tablet when subjected to diametral loading. The AFV values will be large when the resistance to flow is high—a situation which arises as a result of formation of a large number of high strength interparticle bonds. These conditions predominate when plastic deformation of the particles takes place during compaction, thus the results suggest that anhydrous lactose possesses higher plasticity than any of the other direct compression excipients studied. Both AFV and NWF determinations were found to be useful parameters for predicting the plasticity of excipients.

Conclusion. It may be concluded that use of NWF determinations allowed quantitative differences in plasticity of some excipients to be distinguished. Failure viscosity used the time component of tablet loading as a multiplier and was therefore found to have a similar sensitivity to changes in excipient plasticity to that obtained using NWF and it was considered that both AFV and NWF determinations gave a good indication of the plasticity of different excipients. It can be concluded that use of a second new parameter termed 'power of failure', developed to take account of differences in crack progagation rates between tablet excipients lacked the sensitivity required to distinguish changes in plastic behaviour, but may provide a useful method of comparing tablet crushing tablet strengths using test equipment with different rates of platen movement.

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J. Pharm. Pharmacol. 1987, 39: 650–652
Communicated December 30, 1986

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Unloaded polyisobutylcyanoacrylate nanoparticles: efficiency against bloodstream trypanosomes

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The potential use of polyisobutylcyanoacrylate nanoparticles in antiparasitic chemotherapy is described. The nanoparticles on their own proved to have trypanocidal activity against *Trypanosoma brucei brucei* in-vitro but not against *Trichomonas vaginalis* or *Entamoeba histolytica*. The trypanocidal activity was partly confirmed in-vivo with *T. brucei*-infected mice.

Previous studies have demonstrated the interest in polyalkylcyanoacrylate nanoparticles as colloidal carriers of drugs (Couvreur et al 1979, 1982). These ultrafine particles, 100–200 nm in size, were shown to be able to entrap in their polymeric network a wide variety of drugs (i.e. cytostatics, antibiotics, hormones), the tissue distributions of which were modified after administration to animals (Couvreur et al 1980; Grislain et al 1983).

Moreover, because of the lack of success in the treatment of third-world endemic parasitic diseases (Meshnick 1984), drug carriers have been considered as a means of achieving a better specificity of commonly used antiparasitic compounds (Alving et al 1980). In view of such a strategy, we have made preliminary experiments to check the toxicity of nanoparticles against different species of parasitic protozoa in-vitro. This paper describes unexpected antiparasitic activity observed with unloaded nanoparticles against *Trypanosoma brucei brucei* both in-vitro and in-vivo.

Materials and methods

Isobutylcyanoacrylate monomer was obtained from Ethnor (Paris, France). Various chemicals including dextran 70 and D-glucose were purchased from Prolabo (Paris, France). Culture media and foetal calf serum were from GIBCO (Biocult., Paris, France) and culture plates from Falcon (Grenoble, France). Female mice CD1 (Charles River, Cleon, France) were 18–20 g.

The parasite strain *Trypanosoma b. brucei* (Institut Pasteur, Paris, France), conserved in liquid nitrogen with 10% glycerol as cryoprotectant, was administered to the mice by intraperitoneal injection according to Hawking (1963a).

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Nanoparticle preparation. Isobutylcyanoacrylate monomer (100 µL) was added with mechanical stirring to 10 mL of an aqueous polymerization medium (dextran 70, 1% in 10^{-3} M HCl). After polymerization of the monomer (2 h) the milky suspension obtained was neutralized with 0·1 M NaOH, and brought to isotonicity with 5% glucose. Nanoparticles were then freeze-dried at −40 °C using a freeze-drier (Secfroid, type TS 600, Aclens, Lausanne, Switzerland) for 48 h under vacuum (6 × 10 mbar). Resuspension of the nanoparticles was carried out by a single addition of distilled water (10 mL).

The mean size of the nanoparticles by a laser light scattering method (Nanosizer, Coulter, Margency, France) was 130 ± 10 nm.

In-vitro tests. Blood was collected aseptically from the tail vein of 3-day infected mice and then diluted 40-fold with RPMI 1640. The bloodstream form of *Trypanosoma b. brucei* was cultivated in-vitro without loss of infectivity for 24 h (Hawking 1963b) and the number of trypanosomes determined by haemocytometer counting.

The screening assay was performed in flat-bottomed 24 well plastic trays with a well size of 17·8 × 16 mm. Each well received 1·5 mL medium without feederlayer fibroblasts to prevent nanoparticle adsorption to cells. The wells were inoculated with approximately 200 trypanosomes in a volume of 0·12 mL. The culture medium was Hepes (25 mM) buffered RPMI 1640 supplemented with 10% foetal bovine serum. Operating conditions were 37 °C air with CO_2 10%.

Nanoparticle samples (10 µL) were added to trypanosome cultures, each nanoparticle concentration being tested twice. Polymerization medium was used as a control. Both nanoparticles and polymerization medium were compared with untreated trypanosomes as reference. The viability of the trypanosomes was estimated by direct observation of parasitic motility, using an optical microscope. The infectivity of incubated parasites was determined in duplicate, in mice, after intraperitoneal injection of 1 mL of culture medium withdrawn from the bottom of each well, and recording the survival rate after 30 days.

Amoebicidal activity was determined in-vitro using *Entamoeba histolytica* (Rahman strain) which was used to inoculate Pavlova-Jones medium with 10 000 amoebae mL^{-1}. Nanoparticles were then added to the incubation medium at concentrations ranging between 18–830 µg mL^{-1}.

The viability of the parasites was finally estimated microscopically after the amoebae were subcultivated in a fresh incubation medium.

Trichomonas vaginalis (V strain) (30 000 mL^{-1}) was inoculated into thioglycolate medium enriched with horse serum (5%) and that viability was assessed as for *E. histolytica*.

In-vivo test. The in-vivo experiment was performed according to the method of Hawking (1963a), slightly modified. Each mouse received 10^4 trypanosomes, intraperitoneally and, if untreated, died after 3–4 days with an increasing blood parasitaemia.

Nanoparticle samples were administered i.p. into the left flank of mice 10 min after parasite inoculation into the right flank. Survival time was noted and 30 day-survivors were considered to be cured.

Results

In-vitro screen. After 1 and 24 h incubation of *T. b. brucei* with nanoparticles at concentrations of greater than 1·2 µg mL^{-1} of polymer, parasitic mobility disappeared, whereas it was intact after incubation with nanoparticle-free polymerization medium (Table 1). Of the lower polymer concentrations, at 0·6 µg mL^{-1}, parasitic mobility was reduced after 1 h and suppressed after 24 h (Table 1) and at 0·3 µg mL^{-1}, there was no change compared with the control parasites.

Concerning infectivity, all mice survived and remained negative, 30 days after being inoculated with trypanosomes pretreated with 1·2 µg mL^{-1} of nanoparticles or more. However, mice infected with trypanosomes previously exposed to 0·3 or 0·6 µg of polymer became infected and died after 4 and 5 days, respectively. Control mice, which received untreated trypanosomes, as well as those receiving trypanosomes exposed to the polymerization medium, died after 4 days.

Table 1. In-vitro efficiency of polyisobutylcyanoacrylate nanoparticles against *T. b. brucei*.

	Polymer concn (µg mL^{-1})									
	62·5	31·2	15·6	7·8	6·2	1·2	0·6	0·3	P. med.	Cont.
Parasite mobility at 1 h	−	−	−	−	−	−	±	+	+	+
24 h	−	−	−	−	−	−	−	+	+	+
Parasite infectiosity after 1 h incubation	−	−	−	−	−	−	+	+	+	+
Mean survival time of mice (days) (n = 4)	>30	>30	>30	>30	>30	>30	5	4	4	4

P. med. = polymerization medium.
Cont. = untreated control.

Table 2. In-vivo efficiency of polyisobutylcyanoacrylate nanoparticles against *T. b. brucei*-infected mice.

	Volume admin. (mL/inject.)	Polymer dose (mg kg^{-1})	MST (days)	T/C (%)
Control (untreated) (n = 7)	—	0	4 ± 0·25	100
Control (Polymerization medium) (n = 5)	1	0	4 ± 0·25	100
Nanoparticles (n = 5)	1	500	6 ± 0·25	150
	0·5	250	5 ± 0 25	125

n = no. of mice.
MST = median survival time.
T/C = median survival time treated mice/median survival time control mice, percent.

Absence of toxicity to the parasites was recorded after incubation of nanoparticles with *T. vaginalis* and *E. histolytica* up to a concentration of 25 and 180 mg polymer L^{-1} medium, respectively.

In-vivo tests. In-vivo efficiency of drug-free unloaded polyisobutylcyanoacrylate nanoparticles was tested, as a preliminary experiment, on intraperitoneally infected mice. Depending on the dose of the polymer used a moderate trypanocidal activity was observed (Table 2).

Discussion

The present data show evidence of the destruction of trypanosomes incubated with empty nanoparticles, even with polymeric concentrations as low as 1·2 μg mL^{-1}. As the level of cytotoxicity recorded with nanoparticles on mammalian ATCC L929 fibroblastic cells was up to 100 μg mL^{-1} (Guise et al 1986) it is apparent that nanoparticles were about 100 times more toxic to trypanosomes than to mammalian cells.

Nanoparticles were inactive against *T. vaginalis* as well as *E. histolytica* which could indicate that the carrier may display a certain specificity against members of the Kinetoplastida.

Although less pronounced than that in-vitro, the trypanocidal activity of nanoparticles in-vivo was significant. Protection was not complete probably because some of the parasites escaped the trypanocidal activity of the nanoparticles, as after intraperitoneal administration, the parasites are rapidly disseminated throughout the body and that strain of *T. b. brucei* is very infective in experimental mice.

Whatever the mechanism of action of the effects described here, considering the low toxicity of cyanoacrylic nanoparticles (Couvreur et al 1986), their use as antiparasitic drug carriers should be of potential interest.

This work was supported by l'Institut National de la Santé et de la Recherche Médicale (INSERM-Contrat Extérieur no. 862008).

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