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THE INFLUENCE OF A COMPOSITE TREATMENT FOR INTERNAL CONTAMINATION BY SEVERAL RADIONUCLIDES ON CERTAIN HEALTH PARAMETERS IN RATS

B. Kargačin, T. Maljković, M. Blanuša and K. Kostial

Institute for Medical Research and Occupational Health, Zagreb

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The health effects of a composite treatment for the early and late therapy of internal contamination with several radionuclides were evaluated in rats. The rats were given a mixture of calcium alginate, ferrihexacyanoferrate(II) and potassium iodide orally for 10 days together with the intraperitoneal administration of Ca-DTPA in the first three days and the oral administration of Zn-DTPA during the remaining seven days. The health effects of this treatment were evaluated by body weight measurements, haematological and histopathological examinations and trace element analysis. The treated animals had a slightly lower number of erythrocytes, slightly lower haemoglobin and haematocrit values and very mild histopathological changes in the kidneys in the form of hydropic degeneration of proximal convoluted tubules. The observed changes were consistent with the known effects of individual therapeutic agents (alginate, Ca-DTPA) and therefore the simultaneous administration of several agents did not alter toxicity or produce new adverse effects.

The results of our previous studies showed that the simultaneous oral administration of a mixture of calcium alginate, ferrihexacyanoferrate (II) and potassium iodide (antidotes for radioactive strontium, caesium and iodine) and intraperitoneal administration of Ca-DTPA (antidote for transuranium elements) presents a very efficient method for the early therapy of internal contamination with several radionuclides (1). Oral administration of the same mixture together with orally administered Zn-DTPA proved effective for delayed and prolonged therapy (2). There could be situations when early as well as delayed treatment after an in-

ternal contamination with several radionuclides would be necessary. The health effects of such treatment consisting of several different therapeutic agents are, however, unknown.

Alginates (3, 4), ferrihexacyanoferrate(II) (5, 6) and potassium iodide (7) have been found non-toxic if administered singly or together in the form of a mixture (8). Numerous studies on animals have shown Ca-DTPA to cause severe lesions of the kidneys (9 . . 12), the intestinal mucosa (13, 14), and the liver (15), to impair haematopoietic proliferation (16, 17) and to be embriotoxic (18). The observed lesions were, however, caused by doses much higher than those recommended for human use. Ca-DTPA was shown to increase urinary excretion of zinc and manganese causing decreased zinc concentration in the small intestine, skeleton, pancreas, testes and increased zinc concentration in the kidneys (19) and decreasing manganese concentration in the small intestine (20). The toxic action of Ca-DTPA has been therefore ascribed to mobilization or binding of endogenous essential trace metals in exchange for calcium and a consequent impairment of metal-controlled or activated systems (e. g. DNA synthesis — 21 . . 23). The chelates with a higher stability than calcium chelates proved less toxic. Thus, Zn-DTPA has lower toxicity shown by a lower mortality (24), absence of kidney, liver or small intestine lesions (14, 17) and lower embryotoxicity (18, 25). Its toxicity does not depend on the treatment schedule and even high doses given over a period of time are well tolerated (26).

Based on our pharmacokinetic studies (1, 2), the need for early use of the highly efficient Ca-DTPA therapy against transuranium elements (27), the need for the less toxic Zn-DTPA in prolonged treatment (28) which gives good results when administered orally (29) in present experiment the following treatment was used: the mixture of calcium alginate, ferrihexacyanoferrate(II) and potassium iodide was given as a supplement to food for 10 days, Ca-DTPA was administered intraperitoneally on the first three days and Zn-DTPA orally during the remaining seven days of the experiment. Such treatment imitates the possible use of these therapeutic agents in cases of prolonged simultaneous exposure to several radionuclides in the environment. The aim of this work was to find out whether simultaneous administration of these therapeutic agents, because of their mutual interference, could cause untoward health effects. The effects of such combined treatment were evaluated by measurements of body weights, haematological and histopathological examinations and trace element analysis.

MATERIALS AND METHODS

The experiment was performed on six-week-old female outbred albino rats, 119 ± 2 g body weight, from the Institute's breeding farm. It lasted 10 days. The animals were divided into two groups. The controls were on standard rat diet and for the first three days of the experiment they

received 0.9% saline solution intraperitoneally. The experimental group was on food supplemented with a mixture of 15 g calcium alginate (Alginate Industries Ltd., London), 2.5 g ferrihexacyanoferrate(II) (Heyl and Co., Berlin) and 0.015 g potassium iodide (Kemika, Zagreb) throughout the experiment. An intraperitoneal dose of 100 μmol/kg b. w. Ca-DTPA was given on the first three days of the experiment and during the remaining seven days Zn-DTPA was added to the diet together with the above mixture in a dose of 3.3 mmol per 100 g of food. The complexing agents Ca-DTPA and Zn-DTPA were prepared by dissolving H_5DTPA (diethylenetriaminepentaacetic acid, Fluka A. G., Buch S. G., Switzerland) in distilled water in the presence of an equimolar amount of $CaCl_2$ or $ZnCl_2$ and neutralizing it with 20% NaOH to pH 6.4.

The body weight of animals was determined on the first, fifth and eleventh day of the experiment. Other health parameters were determined at the end of the experiment. Haematological examination was carried out on 10 animals from each group. These animals were later subjected to trace element analysis. Histological examination was performed in six control and seven experimental animals.

Haematological examination. Blood samples were taken from the tip of the tail. Leucocyte and erythrocyte counts were made with standard diluting pipettes and counting chambers. Packed cell volume (PCV) was determined by the microhaematocrit method in heparinized microhaematocrit tubes, centrifuged for two minutes at 12000 rpm. Haemoglobin concentration was determined by standard acid haematin method (with haemiglobincyanide solution — International Reference Preparation, RIJKS Institute, Bilthoven the Netherlands — as a standard) on a Unicam SP-600 spectrophotometer at 540 nm wavelength. Mean corpuscular volume (MCV), mean corpuscular haemoglobin level (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated.

Trace element analysis. Animals were anaesthetized and exsanguinated from the abdominal aorta. The liver, kidneys, femur and a 20 cm long segment of the small intestine were dissected, blotted on filter paper and wet weights were determined. The samples were then ashed with nitric acid or stored at —18 °C before wet ashing. Femurs were first dried (105 °C) and then ashed (450 °C). Trace elements (iron, zinc and manganese) were determined by flame atomic absorption spectrophotometry (Varian Instrument, Model AA-375). The results are expressed as micrograms per gram of wet weight of the respective tissues.

Histopathological examination. Animals were anaesthetized and exsanguinated from the abdominal aorta. Gross necropsy observation was performed on all the organs. The following tissues: lung, heart, liver, spleen and both kidneys were histologically analysed from HE-stained paraffin sections (7—8 μm) by means of light microscopy.

RESULTS

The weight increase was 23% in control and 18% in experimental animals (Table 1).

Table 1.

Body weights (g)

Days	Group	Body weight	P	Increase	%
1	C	120.88±1.93	>0.1		
	E	116.94±2.86			
5	C	137.35±1.49	<0.1	16.18±0.81	14
	E	131.67±2.94		14.72±1.03	13
11	C	149.12±2.07	<0.02	28.24±1.82	23
	E	138.33±3.86		22.94±1.61	18

Results are presented as arithmetic mean ± SE of 18 animals in each group.
C — control group — standard rat diet and saline i. p.
E — experimental group — mixture of calcium alginate, ferrihexacyanoferrate (II) and potassium iodide supplemented to food during 10 days, Ca-DTPA i. p. for the first three days of the experiment and Zn-DTPA in food during the remaining seven days.

Table 2.

Haematological parameters

	Group	
	C	E
Leucocytes ($X10^3/\mu l$)	12.7±1.1	10.8±0.9
Erythrocytes ($X10^6/\mu l$)	6.5±0.1	6.0±0.1[a]
PCV (%)	47.3±0.7	44.9±0.8[b]
Haemoglobin (%)	13.8±0.3	12.9±0.3[b]
MCV (μm^3)	73.1±1.3	75.2±1.9
MCH (pg)	21.3±0.1	21.6±0.4
MCHC (%)	29.8±0.5	28.8±0.5

Results are presented as arithmetic mean ± SE of 10 animals in each group.
[a] Difference between the control and experimental group — level of significance, $P < 0.01$.
[b] $P < 0.05$.

The number of erythrocytes ($P < 0.01$), haemoglobin ($P < 0.05$) and packed cell volume (PCV) values ($P < 0.05$) were slightly lower in experimental animals (Table 2). The concentrations of iron ($P < 0.05$) and zinc ($P < 0.001$) in the kidneys, manganese in the liver ($P < 0.02$) and

Table 3.

Iron, zinc and manganese concentration in the liver, kidneys, small intestine and femur

Group	Element (μg/g)	Liver	Kidneys	Small intestine	Femur
C	Fe	254±10 (209—315)	53.3±2.9 (42.4—67.7)	31.8±2.1 (24.5—45.1)	57.3±1.8 (50.0—69.1)
E		282±10 (225—341)	60.3±1.1 (53.6—65.8)[a]	28.5±1.4 (22.3—37.2)	55.3±1.9 (47.0—65.2)
C	Zn	28.1±0.5 (25.7—31.7)	24.7±0.5 (23.0—28.2)	26.4±1.4 (21.6—38.0)	124±55 (107—159)
E		29.8±1.0 (25.6—37.9)	31.7±1.0 (27.9—37.6)[c]	31.1±1.2 (24.9—35.8)[b]	120± 7 (93—178)
C	Mn	2.12±0.06			
E		2.38±0.07[b]			

Results presented as arithmetic mean ± SE (range) of 10 animals in each group.

[a] Difference between the control and experimental group — level of significance, $P < 0.05$.

[b] $P < 0.02$

[c] $P < 0.001$

zinc ($P < 0.02$) in the small intestine were higher in experimental than control animals. Manganese concentrations, with the exception of the liver, were less than could be detected. Other iron and zinc values did not differ in control and experimental animals (Table 3).

The histopathological examination showed changes only in the kidney tissue, confined to proximal convoluted tubules. These changes were in the form of granular-hydropic degeneration of a very mild grade.

DISCUSSION

The results of our previous studies showed that the mixture of calcium alginate, ferrihexacyanoferrate(II) and potassium iodide administered in food for four weeks produced no adverse health effects (8). The only differences found between control and treated animals were slightly lower haemoglobin values in blood and slightly lower iron concentration in the liver. This was related to the possible inhibition of iron absorption caused by alginate (30, 31). *Ebel* (16) showed that toxic doses of Ca-DTPA caused inhibition of DNA-synthesis in the lymphocytes, erythro- and myelopoietic cells of the rat and impaired the incorporation of ^{59}Fe into the erythrocytes. Lower erythrocyte, haemoglobin and PCV values observed in the present experiment may be therefore ascribed partly to the effect of alginate on iron absorption and partly to the influence of chelation therapy on haematopoiesis.

In the present experiment we did not observe a decrease but a slight increase in trace element concentrations. Decreases in zinc and manganese concentration observed by other authors were always caused by very high doses of Ca-DTPA (2 mmol/kg b. w.; 19, 20). In the present experiment Ca-DTPA doses were much lower, and Zn-DTPA given for seven days probably contributed the observed increases in zinc concentration. The mild pathological changes in the kidney tissue corresponded to those described after Ca-DTPA treatment (12). It is known that these changes are reversible as shown by complete recovery after the termination of treatment (32) and therefore they do not elicit the long-term health prospect of animals. The Ca-DTPA dose used in the present experiment was about three times higher than the recommended dose for humans. No lesions were observed when Ca-DTPA was administered at doses within the therapeutic range (33, 34). *Planas-Bohne and Lohbreier* (26) administered 100 μmol/kg b. w. Ca-DTPA or Zn-DTPA twice weekly for 44 weeks and observed no adverse effects in treated animals or their offspring.

We may conclude that the observed decrease in erythrocyte counts, haemoglobin and PCV values as well as the small changes in the kidney tissue may be related to the influence of alginate and Ca-DTPA which are known and are described in the literature. It is important to note that no additional deleterious effects were caused by the simultaneous admi-

nistration of the different therapeutic agents. On balance, the significance of the small changes found in this experiment is negligible when compared to the risk of internal contamination with radionuclides. The composite treatment, being highly efficient in reducing body burden of several radionuclides, should be considered as the therapy of choice in cases of simultaneous exposure to several radionuclides in the environment.

References

1. *Kostial, K., Kargačin, B., Šimonović, I.:* J. Appl. Toxicol., *3* (1983) 291.
2. *Kargačin, B., Kostial, K.:* Health Phys., 1985, in press.
3. *Waldron-Edward, D., Paul, T. M., Skoryna, S. C.:* Can. Med. Assoc. J., *91* (1964) 1006.
4. *Harrison, G. E.:* Diagnosis and Treatment of Deposited Radionuclides, Excerpta Medica Foundation, Amsterdam 1968, p. 333.
5. *Nigrović, V., Bohne, F., Madshus, K.:* Strahlentherapie, *130* (1966) 413.
6. *Dvořák, P., Günther, M., Zorn, U., Catsch, A.:* Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol., *269* (1971) 48.
7. *Wolff, J.:* J. Mol. Med., *4* (1980) 151.
8. *Kostial, K., Kargačin, B., Rabar, I., Blanuša, M., Maljković, T., Matković, V., Ciganović, M., Simonović, I., Bunarević, A.:* Sci. Total Environ., *22* (1981) 1.
9. *Foreman, H., Finnegan, C., Lushbaugh, C. C.:* J. Am. Med. Assoc., *160* (1956) 1042.
10. *Foreman, H., Nigrović, V.:* Diagnosis and Treatment of Deposited Radionuclides, Excerpta Medica Foundation, Amsterdam 1968, p. 419.
11. *Doolan, P. D., Schwartz, S. L., Hayes, J. R., Mullen, J. C., Cummings, N. B.:* Toxicol. Appl. Pharmacol., *10* (1967) 481.
12. *Weber, K. M.:* Virchows Arch. Abt. B. Zellpathol., *5* (1970) 39.
13. *Weber, K. M.:* Z. Gesamte Exp. Med., *150* (1969) 354.
14. *Taylor, G. N., Williams, J. L., Roberts, L., Atherton, D. R., Shabestari, L.:* Health Phys., *27* (1974) 285.
15. *Weber, K. M.:* Experientia, *25* (1969) 509.
16. *Ebel, H.:* Strahlentherapie, *149* (1975) 450.
17. *Planas-Bohne, F., Ebel, H.:* Health Phys., *29* (1975) 103.
18. *Bömer, H.:* Strahlentherapie, *142* (1971) 347.
19. *Auth, U.:* Strahlentherapie, *146* (1973) 490.
20. *Nadolny, W.:* Strahlentherapie, *141* (1971) 100.
21. *Taylor, D. M., Jones, J. D.:* Biochem. Pharmacol., *21* (1972) 3313.
22. *Gabard, B.:* Biochem. Pharmacol., *23* (1974) 901.
23. *Weber, K. M., Bohne, F., Rabe, U.:* Eur. J. Pharmacol., *11* (1970) 117.
24. *Catsch, A., von Wedelstaedt, E.:* Experientia, *21* (1965) 210.
25. *Fisher, D. R., Mays, C. W., Taylor, G. N.:* Health Phys., *29* (1975) 780.
26. *Planas-Bohne, F., Lohbreier, J.:* Diagnosis and Treatment of Incorporated Radionuclides, International Atomic Energy Agency, Vienna 1976, p. 505.
27. *Seidel, A.:* Diagnosis and Treatment of Incorporated Radionuclides, International Atomic Energy Agency, Vienna 1976, p. 323.

28. *Lloyd, R. D., Mays, C. W., McFarland, S. S., Taylor, G. N., Atherton, D. R.:* Health Phys., *31* (1976) 281.
29. *Taylor, D. M., Volf, V.:* Health Phys., *38* (1980) 147.
30. *Wölbling, R. H., Becker, G., Forth, W.:* Digestion, *20* (1980) 403.
31. *Hodgkinson, A., Nordin, B. E. C., Hambleton, J., Oxby, C. B.:* Can. Med. Assoc. J., *97* (1967) 1139.
32. *Catsch, A.:* Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmacol., *246* (1964) 316.
33. *Morgan, R. M., Smith, H.:* Toxicology, *2* (1974) 43.
34. *Morgan, R. M., Smith, H.:* Toxicology, *2* (1974) 153.

Sažetak

UTJECAJ KOMBINIRANE TERAPIJE KOD INTERNE KONTAMINACIJE S VIŠE RADIONUKLIDA NA NEKE ZDRAVSTVENE PARAMETRE U ŠTAKORA

U radu su određeni zdravstveni učinci kombinirane terapije za ranu i kasnu primjenu kod interne kontaminacije s više radionuklida u štakora. Terapija se sastojala u istodobnoj oralnoj primjeni mješavine kalcijeva alginata, feri-ferocijanida i kalijeva jodida tijekom 10 dana, intraperitonealnoj primjeni Ca-DTPA tijekom prva tri dana pokusa te oralnoj primjeni Zn-DTPA tijekom preostalih 7 dana pokusa. Zdravstveni učinci takvog tretmana određeni su mjerenjem tjelesne težine, hematoloških parametara, analizom elemenata u tragovima i histopatološkom pretragom.

Tretirane životinje su imale nešto niži broj eritrocita, nešto niže vrijednosti hemoglobina i hematokrita i vrlo diskretne promjene u tkivu bubrega karakterizirane kao granularno-hidropska degeneracija zavinutih kanalića. Takve promjene mogu se pripisati učinku pojedinih terapijskih sredstava (alginata, kelatogene terapije) koji su poznati i opisani u literaturi, dok nova djelovanja odnosno štetni učinci na zdravlje koji bi nastali zbog istodobne primjene više terapijskih sredstava nisu opaženi.

Institut za medicinska istraživanja i medicinu rada, Zagreb

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