OF SARCOMA 180 IN MICE TREATED WITH HYPERCHLORINATED WATER

FAUSTO EDMUNDO LIMA PEREIRA
FABIO BENEZATH CHAVES

Mice treated with hyperchlorinated water (50 ppm of chlorine) and control mice, drinking tap water (1-3 ppm of chlorine) were inoculated with 2.5 x 10^6 sarcoma 180 cells, by intraperitoneal route. Tumor evolution was measured by enumeration of tumor cells in peritoneal cavity and by evaluation of weight gain at different time intervals after tumor implantation. In mice treated with excessive amounts of chlorine there was enhancement of tumor growth demonstrated by: (a) shorter incubation period and increased weight gain (ascites formation) after tumor implantation; (b) increased number of tumor cells in the peritoneal cavity 2, 3 and 4 days after tumor challenge. The number of peritoneal cells exsudated after tumor implantation was lower in mice treated with hyperchlorinated water than in controls. The tumor enhancement observed after excessive chlorine ingestion would be due to: (a) reduction of the number of peritoneal macrophages that migrate to the peritoneal cavity and (b) reduction of the tumoricidal capacity of peritoneal macrophages induced by the direct effect of chlorine or by the reduction of the amount of endogenous endotoxins due to the bactericidal effect of chlorine.

Chlorination of drinking water in colonies of experimental rodents has been carried out to avoid infection with enteric bacteria, such as peseudomonas, mainly when the animals are submitted to immunossupressive procedures. The level of chlorine for this purpose is around 15 parts per million (ppm). Fidler (1977) showed that hyperchlorination of water could damage macrophage function, regarding their tumoricidal capacity. This author used an *in vitro* system to demonstrate the reduced tumoricidal effect of peritoneal macrophages from mice drinking water with 30 ppm of chlorine.

In this paper we showed that hyperchlorination of water enhances the evolution of the sarcoma 180 (ascitic tumor) in mice.

MATERIAL AND METHODS

Male, albino, outbred mice, weighting between 25 and 27 g, were used for the experiments. The mice were cage-conditioned and received a commercial diet *ad libitum.*

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Departamento de Patologia do Centro Biomédico da Universidade Federal do Espírito Santo. CP. 780 – 29000 Vitória, ES, Brazil.

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One group of mice (20 in each experiment) received tap water for drinking (1 to 2 ppm of chlorie). Another group (20 mice in each experiment) received water with 1% dilution of a commercial product used for chemical sterilization (Milton, R. Merriel – Moura Brasil, São Paulo). The water used contains about 50 ppm of chlorine, evaluated by the ortho toluene method.

The sarcoma 180 cells were inoculated by intraperitoneal route four weeks after the beginnig of chlorine ingestion. Each animal received 2.5×10^6 cells of the sarcoma – 180, freshly removed from a mouse with ascitic tumor.

After the tumor implantation, the mice were weighted daily until the 15th day, when they began to die. The difference between the initial weight (Po, at the day of tumor inoculation) and the weight in the following days (P) indicates the weight gain after tumor implantation and thus the ascites production, as demonstrated by Lubiniecki & Cypess (1975).

For counting the tumor cells in peritoneal cavity after tumor implantation, groups of five mice were killed after ether anesthaesia, and peritoneal cells were colleted after injection of 3ml of cold Ringer solution. The cells were counted in a Newbauer chamber, after dilution in gentian violet 1:1000 in 5% acetic acid solution. During the counting of tumor cells, the resident exsudated peritoneal cells (macrophages, lymphocytes and neutrophils) were also counted. The differentiation of tumor cells from peritoneal cells was easy because of the difference in size of these cells. The counting of tumor cells in peritoneal cavity was performed two, three and four days after tumor implantation.

For the comparison of the results the Student's t-test was used.

RESULTS

There was a significant difference in weight gain between the two groups. The control group showed weight gain that was a linear function of time after the 5th day of tumor implantation. The mice drinking hyperchlorinated water showed a slight reduction in body weight in the three initial days after tumor challenge but showed an increased rate of weight gain, higher than in control group, after the 6th day of tumor implantation. The difference in the weight gain between the two groups was significant after the 6th day (Figure I and Table III).

For cheking the evolution of tumor in the initial phase after tumor implantation, tumor cells were enumerated in the peritoneal cavity 2, 3 and 4 days after tumor implantation. The number of tumor cells was higher in mice drinking hyperchlorinated water than in controls (Table I).

The number of exsudated cells in peritoneal cavity 2, 3 and 4 days after tumor implantation was lower in mice drinking hyperchlorinated water (Table II). In control mice the number of mononuclear cells with morphological characteristics of macrophages increased after tumor implantation but did not in mice treated with hyperchlorinated water.

DISCUSSION

Our results showed that the mice drinking excessive amounts of chlorine have a reduced resistance against the sarcoma 180. This observation is in agreement with data of Fidler (1977) that showed *in vitro* redution cytotoxicity of peritoneal macrophages from mice drinking a high dose of chlorine, against B16 melanoma and UV – 112 fibrosarcoma cells.

The fast evolution of the sarcoma 180 in mice drinking hyperchlorinated water is demonstrated by: (1) a higher number of tumor cells in peritoneal cavity 2, 3 and 4 days after tumor inoculation; this is a demonstration that the number of survivor cells after the inoculation was higher than in controls; (2) the increased amount of ascitic fluid produced, indicated by the weight gain after the 6th day of tumor implantation; the weight gain after tumor inoculation is a linear function of time, after a variable incubation period, and is related to the ascitic fluid produced (Lubiniecki & Cypess, 1975). The incubation period (the time interval between the tumor inoculation and the start of ascites formation) is a function of the number of tumor cells implanted. In our experiments the incubation period of control mice was 6.4 ± 0.5 days and was 5.1 ± 0.4 days in mice treated with hyperchlorinated water (Table III).

The slight weight loss observed in the three initial days after tumor challenge in the group of mice drinking hyperchlorinated water was due to the greater toxic effect of tumor cells. In fact, the number of survivor cells in this group was higher than in control group, as demonstrated by counting the tumor cells 48h after tumor challenge.

There are several possible explanations for this reduced resistence against a transplantable tumor observed in mice drinking an excessive amount of chlorine.

One explanation is the reduced tumoricidal capacity of peritoneal macrophages induced by chlorine ingestion, as demonstrated *in vitro* by Fidler (1975). There are demonstrations that chlorine has an inhibitory effect on the hexose monophosphate shunt (Eaton et al, 1973) and this oxidative pathway of glucose is important for the tumoricidal capacity of macrophages (Fidler et al, 1976).

Another mechanism would be related to the reduction in the number of macrophages that migrate into the peritoneal cavity after tumor implantation. In fact we showed that the number of peritoneal cells exsudated after tumor challenge was lower in mice treated with hyperchlorinated water than in controls. Fidler (1977) observed reduction in migration of macrophages to the peritoneal cavity after thioglycolate injection in mice drinking hyperchlorinated water. The reduction of macrophages in the peritoneal cavity after tumor implantation would be an important factor that diminishes the number of tumor cells that are killed after tumor inoculation.

On the other hand an excessive chlorine ingestion reduces the number of intestinal bacteria, reducing the amount of endogenous endotoxin. There are several demonstrations that endotoxin could render macrophages cytotoxic for tumor cells *in vitro* (Alexander & Evans, 1971; Currie & Bashman, 1975; Doe & Henson, 1978 and Meltzer et al, 1979). The induction of tumoridicidal activity on macrophages appear to be a general property of endotoxins, and determinant event of macrophage activation is the lipid A-macrophage interaction (Doe et al, 1978). Therefore it is possible that low levels of endotoxin absorbed from the gut decreases the tumoricidal activity of macrophages in mice treated with chlorine.

RESUMO

Camundongos tratados com água hiperclorada (50 ppm de cloro) e camundongos controles, ingerindo água de torneira (3 ppm de cloro) foram inoculados por via i.p. com 2.5×10^6 células do sarcoma 180. O desenvolvimento do tumor foi medido pela contagem de células tumorais na cavidade peritoneal e pela avaliação da produção de líquido ascítico através da verificação do ganho de peso em diferentes dias após a implantação das células tumorais. Nos camundongos tratados com água hiperclorada houve maior desenvolvimento do tumor, demonstrado por: (a) menor período de incubação e maior ganho de peso (maior formação de líquido ascítico) após a implantação do tumor; (b) maior número de células tumorais na cavidade peritoneal 2, 3 e 4 dias após a implantação do tu-

mor. O número de células peritoneais exsudadas depois da implantação do tumor foi menor nos camundongos tratados com água hiperclorada do que nos animais controles. O maior desenvolvimento do tumor nos animais tratados com água hiperclorada poderia estar relacionado a: (a) redução do número de macrófagos que migram para a cavidade peritoneal e (b) reduçao da capacidade tumoricida dos macrófagos peritoneais induzida pela ação direta do cloro ou indiretamente, pela redução dos níveis de endotoxina devida ao efeito bactericida do cloro.

REFERENCES

ALEXANDER, P. & EVANS, R., 1971. Endotoxin and double stranded RNA render macrophages cytotoxic. *Nature, 232* :76-78.

CURRIE, G.A. & BASHAM, C., 1975. Activated macrophages release a factor which lyses malignant cells but not normal cells. *J Exp Med, 142* :1600-1605.

DOE, W.F. & HENSON, P.M., 1978. Macrophage stimulation by bacterial lipopolysacharides. I-Cytolitic effect on tumor target cells. *J Exp Med, 148* :544-556.

DOE, W.F.; YANG, S.T.; MORRISON, D.C.; BETZ, S.J. & HENSON, P.M., 1978. Macrophage activation by bacterial lipopolysacharides. II-Evidence for independent differentiation signal delivered by lipid A and protein rich fraction. *J. Exp. Med, 148* :557-568.

EATON, J.W.; KOLPIN, C.F.; SWOFFORD, J.S.; KJESLLSTRAND, C.M. & JACOBS, H.S., 1973. Chlorinated urban water: a cause of dialysis induced hemolytic anemia. *Science, 183* :463-464.

FIDLER, I.J.; DARNELL, J.M. & BUDMEN, M.B., 1976. Tumoricidal properties of mouse macrophages activated mediators from rat lymphocytes stimulated with concanavalin A. *Cancer Res, 36* :3608-3615.

FIDLER, F.J., 1977. Depression of macrophages in mice drinking hyperchlorinated water. *Nature, 270* :735-736.

LUBINIECKI, A.S. & CYPESS, R.H., 1975. Quantitative study of the effect of previous *Trichinella spiralis* infection on Sarcoma 180 ascitic tumor formation in mice. *Tropenmed Parasit, 26* :329-334.

MELTZER, M.S.; RUCCO, L.P.; BORASCHI, D. & NACY, C.A. 1979. Macrophage activation for tumor citotoxicity: analysis of intermediary reactions. *J Reticuloendot Soc, 26* :403-415.

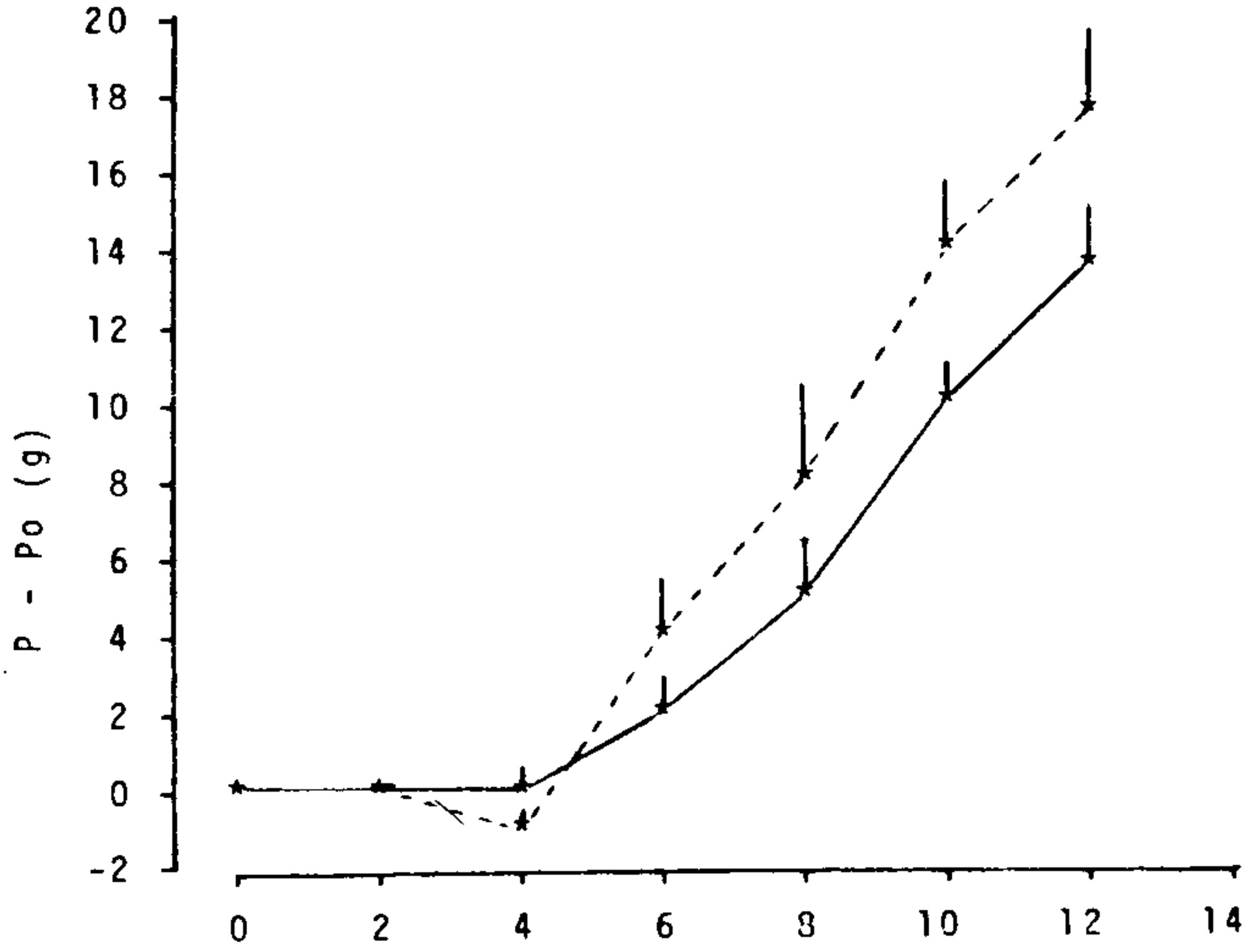

Fig. 1 – Evolution of sarcoma 180 in mice drinking hyperclorinated water (50 ppm of chlorine) or tap water (1-3 ppm of chlorine). The mice (20 in each group) were inoculated with 2.5×10^6 cells of sarcoma 180, by i.p. route, four weeks after chlorine treatment. Tumor evolution was measured by evaluation of weight gain (ascites formation) on different days after tumor inoculation. The differences observed after the 6th day are significant ($p < 0.05$).

_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ = mice treated with hyperchlorinated water

_____ = mice treated with tap water

TABLE I

Number of tumor cells in the peritoneal cavity of control mice and of mice treated with an excessive amount of chlorine, in different time intervals after tumor inoculation.

Time after tumor inoculation (days)	*2*	*3*	*4*
Control	4.90 ± 1.39	9.03 ± 2.54	17.32 ± 3.91
Chlorine	9.33 ± 2.56	17.25 ± 3.20	31.36 ± 6.40

The results are the mean ($X\ 10^6$) ± one standart deviation of five mice per group. The differences observed between the two groups are significant ($p < 0.05$).

TABLE II

Number of peritoneal cells (macrophages, lymphocytes and neutrophils) exsudated into the peritoneal cavity in different time intervals after the inoculation of 2.5×10^6 cells of the sarcoma 180 in control mice and in mice treated with hyperchlorinated water.

Time after tumor inoculation (days)	*2*	*3*	*4*
Control	5.2 ± 0.7	$8.7 \pm 1.2^*$	$13.8 \pm 1.1^*$
Chlorine	5.1 ± 0.8	6.2 ± 0.8	7.4 ± 1.4

The results are the mean ($X\ 10^6$) peritoneal cells ± one standard deviation of five mice per group. The differences observed between the two groups are significant on days 3 and 4 after tumor inoculation ($p < 0.5$).

TABLE III

Incubation period and mean weight gain/day observed after inoculation of 2.5×10^6 sarcoma 180 cells in the peritoneal cavity of mice drinking tap water (control) and mice drinking hyperchlorinated water (chlorine).

	Incubation period (days)	*Mean weight gain/day (grams)*
Control	6.4 ± 0.5	2.3 ± 0.4
Chlorine	5.1 ± 0.4	3.7 ± 0.7

All the differences observed are significant ($p < 0.05$).