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Successful Antidote of Multiple Lethal Infections Using Sustained Delivery of Difluoromethylornithine by Means of Ceramic Drug Delivery Devices

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Abstract: The objectives of this study were (1) to cure multiple infections of trypanosomiasis in rats by the sustained release of DFMO from biodegradable tricalcium phosphate (TCP) and aluminum–calcium–phosphorous oxide (ALCAP) delivery systems, and (2) to determine if the side effects associated with oral administration of DFMO can be avoided by using TCP and ALCAP capsules. Sixty-eight SD male albino rats (235–270 g) were divided randomly into five groups. Each rat in group I ($n = 16$) was implanted subcutaneously (s.c.) with four TCP capsules (two large TCP (L-TCP), one PLA-impregnated large TCP (IL-TCP) and one thin TCP capsule (TN-TCP)). Rats in group II ($n = 16$) were implanted s.c. with four ALCAP ceramics (two large ALCAP (L-ALCAP), one PLA-impregnated large ALCAP (IL-ALCAP) and one thin ALCAP capsule (TN-ALCAP)). Rats in groups III ($n = 16$), IV ($n = 4$) and V ($n = 16$) were left without implants. Rats in group III ($n = 16$) were given 4% (w/v) DFMO (pH 7) in drinking water at the day of inoculation and continued up to 7 days postinoculation. Rats in group IV ($n = 4$) served as a nontreated group. Rats in group V ($n = 16$) served as normal controls. The results showed that all rats implanted with TCP or ALCAP implants had no intoxication symptoms or side effects such as diarrhea during the treatment period. In contrast, rats given DFMO in drinking water exhibited foul-smelling diarrhea during the treatment period. Microscopic evaluation of blood smears collected from rats receiving DFMO chemotherapy showed an occasional or limited number of stumpy shape (SS) trypanosomes. This study suggests that (1) ceramic drug delivery systems are capable of delivering DFMO in a sustained manner for two months, and were able to cure repeated infections of trypanosomiasis; (2) the use of ceramic implants avoided widely fluctuating, irregular levels of DFMO in the body by keeping sustained levels above minimal effective concentrations; (3) ceramic drug delivery systems provide a pharmacological potentiality for drugs such as DFMO which have been withheld from the market because of severe side effects when administered using conventional methods of drug administration; and (4) DFMO-filled ceramic devices can be implanted subcutaneously in animals that face a threat of lethal protozoal infections in highly infested areas of the world.

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INTRODUCTION

Members of the *Trypanosoma brucei* subspecies are the causative agent of African sleeping sickness in humans and nagana in nonhuman mammals which makes some 10 million square miles of Africa useless for the breeding of domestic livestock and poses a health threat to 6 million humans.[1] The ability of the trypanosome to undergo antigenic variation and constantly alter its glycoprotein coat enables the parasite to evade the host's immune system,[2] making immunological control of this disease an impossibility.

The polyamines are aliphatic organic cations which are involved in cellular growth processes and differentiation.[3] Recent studies[4–9] have shown that perturbations in intracellular polyamines following exposure of long slender (LS) bloodstream trypanosomes to difluoromethylornithine (DFMO) resulted in morphological alterations to the short stumpy (SS) forms. DFMO, the irreversible inhibitor of ornithine decarboxylase (the first enzyme in the polyamine biosynthetic pathway), rapidly depletes the intracellular content of polyamines (Fig. 1). This results in decreased rates of DNA and RNA synthesis and a subsequent block to cell division.

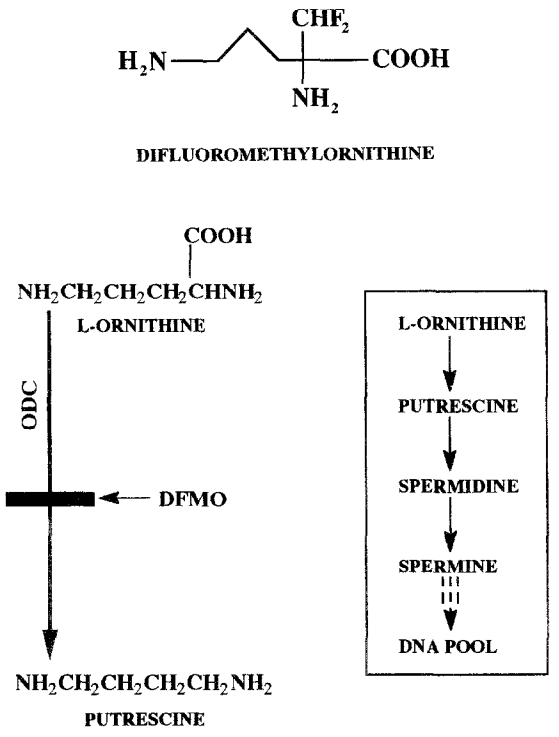

Fig. 1. The chemical structures of L-ornithine and DL-α-difluoromethylornithine (DFMO). DFMO is an analogue of ornithine and is an enzyme-activated irreversible inhibitor of ornithine decarboxylase, the first enzyme in the polyamine biosynthetic pathway.

Both the morphological alterations and the cytostatic effect can be abrogated by coadministration of putrescine, the first polyamine in the biosynthetic pathway. Complete cures of *T. brucei* infections in rodents have been demonstrated,[9] but the administration of the drug (4% w/v) in drinking water has been shown to be an inefficient route of administration owing to the short half-life of DFMO and the continuous diarrhea, soft stools, fluid loss, emesis and anorexia which results.[6–9]

DFMO has also been administered intravenously every 6 h to maintain a sustained level of the drug in circulation; however, this mode of drug administration is extremely difficult to maintain under field conditions. Methods that provide a means of sustained release of drugs into the plasma overcome the problems associated with repeated intravenous injections for drugs with a short half-life and compensate for rapid elimination of the drug. Among the drug delivery systems that hold promise for overcoming some of the abovementioned limitations is the ceramic drug delivery system (CDDS). These systems have been shown to have the potential to deliver various chemicals and biologicals (C/B) directly to the systemic circulation in a less risky and continuous manner for long duration.[10–28] Furthermore, the use of CDDS alleviates several complications observed when the drug is given by conventional methods. Most importantly, use of CDDS plays a role in (i) reduction of the rate of drug metabolism by initial bypassing of the liver, and (ii) improved control of the concentrations of C/B with small therapeutic indices. To date, CDDS have been used effectively to deliver steroids,[10,11,28] enzymes,[24] amino acids,[25] phenolics,[22] proteins[18] and nucleosides[23] over long intervals. Previous studies have shown that ALCAP ceramic capsules are capable of delivering DFMO *in vitro* and *in vivo* in intact male rats.[26] Preliminary results suggested that, owing to the high clearance rate of DFMO, larger doses and the use of multiple implants (which provide a larger surface area for drug release) are required to maintain an effective therapeutic level. Thus, the objectives of this study were: (1) to cure multiple infections of trypanosomiasis in rats by the sustained release of DFMO from tricalcium phosphate (TCP) and aluminum–calcium–phosphorous oxide (ALCAP) ceramic delivery systems; (2) to investigate the differences in the release rate of DFMO from TCP and ALCAP ceramic reservoirs; and (3) to determine if the side effects associated with oral administration of DFMO can be avoided by using CDDS.

MATERIALS AND METHODS

Chemicals

Difluoromethylornithine (DFMO) was provided as a generous gift by the Merrell Dow Research Institute (Cincinnati, OH, USA). Collidine, ninhydrin and hydrindantin were obtained from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals used in this investigation were analytical grade and were obtained from standard commercial sources.

Growth and isolation of trypanosomes

The growth and isolation of trypanosomes was conducted by following the procedure of Giffin *et al.*[6] The EA TRO 110 isolate of *T. brucei brucei* was used for the experiments in this study. Frozen stabilates were prepared, stored under liquid nitrogen, and used to inoculate intraperitoneally 250 g male Sprague Dawley rats with 2×10^7 trypanosomes. Three days after inoculation, animals were anesthetized with a mixture of ether–CO_2 and bled by cardiac puncture into syringes containing 0·5 ml of 0·1 M EDTA, pH 7·4. Trypanosomes were separated from blood elements by density centrifugation[7] or by DEAE ion-exchange chromatography.[8]

Ceramic fabrication

TCP ceramic fabrication

The microcrystal of tricalcium phosphate powder was prepared by mixing 70% calcium nitrate (210 g calcium nitrate + 300 ml water) with 100 ml of ammonium hydroxide (solution A). In a separate flask, 16% (w/v) of ammonium hydrogen phosphate was added to 27% ammonium hydroxide (solution B). Using a separatory funnel, solution A was added to solution B over a period of 20 min. The precipitate resulting from the reaction was harvested by centrifuging for 5 min at 1800 rpm. The precipitate was then washed three times with distilled water, and resuspended in 3% ammonium sulfate for 5 min. The precipitate was collected and dried at 75 °C for 4 h. This material was calcined at 1150 °C for 1 h. The calcined TCP was then placed on an automatic Tyler sieve stack to obtain particles 1–38 μm in size. The calcined material was then pressed into cylindrical form using a $\frac{5}{16}$in (7·9 mm) die set (French Pressure Cell) at a compression load of 715 kg. The pressed capsules were sintered at 1200 °C for 36 h to achieve the appropriate mechanical strength (Table 1).

Table 1. Dimensions of sintered TCP capsules pressed at 715 kg compression load

Dimensions (cm)	*TN-TCP (1·0 g)*	*L-TCP (1·5 g)*
Outer diameter	0·75 ± 0·023	0·78 ± 0·012
Inner diameter	0·49 ± 0·019	0·38 ± 0·015
Inner surface area	2·66 ± 0·041	3·24 ± 0·056
Outer surface area	4·48 ± 0·021	6·33 ± 0·182
Height	1·74 ± 0·070	2·49 ± 0·079
Total volume (cm^3)	0·57 ± 0·011	0·95 ± 0·035
Capacity (mg)	200	300
Density (g/cm^3)	1·56 ± 0·066	1·58 ± 0·072

ALCAP ceramic fabrication

ALCAP ceramic capsules were fabricated by calcining a 50 : 34 : 16 (by weight) mixture of aluminum oxide, calcium oxide and phosphorous pentoxide powders (Fisher Scientific Co., Fairlawn, NJ, USA) at 1350 °C in a high temperature furnace (Leco Corp., St Joseph, MI, USA) for 12 h. The calcined material was ground in a roller mill and sieved (Tyler Sieve Stacks) to obtain particles of < 38 μm size.[10] The ceramic capsules were then pressed into cylindrical forms (green shape) using a $\frac{5}{16}$in (7·9 mm) die set with the aid of a French Pressure Cell (American Instrument Co., Silver Springs, MD, USA). The pressed capsules were sintered at 1400 °C for 36 h to increase mechanical strength (Table 2).

Ceramic impregnation and loading

A total of 64 sintered capsules (32 IL-TCP and 32 IL-ALCAP) was impregnated *in vacuo* with a solution of polylactic acid (Orthomatrix Inc., Dublin, CA, USA) in chloroform (2% L-PLA w/v,

Table 2. Dimensions of sintered ALCAP capsules pressed at 715 kg compression loads

Dimensions (cm)	*TN-ALCAP (1·0 g)*	*L-ALCAP (1·5 g)*
Outer diameter	0·77 ± 0·017	0·79 ± 0·000
Inner diameter	0·48 ± 0·009	0·39 ± 0·008
Inner surface area	2·53 ± 0·035	3·12 ± 0·143
Outer surface area	4·31 ± 0·064	6·29 ± 0·202
Height	1·81 ± 0·110	2·54 ± 0·081
Total volume (cm^3)	0·54 ± 0·024	0·94 ± 0·027
Capacity (mg)	200	300
Density (g/cm^3)	1·68 ± 0·071	1·56 ± 0·065

mol. wt 500 K) for 1 h and subsequently air dried for 24 h. Previous studies[15,16,23,26] have shown that an insignificant amount of chloroform (0–4 ppm) was detected in PLA-impregnated capsules. Furthermore, hematological observation (hematocrit, RBC, WBC, etc.) and growth and histological evaluation of vital and reproductive organs revealed that there was no significant difference between sham-operated and control animals.[16,23,26] Polylactic acid (PLA) impregnation was used to decrease the porosity of the ceramic.[18] PLA-impregnated and nonimpregnated ceramic reservoirs were loaded with DFMO powder (Tables 1 and 2). Each ceramic capsule was sealed at both ends with Silastic Medical Adhesive, Silicon Type A (Dow Corning, Midland, MI, USA). DFMO-containing and empty ceramics were sterilized by exposure to ethylene oxide gas for 72 h.

Animals and housing

Sixty-eight Sprague Dawley male albino rats (235–270 g) obtained from Holtzman Co. (Madison, WI, USA) were divided randomly into five groups. Each rat in group I ($n = 16$) was implanted subcutaneously (s.c.) with four TCP capsules (two large TCP (L-TCP), one PLA-impregnated large TCP (IL-TCP) and one thin TCP capsule (TN-TCP)) (Table 1). Rats in group II ($n = 16$) were implanted s.c. with four ALCAP ceramics (two large ALCAP (L-ALCAP), one PLA-impregnated large ALCAP (IL-ALCAP) and one thin ALCAP capsule (TN-ALCAP)) (Table 2). Rats in groups III ($n = 16$), IV ($n = 4$) and V ($n = 16$) were left without implants. Previous reports have shown that surface area, thickness and density of the ceramic capsules are instrumental keys in determining the rate of release of biologicals from the ceramic capsules.[10,12,17–19,26] Rats in group III ($n = 16$) were given 4% (w/v) DFMO (pH 7) in drinking water at the day of inoculation and continued up to 7 days postinoculation. Rats in group IV ($n = 4$) served as an unimplanted and nontreated group. Rats in group V ($n = 16$) served as normal controls. During the entire duration of this study, the animals were kept on a 12 h day : night cycle and fed Purina Rodent Lab Chow 5001 (Ralston Purina, St Louis, MO, USA) and water *ad libitum*.

Ceramic implantation and retrieval

Rats were anesthetized with sodium pentobarbital (Butler Co., Columbus, OH, USA) or diethyl ether, and the abdomens shaved, scrubbed with providone iodine and the sterilized ceramics inserted s.c. using standard aseptic surgical techniques. After implantation, each rat was injected intramuscularly with 0·1 ml of 200 000 units/ml Penicillin G Proccaine (Veticare, Baltimore, MD, USA). Four rats from each group were euthanized at 2, 4, 6 and 8 weeks by using an overdosage of diethyl ether. Ceramic cylinders were retrieved from the animals and the contents of each ceramic washed and diluted in phosphate-buffered saline (PBS) adjusted to pH 7·4. Complete recovery of DFMO from each capsule was ensured by crushing and agitating each ceramic in its respective PBS solution. The amount of DFMO recovered in the PBS solution was estimated by a modified ninhydrin hydrindantin method.[29] A standard curve was constructed using known amounts of DFMO dissolved in PBS. Statistical analysis of the data was performed using analysis of variance and is expressed as means ± standard deviation (SD) in the text.

Inoculation of experimental rats

Each of 52 rats was inoculated intraperitoneally with $1{\cdot}8 \times 10^7$ trypanosomes 7 days after ceramics were implanted. At 28 days postimplantation the experimental rats were inoculated again with $2{\cdot}2 \times 10^7$ trypanosomes. Blood samples were collected via the tail artery both prior to and following implantation of the DFMO-filled ceramic. Blood smears were made and evaluated for trypanosomes.

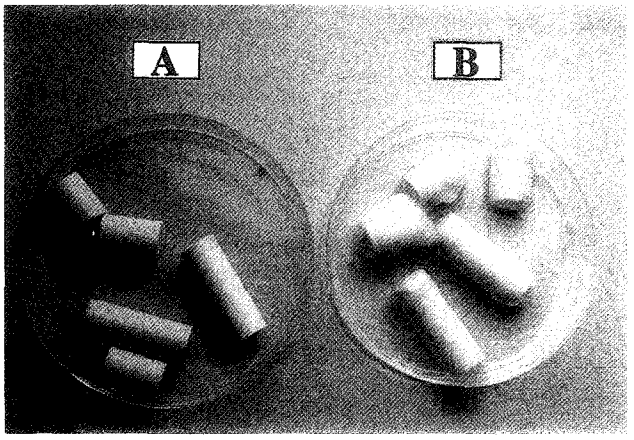

Fig. 2. Representative photograph of ceramic implants loaded with DFMO at $t = 0$ (A), and DFMO-filled ceramic implants retrieved from rats at $t = 8$ weeks (B).

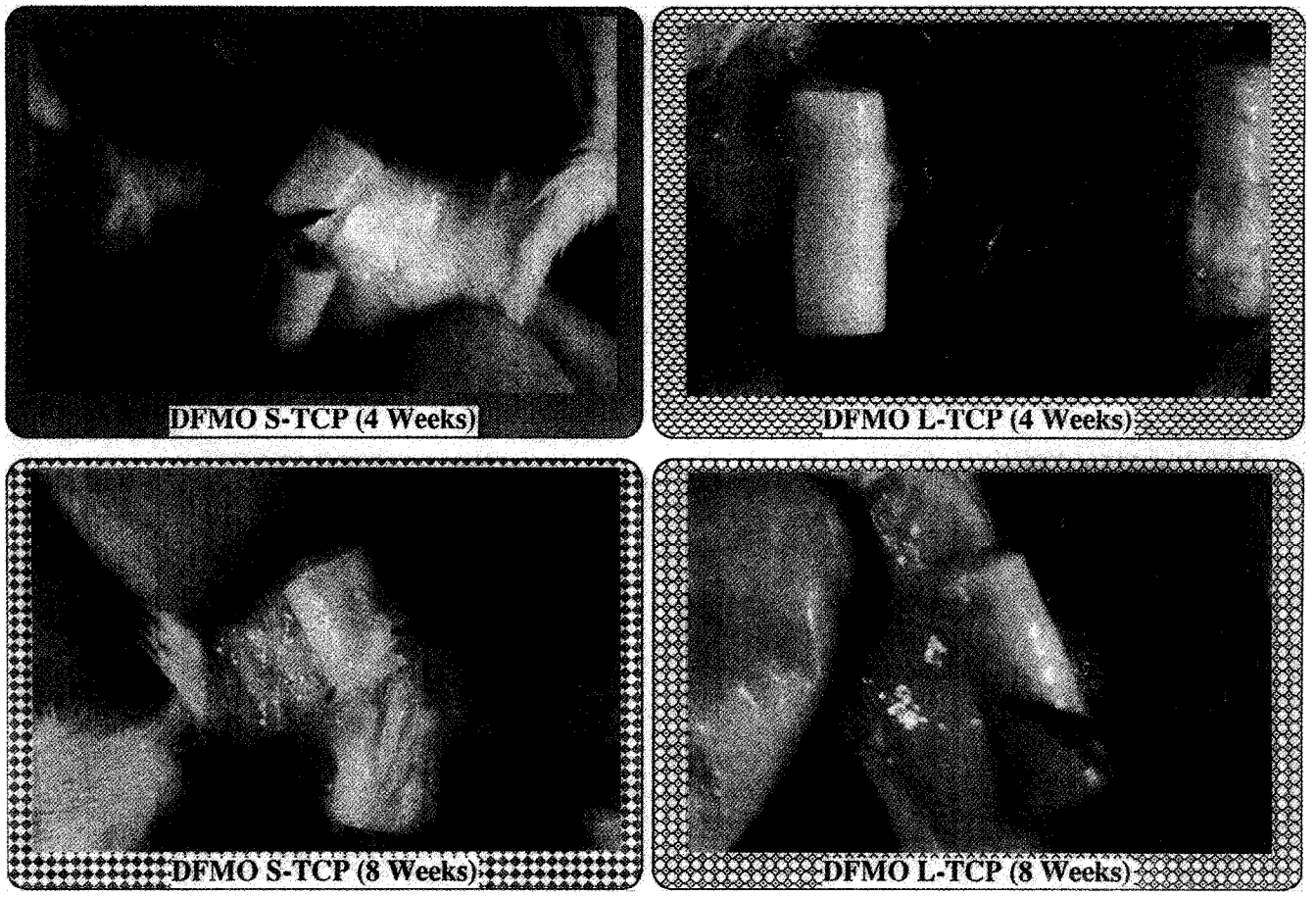

Fig. 3. Representative photographs of ceramic capsules implanted s.c. in male rats showing encapsulation at the end of 4 weeks and at the end of 8 weeks postimplantation.

RESULTS

DFMO-filled TCP or ALCAP ceramics implanted in male rats were capable of protecting against multiple infections of trypanosomiasis. All rats implanted with TCP or ALCAP showed no drug intoxication symptoms or side effects such as diarrhea during the treatment period. In contrast, rats given DFMO orally in drinking water exhibited foul-smelling feces, and evaluation of the blood smears collected from rats receiving DFMO chemotherapy showed an occasional or limited number of stumpy shape (SS) trypanosomes (Figs 2–4). The LS forms were not seen during the entire investigation. In contrast, analysis of infected rats receiving no DFMO chemotherapy treatment (group IV) showed a total of $2{\cdot}3 \times 10^4 \pm 987$, $1{\cdot}89 \times 10^6 \pm 732$ and $1{\cdot}2 \times 10^8 \pm 1711$ trypanosome cells/ml blood at the end of 24, 48 and 72 h of inoculation. All parasitemic rats in this group died at the end of the third day or at the beginning of the fourth day following inoculation.

Figure 5 shows that the rate of DFMO released from the CDDS reservoirs *in vivo* was altered by impregnating the ceramic materials with 2% PLA. The rate of DFMO released from PLA-impregnated TCP ($3{\cdot}20 \pm 0{\cdot}24$ mg/day) or PLA-impregnated ALCAP ($3{\cdot}79 \pm 0{\cdot}27$ mg/day) ceramic devices was lower than the rate of DFMO released from noncoated TCP ($4{\cdot}19 \pm 0{\cdot}21$ mg/day) or noncoated ALCAP ($5{\cdot}51 \pm 0{\cdot}33$ mg/day) ceramic capsules. Figure 5 shows that the surface area (SA) (size and thickness) of the ceramic capsules can also affect the rate of release of DFMO. Regardless of the SA of the ceramic, the data presented in Fig. 5 show that the amount of DFMO released from TCP capsules was less than the amount of DFMO released from ALCAP ceramic capsules. However, sustained release of DFMO from the ceramic capsules in this investigation did not affect the growth of testes, seminal vesicles, prostates (Fig. 6), spleen, heart, kidneys or adrenals. Mean body weights of rats implanted with DFMO-filled TCP or ALCAP ceramic capsules

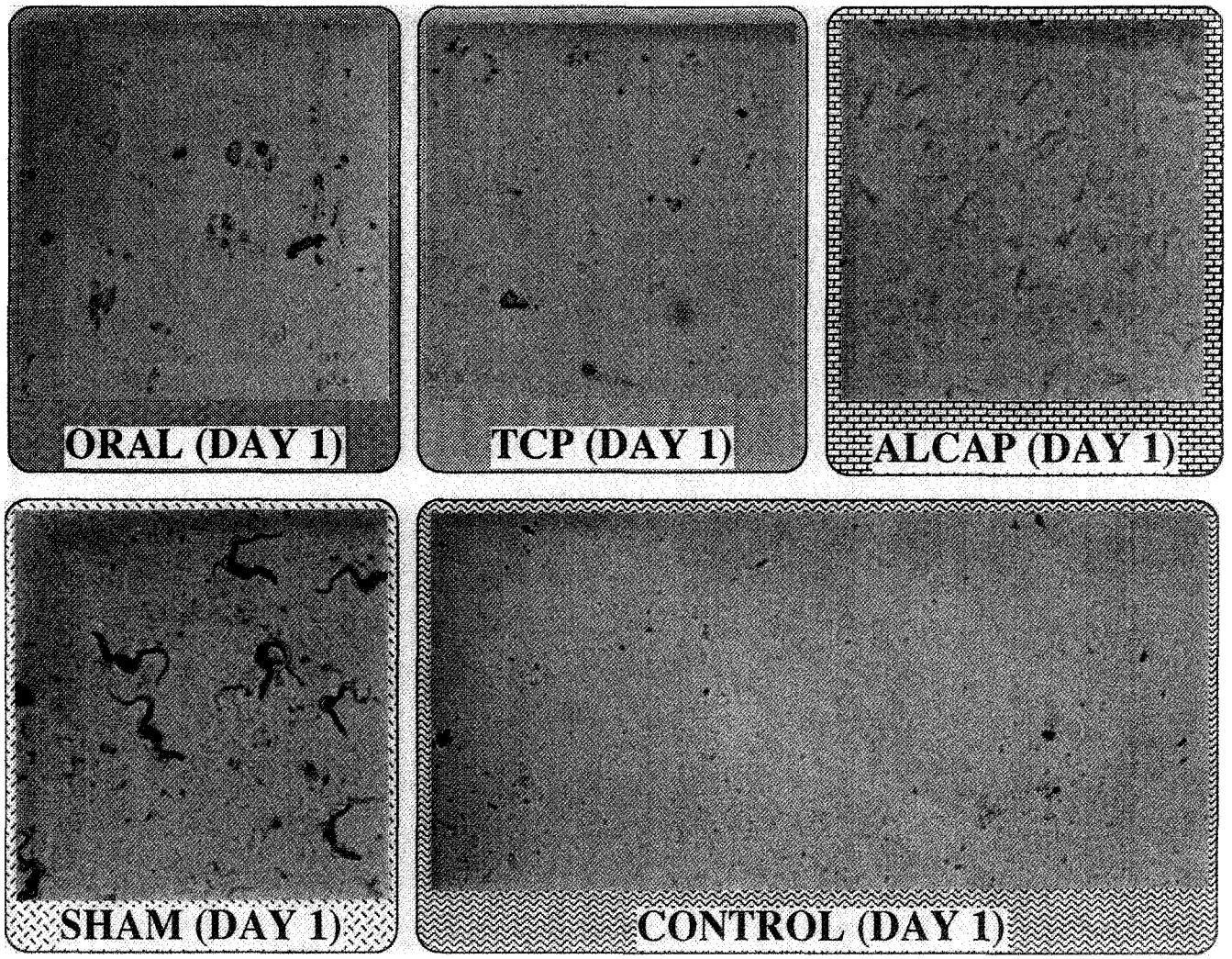

Fig. 4A. Photographs of blood smears obtained from rats infected with *Trypanosoma brucei brucei* (Giemsa stained) showing the effects of DFMO treatment orally, treatment with DFMO-filled TCP capsules, treatment with DFMO-filled ALCAP capsules, non-treated animals at the end of 24 h postinoculation with trypanosomes, and nontreated and uninfected animals.

were not significantly different from those of control rats (Fig. 7). In contrast, mean body weights of intact rats given DFMO in drinking water were significantly lower than the mean body weights of intact rats at the end of 2, 4, 6 and 8 weeks.

DISCUSSION

Bacchi and McCann[9] reported that when trypanosomes are exposed to DFMO there is an inhibition of cell division and a change in cellular morphology. Data collected in this investigaton showed that DFMO-filled TCP or ALCAP ceramics implanted s.c. in adult male rats were capable of curing multiple infections of trypanosomiasis. This was supported by the fact that all parasitemic rats in the nontreated group died at the end of the third day or at the beginning of the fourth day following inoculation. The results of this investigation provide promising findings that all rats implanted with CDDS showed no intoxication symptoms or side effects such as diarrhea during the treatment period. In contrast, rats given DFMO orally in drinking water exhibited foul-smelling diarrhea during the treatment period. Microscopic evaluations of blood smears collected from rats receiving DFMO chemotherapy have shown an occasional or limited number of SS trypanosomes. The LS forms were not seen during the entire investigation. Several investigators have suggested that, upon continuous exposure to DFMO, the trypanosome population changes morphologically from LS to SS by passing through an intermediate (INT) stage and finally becomes a population of predominantly SS forms.[5,9] However, true SS forms were not

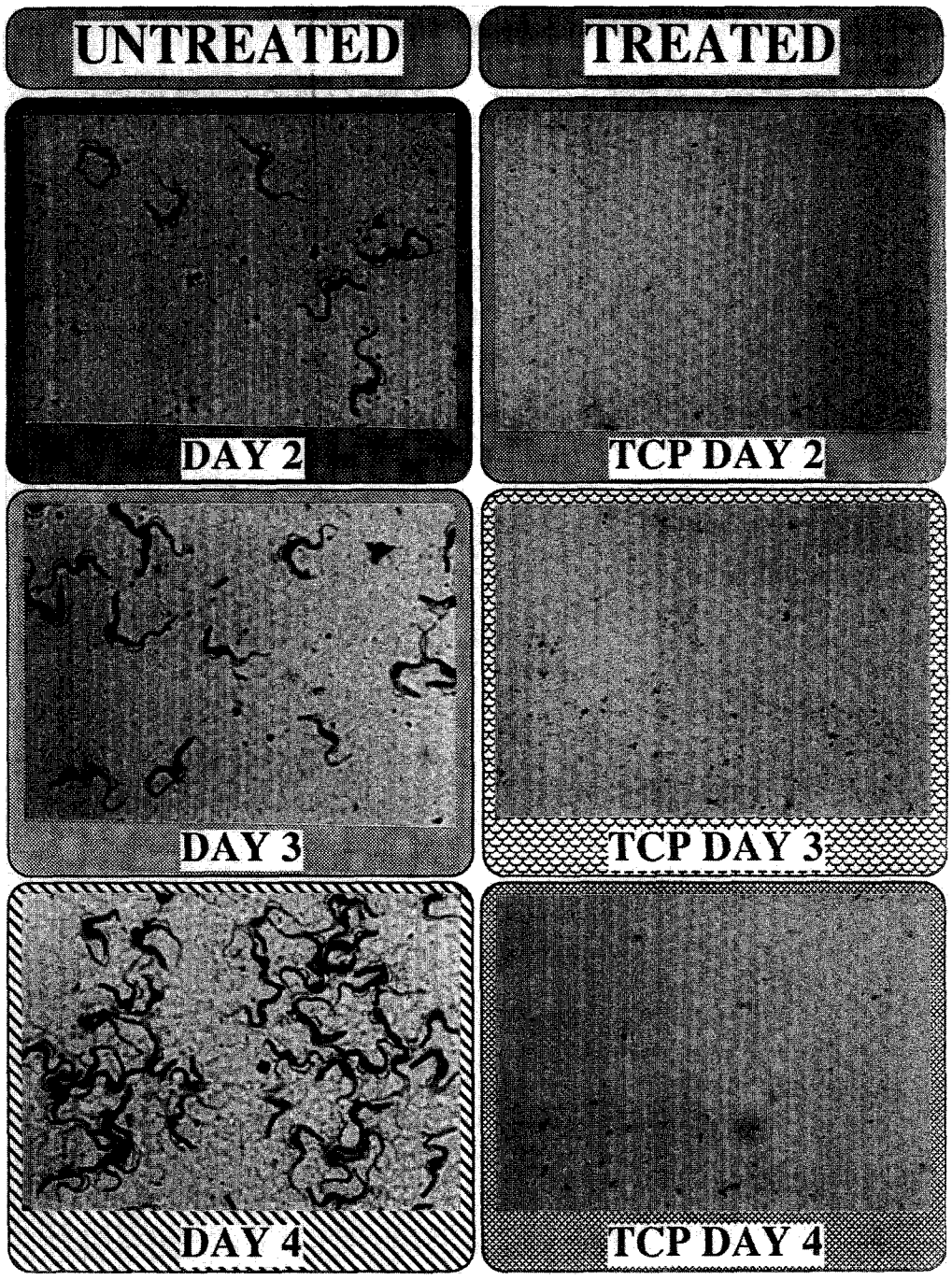

Fig. 4B. Photographs of blood smears obtained from rats infected with *Trypanosoma brucei brucei* (Giemsa stained) showing the effects of treatment with DFMO-filled TCP capsules on the proliferation rate of trypanosomes.

detected in this experiment, which could be due to complete elimination of all parasites by the immune system.

The data obtained in this study showed that thickness, size and coating of the CDDS with PLA all affect the delivery rate of hydrophilic compounds such as DFMO from the ceramic reservoirs. These observations confirmed our previous studies using steroids.[10] Regardless of the SA of the ceramic, DFMO release rate from TCP capsules was less than DFMO released from ALCAP ceramic capusles. The variation in the rate of release of DFMO from these two ceramic systems could be due to (i) differences in the ionic interaction between the ceramic surface and DFMO molecules, and/or (ii) differences in the lattice structure of the different ceramic materials. Several researchers have studied the behavior of these ceramic materials in clinical

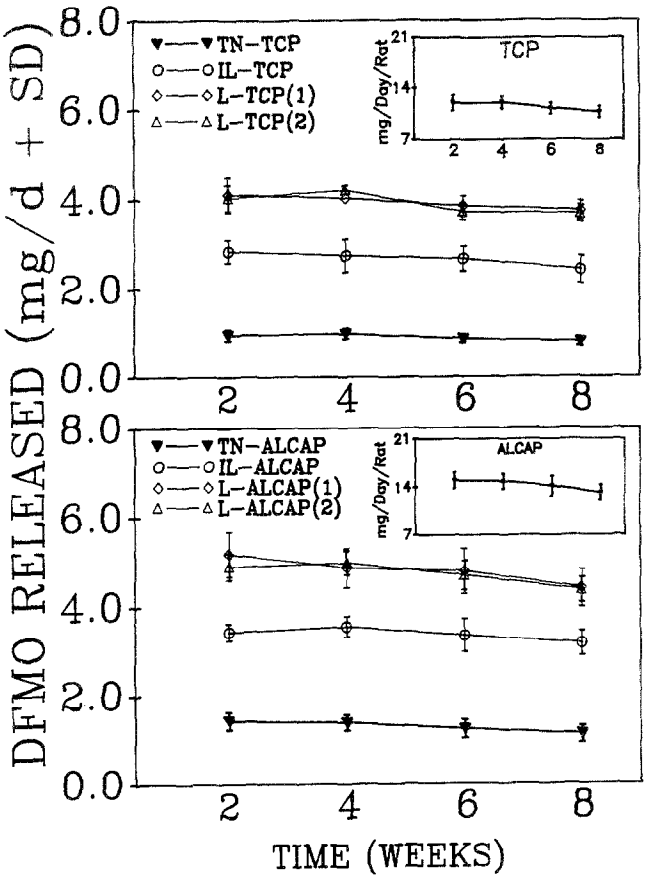

Fig. 5. The net amount (mean ± SD) of DFMO released from TCP ceramic implants (top), and the net amount (mean ± SD) of DFMO released from ALCAP ceramic implants (bottom).

and nonclinical environments and especially from the standpoint of orthopedic applications.[10] Thus, the physiochemical differences between TCP and ALCAP are also probably responsible for the differences in the rate of delivery of DFMO. Comparison of the results of this investigation with data obtained from *in vitro* studies reported from our laboratory[25,26] showed that the release rate of DFMO in an *in vivo* environment is much slower than the release rate of DFMO in an *in vitro* environment (approx. 27-fold). Analysis of retrieved capsules shows expected benign encapsulation around the ceramic reservoir by hyaline fibrous tissue. This could be the main factor which is responsible for the decrease in the rate of DFMO from ceramic implants *in vivo*. Furthermore, since DFMO was loaded in a powdered state within the ceramic reservoir, the release of DFMO from the ceramic reservoirs was achieved by (i) dissolution of the powder, (ii) exclusion of the dissolved DFMO from the ceramic pores and (iii) slow erosion of the ceramic (biodegradation). Representative scanning electron micrographs of TCP and ALCAP capsules, retrieved at the end of several phases,

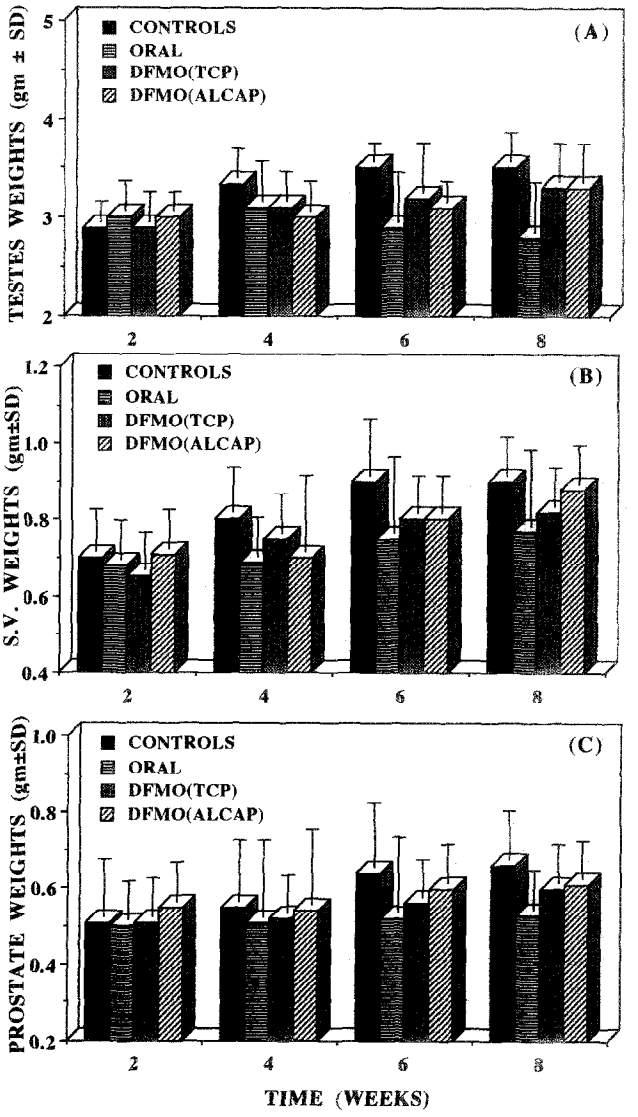

Fig. 6. The effect of oral intake of DFMO and DFMO released from TCP or ALCAP implanted ceramic capsules on weights of (A) testes, (B) seminal vesicles and (C) prostate.

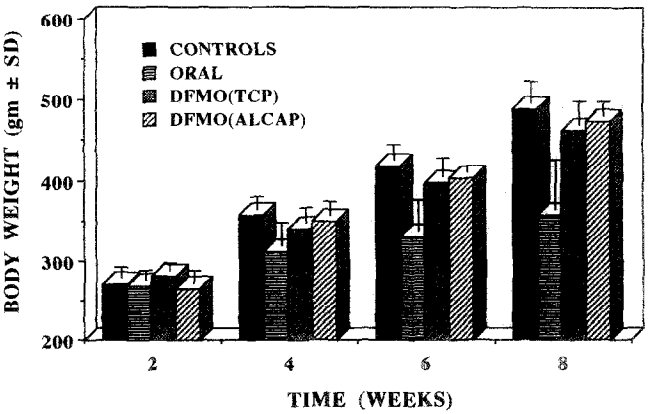

Fig. 7. The effect of DFMO (mean ± SD) on the body weights of rats surgically implanted with DFMO-filled TCP, DFMO-filled ALCAP ceramic capsules, and orally treated DFMO animals (oral) and intact nontreated animals (controls).

confirmed that the degree of biodegradation of the capsule is directly proportional to the duration of implantation. Since DFMO inhibits the activity of ornithine decarboxylase, it was thought that DFMO could affect the growth of reproductive as well as vital tissues. Several reports have suggested that administration of DFMO has been shown to arrest embryonic development in the early phases of murine gestation,[30] synaptogenesis in the cerebellar cortex in rats[31] and carcinogenesis in the urinary bladder.[32] However, sustained release of DFMO from ceramic drug delivery systems in this investigation did not affect the growth of testes, seminal vesicles, prostate, spleen, heart, kidneys or adrenals. In contrast, mean body weights of intact rats given DFMO in drinking water were significantly lower than the mean body weights of intact rats at the end of 4, 6 and 8 weeks. The mean body weights of rats implanted with DFMO-filled TCP or ALCAP ceramic capsules were lower than the weights of control rats but were not significantly different. The role of polyamine in regulating the differentiation of mammalian cells is well documented.[3] In some cell systems, polyamine inhibition prevented differentiation.[3,7] These observations have led us to suggest that the reduction in body weights of DFMO-treated animals is due to a slowing of mitotic activity in all cells leading to a reduction in the rate of total body growth.

CONCLUSIONS

Overall conclusions: (1) ceramic drug delivery systems (CDDS) are capable of delivering DFMO at sustained therapeutic levels for two months and protected against repeated infections of trypanosomiasis; (2) CDDS avoided the adverse side effects observed by the use of more conventional methods of DFMO administration, an observation that may be of considerable interest not only for DFMO but for other efficacious drugs with deleterious side effects; and (3) subcutaneous implants of DFMO-filled CDDS can be used to treat effectively animals threatened with lethal protozoal infections in highly infested areas of the world.

REFERENCES

1. Englund, P. T. & Sher, A., in *The Biology of Parasitism*. Alan R. Liss, Inc., New York, 1988, p. 349.
2. Borst, P. & Cross, G. A. M., Molecular basis for trypanosome antigenic variation. *Cell*, **29** (1984) 291.
3. Pegg, A. E. & McCann, P. P., Polyamine metabolism and function: a brief review. *Am. J. Physiol.*, **243** (1982) 212.
4. Bacchi, C. J., Garafalo, J., Mochenhaupt, D., McCann, P. P., Diekema, K. A., Pegg, A. E., Nathan, H. C., Mullaney, E. A., Chunosoff, L., Sjoerdsma, A. & Hunter, S. H., *In vivo* effects of DL-α-difluoromethylornithine on the metabolism and morphology of *Trypanosoma brucei brucei*. *Mol. Biochem. Parasitol.*, **7** (1983) 209.
5. deGee, A. L. W., Carstens, P. H. B., McCann, P. P. & Mansfield, J. M., Role of antibody in the elimination of trypanosomes after DL-α-difluoromethylornithine chemotherapy. *J. Parasitol.*, **69** (1984) 818.
6. Giffin, B. F., McCann, P. P., Bitanti, A. J. & Bacchi, C. J., Polyamine depletion following exposure to DL-α-difluoromethylornithine both *in vitro* and *in vivo* initiates morphological activation in a monomorphic strain of *Trypanosoma brucei brucei*. *J. Protozool.*, **33** (1986) 239.
7. Giffin, B. F., The role of polyamines in the growth and transformation of the African trypanosome. In *Progress in Polyamine Research*, ed. V. Zappia & A. E. Pegg. Plenum Publishing, New York, 1988, p. 651.
8. Giffin, B. F. & McCann, P. P., Physiological activation of the mitochondrion and the transformation capacity of DFMO induces intermediate and short stumpy bloodstream form trypanosomes. *Am. J. Trop. Med. Hyg.*, **40** (1989) 487.
9. Bacchi, F. J. & McCann, P. P., Parasitic protozoa and polyamines. In *Inhibition of Polyamine Metabolism: Biological Significance and Basis for New Therapies*, ed. P. P. McCann, A. E. Pegg & A. Sjoerdsma. Academic Press, Boca Raton, FL, 1987, p. 317.
10. Bajpai, P. K. & Benghuzzi, H. A., Ceramic system for long term delivery of chemicals and biologicals. *J. Biomed. Mater. Res.*, **22** (1988) 1245.
11. Benghuzzi, H. A. & Bajpai, P. K., ALCAP ceramic implantable device and the delivery of steroids, proteins, and amino acids in human plasma. In *Proceedings of the 15th Annual Northeast Bioengineering Conference*, ed. S. Buns. Boston, MA, 1989, p. 57.
12. Benghuzzi, H. A. & Bajpai, P. K., The effects of ALCAP mixed particle sizes on destiny and delivery of proteins *in vitro*. In *Proceedings of the 13th Annual Northeast Bioengineering Conference*, ed. K. R. Foster. Philadelphia, PA, 1987, p. 566.
13. Benghuzzi, H. A. & Bajpai, P. K., The effect of molecular weight on the release of proteins from ALCAP ceramic capsules. In *Proceedings of the 14th Annual Northeast Bioengineering Conference*, ed. J. R. LaCourse. Durham, NH, 1988, p. 96.
14. Benghuzzi, H. A. & Bajpai, P. K., The delivery of danazol, dihydrotestosterone, and testosterone combinations from ALCAP ceramic capsules. In *Trans. 13th Annual Meeting of the Society for Biomaterials*, New York, 1987, p. 214.
15. Benghuzzi, H. A., Beck, S., Arar, H. & Bajpai, P. K., Maintenance of accessory reproductive organs in castrated male rats by means of testosterone delivered from ALCAP ceramics. In *Proceedings of the 14th Annual Northeast Bioengineering Conference*, ed. J. R. LaCourse. Durham, NH, 1988, p. 100.
16. Benghuzzi, H. A. & Bajpai, P. K. Comparison of subcutaneous and intraperitoneal sustained release of testosterone by ALCAP delivery devices. In *Proceedings of the 25th Annual Rocky Mountain Bioengineering Symposium*, ed. H. Valenta & S. Fisher. Pergamon Press, New York, 1986, p. 482.
17. Benghuzzi, H. A. & Bajpai, P. K., Effects of polylactic acid impregnation and variation of aqueous ethanol

concentration on the *in vitro* delivery of testosterone by ALCAP ceramics. In *Biomedical Engineering V, Recent Development*, ed. S. Saha. Pergamon Press, New York, 1986, p. 482.
18. Benghuzzi, H. A. & Bajpai, P. K., Effects of polylactic acid impregnation and compression load on the delivery of proteins, steroids and amino acids from ALCAP ceramic capsules. In *Proceedings of the 17th Southern Biomedical Engineering Conference*, ed. D. Moyle. Greenville, SC, 1988, p. 36.
19. Benghuzzi, H. A. & Bajpai, P. K., Effects of compression loads and polylactic acid on the delivery of low molecular weight substances from ALCAP ceramics. In *Trans. 12th Annual Meeting of the Society of Biomaterials*, Minneapolis St Paul, MN, 1986, p. 102.
20. Benghuzzi, H. A. & Bajpai, P. K., Effects of compression loads on delivery of steroids (testosterone) from ALCAP ceramics. *Fed. Proc.*, **45** (1986) 171.
21. Benghuzzi, H. A. & Bajpai, P. K., A ceramic system for delivering mixtures of steroids. In *Proceedings of the 15th Annual Northeast Bioengineering Conference*, ed. S. Buns. Boston, MA, 1989, p. 61.
22. Strasser, J. & Bajpai, P. K., Polymer impregnated ALCAP ceramics: delivery of gossypol acetic acid. In *Proceedings of the 11th Annual Northeast Bioengineering Conference*, ed. W. Kuklinski & W. Ohley. Worcester, MA, 1985, p. 166.
23. Benghuzzi, H. A., Barbaro, R. M. & Bajpai, P. K., Sustained delivery of ^{3}H-thymidine by means of ceramic capsules in rats. In *Proceedings of the 26th Annual Rocky Mountain Bioengineering Symposium and 26th International ISA Biomedical Science Instrumentation Symposium*, ed. D. Carlson. Ames, IA, 1989, p. 169.
24. Benghuzzi, H. A. & Bajpai, P. K., The effect of isoelectric pH on the rate of delivery of proteins by alumino–calcium–phosphorous oxides (ALCAP) implantable devices *IRCS Med. Sci.*, **14** (1986) 1157.
25. Benghuzzi, H. A. & Bajpai, P. K., Sustained delivery of difluoromethylornithine by alumino–calcium–phosphorous oxide ceramic capsules. *Clin. Res.*, **34** (1986) 395.
26. Benghuzzi, H. A. & Bajpai, P. K., Sustained delivery of difluoromethylornithine by means of implantable ceramic devices. *J. Invest. Surg.*, **2** (1988) 163.
27. McFall, F. & Bajpai, P. K., Fate of resorbable alumino–calcium–phosphorous oxide (ALCAP) ceramic implants in rats. *Trans. Soc. Biomater.*, **7** (1984) 354.
28. Benghuzzi, H. A. & Bajpai, P. K., Danazol delivery by polymer impregnated ALCAP ceramic capsules. *Clin. Res.*, **34** (1986) 926A.
29. Moore, S. & Stein, W. H., A modified ninhydrin reagent for the photometric determinations of amino acids and related compounds. *J. Biomed. Chem.*, **211** (1954) 907.
30. Fozard, J. R., Part, M. L., Prakash, N. J. *et al.*, L-Ornithine decarboxylase: an essential role in early mammalian embryogenesis. *Science*, **208** (1980) 505.
31. Bortolome, J. V., Schweitzer, L., Slotkin, T. A. *et al.*, Impaired development of cerebellar cortex in rats treated postnatally with α-difluoromethylornithine. *Neuroscience*, **15** (1985) 203.
32. Homma, Y., Ozono, S., Numata, I. *et al.*, Inhibition of carcinogenesis by α-difluoromethylornithine in heterotropically transplanted rat urinary bladders. *Cancer Res.*, **45** (1985) 648.